Lectures

L1 (2PA)

The ins and outs of glutamate receptor trafficking during synaptic plasticity

Nicoll, Roger A. (*Department of Cellular and Molecular Pharmacology, University of California at San Francisco, San Francisco, California, USA*)

Glutamate, the major excitatory neurotransmitter in the brain, acts primarily on two types of ionotropic receptors, AMPA receptors and NMDA receptors. Work over the past decade indicates that the number of synaptic AMPA receptors is tightly regulated and may serve as a mechanism for information storage. Recent studies show that stargazin, the mutated protein in the ataxic and epileptic mouse stargazer, is necessary for the expression of surface AMPA receptors in cerebellar granule cells. Stargazin is a small tetraspanning membrae protein and is a member of a family of proteins referred to as transmembrane AMPAR regulatory proteins (TARPs). These proteins are differentially expressed throughout the brain. TARPs control AMPA receptor trafficking through the endoplasmic reticulum and are important for the maturation and proper folding of AMPARs. They are necessary for the delivery of AMPA receptors to the cell surface, as well as to the synapse. Finally TARPs control the gating of synaptic receptors. The role of TARPs is analogous to non-pore forming auxiliary subunits of voltage gated ion channels. Thus TARPs provide the first example of auxiliary subunits of ionotropic receptors. In this talk I will review the pivotal role that TARPs play in the life history of an AMPA receptor.

L2 (3PB)

The Actin Cytoskeleton and Synaptic Plasticity

Matus, Andrew (*Friedrich Miescher Institute, Basel, Swiss Confederation*)

Dendritic spines form the postsynaptic contact elements for most excitatory synapses in the central nervous system. Using time-lapse imaging of living neurons expressing proteins tagged with green fluorescent protein (GFP) we discovered that dendritic spines undergo rapid changes in shape thus identifying them as the major sites of morphological plasticity in neuronal circuits of the brain. This motility is driven by dynamic actin filaments and is differentially regulated by various subtypes of postsynaptic glutamate receptors. Activation of AMPA receptors produces an immediate blockade of spine motility which is reversed as soon as the stimulus is withdrawn. By contrast, blockade of spine motility via NMDA receptors requires 30 min to develop and persists for hours after the stimulus is withdrawn. To understand the cellular mechanisms underlying these effects we examined the influence of actin binding proteins in dendritic spines. Profilin shows activity-dependent targeting to spine heads which depends on activation of NMDA receptors and is induced by electrical stimulation patterns associated with changes in synaptic strength such as LTP. Simulataneously actin dynamics are suppressed and spine motility is blocked for several hours. Conversely, blocking profilin targeting to spines by expressing a peptide that inhibits its binding to VASP family proteins destabilizes spine morphology. Together with data for other actin binding proteins to be presented, this suggests that several distinct receptor dependent mechanisms regulate the dynamic state of the spine actin cytoskeleton and hence morphological plasticity at excitatory synapses.

L3 (1EA)

Water and ion channels in kidney function

Sasaki, Sei (*Dept. Nephrology. Grad. Sch.Tokyo Med Dent Univ. Tokyo, Japan*)

Kidney is the main organ in the maintenance of water and electrolyte homeostasis of extracellular fluid of the body. Extensive physiological research has been performed to understand this important kidney function. The research has developed from the organ level to molecular level and the development was accompanied by advancement of experimental technology, i.e., from the clearance method to molecular biology. Molecular biological studies have identified a wide variety of channel and transporter proteins that exist in renal epithelial cells. Coupled with human genetic studies it has been shown that mutations of the genes encoding renal membrane transport proteins (channels and transporters) cause human hereditary diseases such as water and solute loosing or retaining diseases. At present nearly all genes responsible for relatively popular human hereditary solute and water disorders have been determined and the current research interest is focusing on the mechanisms of regulation of the membrane transport proteins at molecular level. For this aim the disease-causing mutations found in hereditary diseases have provided valuable information, for example, possible phosphorylation sites and protein-protein interaction domains. In this lecture, I will present our data on water channels (aquaporin) and chloride channels and their diseases of the kidney, and will show how molecular and genetic studies work together to open a new field of the transport protein research.

L4 (1EB)

Epegenetics and Regulation of Gene Expression through Nuclear Hormone Receptors

Kurokawa, Riki (*Division of Gene Structure and Function, Research Center for Genomic Medicine, Saitama Medical School*)

Coactivator and corepressor (cofactors) are essential for the transcriptional regulation by nuclear receptor (NR). Coactivator possesses intrinsic histone acetylase (HAT) activity and corepressor is associated with histone deacetylase (HDAC). These enzyme activities are indispensable for the function of these cofactors, and also for modulation of chromatin structures, or epigenetic regulation. Recently, we have shown that dysregulation of the HAT activity causes human diseases such as Rubinstein-Taybi Syndrome (RTS) and Huntington's disease.Our present efforts are to identify the roles of HAT activity in eukaryotic transcriptional regulation. Recently we have found an RNA-binding protein, TLS (translocated in liposarcoma)/FUS, which has potent inhibitory effect on the HAT activity of CREB-binding protein (CBP). This inhibitory molecule has a regulatory role in a CREB-dependent transcription system. Unexpectedly, specific RNA sequences stimulate the inhibitory activity of TLS. Such RNA sequence exists at 94% of coding regions of human genome. The specific RNAs form guanine (G) quartet structures in a K^+-dependent manner. This structure enhanced binding of the RNAs to TLS. Addition of the RNAs in HAT assay of CBP with TLS resulted in more efficient inhibition to the HAT activity. These results have demonstrated that the RNAs with G-quartet structure function as ligands for TLS and that the TLS-RNA complex is a potent repressor for the CREB-dependent transcriptional systems, suggesting that TLS mediates RNA-dependent transcriptional repression.

L5 (1EC)

Genetically engineered rodents in brain research

Yanagawa, Yuchio[1,2] ([1]*Grad. Sch. Med. Gunma Univ., Maebashi, Japan;* [2]*SORST, JST, Kawaguchi, Japan*)

Certain types of neurons in the brain are difficult to study because they cannot be easily identified by location or morphological criteria alone. One approach to identify such neurons is to label them with a reporter protein. GABAergic inhibitory neurons play an important role in the regulation and stabilization of network activities, but they are primarily scattered throughout mammalian central nervous systems and thus can be hardly identified in live brain preparations. GABA is synthesized from glutamic acid by glutamate decarboxylase (GAD) and is accumulated into synaptic vesicles by vesicular GABA transporter (VGAT). Two isozymes of GAD, GAD65 and GAD67 and VGAT are primarily expressed in GABAergic neurons. To facilitate the study of GABAergic neurons, we generated the GAD67-GFP mice using a gene targeting method via homologous recombination in ES cells. EGFP fluorescence was specifically observed in the GABAergic neurons in the GAD67-GFP mice. The GAD67-GFP mice have helped to elucidate the anatomical profile of GABA neuronal network and its electrophysiological activity as well as the development of GABAergic neurons. In addition, we generated bacterial artificial chromosome transgenic rats, which specifically express a modified YFP, Venus protein under the control of the VGAT promoter. In VGAT-Venus rats, Venus fluorescence was sufficiently bright to visualize GABAergic neurons and thus allowed whole-cell patch-clamp recordings from visually identified GABAergic neurons. The detailed morphological and functional analyses of GABAergic neurons in brain slices will greatly benefit from these genetically engineered rodents.

L6 (1EF)

Visualizing Columnar Architectures with High-Resolution fMRI

Cheng, Kang (*RIKEN Brain Science Institute, 2-1 Hirosawa, Wako-shi, Saitama 351-0198, Japan*)

Among the many neuroimaging tools available for studying human brain functions, functional magnetic resonance imaging (fMRI) is the most widely used today. One advantage of fMRI over other imaging techniques is its relatively high spatial resolution. High-field fMRI, with its superb signal-to-noise ratio, has strengthened the capability of fMRI and allowed mapping of fine cortical architectures in human primary visual cortex (V1). In this presentation, I will explain the factors limiting the spatial precision and resolution of fMRI, describe the benefits that high-field fMRI offers in dealing with these issues, and introduce several high-resolution studies that have been conducted in our laboratory on the functional organization of human primary V1, including mapping of ocular dominance columns, temporal frequency dominance domains and orientation selectivity.

L7 (3EC)

Clonal identification of mulltipotent stem/progenitor cells in the developing liver and pancreas using flowcytometric cell sorting

Taniguchi, Hideki[1,2] ([1]*Department of Regenerative Medicin, Graduate School of Medicine, Yokohama City University;* [2]*Research Unit for Organ Regeneration, Center for Divelopmental Biology, RIKEN*)

Using flowcytometry combined with single-cell-based assays, we prospectively identified hepatic stem cells with multilineage differentiation potential and self-renewing capability in the developing mouse liver. c-Met+ CD49f+/low CD29+ c-Kit- CD45- TER119- cells in fetal liver could be clonally propagated in culture, where they continuously produced hepatocytes and cholangiocytes as descendants while maintaining primitive stem cells. When cells that expanded in vitro were transplanted into recipient animals, they morphologically and functionally differentiated into hepatocytes and cholangiocytes, with reconstitution of hepatic cord and bile duct structures. These data indicate that self-renewing multipotent stem cells are retained in midgestational developing liver. The pancreas also contains a population of pancreatic stem cells that generate endocrine, exocrine, and ductal cells during development, neogenesis, and regeneration. By combining flowcytometry and clonal analysis, we show here that stem/progenitor cells of pancreatic endocrine and exocrine cells that reside in the neonatal mouse pancreas. Clonally isolated stem/progenitor cells could be used to reveal the mechanism of cell differentiation in digestive organs, and also could provide new insight into therapies for liver diseases, diabetes mellitus and cancer.

L8 (3EF)

Development of a comprehensive cardiac cell model

Noma, Akinori (*Grad. Sch. Med. Kyoto Univ. Japan*)

To understand mechanisms of various functions of cardiac cells, the whole cell model was developed. The model is composed of ion channels, ion transporters, membrane receptors, coupling between the sarcolemmal Ca channel and the ryanodine receptor channels, sarcoplasmic reticulum with Ca, SERCA, calsequestrin, the contraction machinery, intracellular ion concentrations, mitochondria model, and gap junction channels. The function of these functional units are mostly described with experimenatal equations in literatures, otherwise model adjusted refering to experimental observations on macroscopic levels. The whole cell model, we call 'Kyoto Model', well reconstracts the pacemaker activity in the sinoatrial node cell, the ventricular action potential, the contraction, and homeostasis of the intracellular ion concentrations, energy metabolism and the cell volume regulation, classic regulation by the autonomic nervous transmitters. Responses to various experimental interventions are reversible. Model based new hypotheses were obtained for the pacemaker mechanisms, cell volume regulation via ion fluxes through sarcolemma, and the Ca mediated upregulation of the mitochondrial ATP production on increasing the work load. The model still needs to be revised and implemented with new mechanisms, thereby the integrations of the experimental knowledge will be most efficiently and systematicall achieved through developing the whole cell model. All these model constructions were conducted on simBio, which is newly developed Java package by us for constructing cell models on a large scale (http://www.sim-bio.org/).

L9 (2LB1)

Integrative Physiology of Cardiac Function

Suga, Hiroyuki (*Nat'l Cardiovasc Ctr, Suita, Osaka, Japan*)

I would like to review the four eurekas I have experienced in my cardiac function research over these 40 years. In 1966, the major concepts of cardiac pump function were Frank's ventricular pressure (P)-volume (V) relation, Starling's law of the heart, Sarnoff's ventricular function curve, and Sonnenblick's myocardial force-velocity relation. Since these could not persuade me, I started my own research, using canine in situ beating hearts. I obtained P-V loops of the left ventricle (LV) and their relations with LV contractility. My first eureka suggested in 1967 that the contracting LV could be modeled as a time-varying elastance E(t). I then found that its end-systolic peak (Emax) could serve as a reliable contractility index, later adopted as a core concept in cardiac physiology. My second eureka suggested in 1974 that the E(t) model could provide a specific P-V area (PVA) as a measure of the total mechanical energy generated by an LV contraction. Both Emax and PVA could reliably predict LV oxygen consumption (Suga: Ventricular energetics. Physiol Rev, 1990). My third eureka suggested in 1994 that the total amount of calcium recruited in the excitation-contraction coupling could be calculated from a set of Emax, PVA, and a decay time constant of the post-extrasystolic transient alternans. My forth eureka suggested in 2003 that the sliding length of a crossbridge per ATP could be calculated from a set of PVA and its oxygen cost to be variable up to more than 20 times unit step. These integrative physiological findings seem to have advanced a better integrative understanding of the pump function of a beating heart.

L10 (2LB2)

Exploring the Logic for Olfactory Perception

Mori, Kensaku (*Grad. Sch. Med. Univ. Tokyo, Tokyo, Japan*)

Exploring the Logic for Olfactory Perception Mori, Kensaku (Dept. Physiol., Grad. Sch. Med. Univ. of Tokyo, Japan) The olfactory perception plays a key role in the daily life of human and animals. Since the discovery of odorant receptors in 1991, we have witnessed a rapid progress in the understanding of the olfactory system. However, the recent studies focused on the early olfactory processing at the levels of odorant receptors, sensory neurons and olfactory bulb (OB). The central processing of olfactory information in the mammalian brain is still not well understood. Late Professor Sadayuki Takagi and his colleagues are the pioneers who explored the odorant-response specificity of neurons from the OB through the olfactory cortex to the orbitofrontal cortex in the monkey brain. In the OB, individual glomeruli represent a single odorant receptor, and the glomerular sheet of the OB forms odorant receptor maps. Studies of OB mapping show that (1) individual glomeruli respond to a range of odorants that share a specific combination of molecular-features, that (2) each glomerulus appears to be unique in its molecular receptive range property, and that (3) glomeruli with similar molecular receptive range properties are located in proximity and form molecular-feature clusters. The olfactory cortex reads the molecular-feature maps in the OB and is thought to integrate information from different molecular-feature detecting glomeruli to form the olfactory image of objects. We discuss also behavioral-state-dependent gating of olfactory information flow at the level of the olfactory cortex.

L11 (3LA1)

Ca^{2+} signaling and regulation of cell cycle in mammalian fertilization

Miyazaki, Shunichi (*Dept. Physiol., Tokyo Women's Med. Univ. Sch. Med., Tokyo, Japan*)

Late Professor Susumu Hagiwara (1922-89) is a great physiologist who discovered Ca^{2+}-dependent action potentials in barnacle muscle fibers in 1964, distinct from Hodgkin/Huxley-type Na^{+} spikes. Thereafter, he characterized Ca^{2+} and other ion channels in a wide variety of cells in relation to functions. He was interested in Ca^{2+}-regulated cell functions. Fertilization is one of such phenomena. A dramatic increase in intracellular Ca^{2+} occurs at fertilization in eggs of all species examined to date, and it is a pivotal signal for egg activation seen in resumption of meiosis and cell cycle progression. Ca^{2+} signaling at fertilization was first investigated in sea urchin and fish eggs in mid-70s. We began to address the mechanism in mammals in 1980. Mammalian eggs exhibit repetitive Ca^{2+} increase mainly due to Ca^{2+} release from the ER via type 1 IP_3 receptor/Ca^{2+} channel. Accumulated evidence indicates that a cytosolic sperm factor is driven into the ooplasm upon sperm-egg fusion and induces repetitive Ca^{2+} release. Recent studies have shown that a novel isozyme of phospholipase C, PLCζ, is a strong candidate of the sperm factor. Fertilization-like Ca^{2+} oscillations are induced by injection of sperm extract, PLCζ RNA, or recombinant PLCζ into mouse eggs. PLCζ has extremely high Ca^{2+}-sensitivity in PLC activity and nuclear translocation ability. These properties qualify PLCζ as the sperm factor that initiates and drives cell cycle-dependent Ca^{2+} oscillations. Ca^{2+} activates CaMK II and thereby ubiquitin/proteasome system, leading to degradation of cyclin, inactivation of metaphase promoting factor, and resumption of meiosis.

Symposia

SYMPOSIA
Temporal organization of physiology and behaviors—Functions of multi-oscillatory hierarchical system [IUPS Symposium in the 83rd Annual Meeting of the Physiological Society of Japan]

S1 (1S-01A1)
SCN vs Non-SCN clocks

Honma, Ken-ichi; Abe, Hiroshi; Honma, Sato (*Department of Physiology, Hokkaido University Graduate School of Medicine*)

The circadian system in mammals entrains to several oscillating factors in the environment. Among them, a light-dark cycle is the common as well as most potent factor so far examined. Entraining light signals enter the brain through the retinohypothalamic tract and reach the suprachiasmatic nucleus (SCN) where the master clock is located. The circadian system in humans is able to entrain to non-photic factors as best exemplified by entrainment of totally blind persons. However, it is not known whether the non-photic entrainment is achieved by the clock in the SCN or other clocks located somewhere outside the SCN. Methamphetamine, a CNS stimulant, is known to produce robust activity rhythms in bilaterally SCN lesioned rats and aperiodic clock mutant or *Cry1/Cry2* double knockout mice, indicating the existence of a behavior related oscillator(s) outside the SCN. Interestingly, the activity rhythm produced by methamphetamine shows similar characteristics to those observed in the human sleep rhythm, such as internal desynchronization from the circadian rhythms, a long endogenous period, circabidian (ca. 48 h) rhythm and entrainment to non-photic factors. The underlying mechanism is regarded as a non-SCN clock. A feeding-associated oscillator is another example of non-SCN clock. Activity rhythms in the circadian domain were developed in rats and mice, when feeding is restricted to a fixed time of day. The feeding-associated oscillation persists for several weeks after the termination of restricted feeding. Using these model animals, we are attempting to find out the site of oscillation of non-SCN clock.

S2 (1S-01A1)
Luminescence reporter techniques have uncovered complexity among oscillatory structures in the mammalian circadian system.

Yamazaki, Shin[1]; Davidson, Alec J.[2] ([1]*Department of Biological Sciences, Vanderbilt University, Nashville TN, USA;* [2]*Departmenf of Biology, University of Virginia*)

Luminescence reporters have been used successfully in studies of circadian rhythms in many organisms. Ever since luciferase was introduced for real-time monitoring of gene expression rhythms in plants and cyanobacteria, luminescence reporter techniques have become a powerful tool for noninvasive assays of circadian oscillations. Using a real-time light detection system from cultured rodent tissues, we were able to record a circadian oscillation from the cultured suprachiasmatic nucleus (master pacemaker in the brain) for more than 16 month. We also discovered that most peripheral tissues were rhythmic in culture with distinct phases relative to the light dark cycle to which the animal had been exposed. Daytime restricted-feeding uncoupled the rhythms in digestive tissue from the environmental light cycle. Therefore the mammalian circadian system consists of at least two oscillatory systems; one is coupled with environmental light and another is coupled with food. This might have significant meaning for the adaptation of the circadian system to the natural environment. The study of multiple oscillatory systems also has had significant impact on medicine. We have begun to understand the circadian relationship between tumor cells and normal cells. Management of circadian rhythms may provide a new therapeutic approach for combating human disease.

S3 (1S-01A2)
Reconfiguring Cellular Ensembles Within the Suprachiasmatic Nucleus

Schwartz, William J. (*Department of Neurology, University of Massachusetts Medical School, Worcester, Massachusetts, Japan*)

The circadian clock in the suprachiasmatic nucleus (SCN) is composed of multiple single-cell circadian oscillators, and a challenge now is to learn how individual cells are assembled to create an integrated tissue pacemaker that can orchestrate the temporal programs of whole organisms. By measuring SCN gene expression (in situ hybridization) as an assay of clock activity, we have found that assembled cellular oscillators can assume different configurations within the SCN, giving rise to unusual locomotor activity patterns. Thus, in hamsters maintained in constant light, splitting of the single circa-24 hr activity bout into two circa-12 hr components appears to be the consequence of a paired SCN that is reorganized into two oppositely-phased, left- and right-sided circadian pacemakers. In rats exposed to an artificially short light-dark cycle, the simultaneous expression of two stable circadian motor activity rhythms with different period lengths corresponds to the desynchronization of circadian pacemakers in the ventrolateral and dorsomedial subdivisions of the SCN (as previously defined by regional differences in their cyto- and chemo-architecture and topography of afferents and efferents). These kinds of reconfigurations (left/right, dorsal/ventral) of regional oscillators should provide a powerful approach for understanding intercellular coupling, tissue organization, and differential outputs of the SCN in intact, behaving animals.

S4 (1S-01A3)

An Important Role of Circadian Phosphorylation of CRY Proteins in the Mouse Peripheral Clock

Fukada, Yoshitaka (*Dept. Biophys. & Biochem., Graduate School of Science, University of Tokyo, Tokyo, Japan*)

Protein phosphorylation plays a crucial role for time-keeping mechanism of circadian clock systems. Several clock proteins undergo temporal change in phosphorylation in the mouse liver, a well-characterized peripheral clock tissue, but it was unclear as to whether the central negative regulator CRYs are phosphorylated *in vivo*. We found that mCRY1 and mCRY2 are phosphorylated by MAPK *in vitro* and identified Ser265 and Ser557 of mCRY2 as *in vitro* phospho-acceptor residues. Similarly, MAPK phosphorylated mCRY1 at Ser247, a site corresponding to Ser265 of mCRY2. An effect of the Ser phosphorylation was investigated by mutating Ser247 of CRY1 and Ser265 of CRY2 to Asp, which resulted in attenuation of each CRY's ability to inhibit BMAL1:CLOCK-mediated transcriptional activation. On the other hand, we found Ser557-phosphorylated CRY2 accumulated in the liver during the subjective night in parallel with CRY2 protein, and the phosphorylated form reached its maximal level at late night preceding the peak-time of the protein abundance by approximately 4 hrs in LD and DD conditions. The Ser557-phosphorylated form of CRY2 was localized in the nucleus, whereas CRY2 protein was located in both the cytoplasm and nucleus. Functionally, Ser557-phosphorylation of CRY2 allowed subsequent phosphorylation of the protein by GSK3β, resulting in efficient degradation of CRY2 by a proteasome pathway. These results demonstrate the important role of priming phosphorylation at Ser557 for destabilization of CRY2 and illustrates a model that the circadian phosphorylation of CRY2 contributes to its rhythmic degradation.

S5 (1S-02B1)

Commemorative and illustrious individuals devoting to physiological sciences in Japan: Kojiro Matsuda and Yasuyoshi Nisimaru

Arita, Makoto[1,2]; Kameyama, Masaki[1,3]; Nisimaru (Yamada), Naoko[1,4] (*[1]SCCRE:The supporting Center for Clinical Research and Education, Osaka, Japan; [2]Yufuin Kohseinenkin Hosp., Oita, Japan; [3]Dept. Physiol., Grad. Sch. Med. and Dent. Sci., Kagoshima Univ., Kagoshima, Japan; [4]Dept. Physiol., Oita Univ.Facul. Med., Oita, Japan*)

1. Kojiro Matsuda (1908-1993): Dr. K. Matsuda devoted his academic life to the development of cardiac electro-physiology/pathophysiology and circulation physiology. He and his group have recorded, for the first time, action potentials in specialized cardiac muscle tissues including atrioventricular node and established physiological basis of impulse conducting system in the heart. (M. Kameyama)

2. Yasuyoshi Nisimaru (1897-1990): Dr. Y. Nisimaru established with his numerous pupils (over 140 members) the concept of body fluid circulation; blood (William Harvey), lymph (Claude Bernard) and tissue fluid are linked and essentially one and the same extracellular fluid. He was a great teacher and encouraged many, even outside his immediate circle, for reasearch. He was one of the members who founded the Physiological Society of Japan in 1922 (Naoko Nisimaru).

SYMPOSIA
Commemorative and illustrious individuals devoting to physiological sciences in Japan: Kojiro Matsuda and Yasuyoshi Nisimaru [IUPS Symposium: History of Physiologist in Japan (held in Japanese)]

SYMPOSIA
Endocytosis of receptor tyrosine kinase: Novel mechanisms by new molecules

S6 (1S-03C1)
Introduction: Regulation of endocytosis of receptor tyrosine kinase

Shimokawa, Noriaki; Londono, Marina; Qiu, Chun-Hong; Koibuchi, Noriyuki (*Gunma Univ. Grad. Sch. Med., Gunma, Japan*)

Cells are constantly exposed to various extracellular signals, which coordinate their growth, proliferation, differentiation, mortality and survival. Receptor tyrosine kinases (RTKs) are critical mediators between ligands and cell interior. Such receptors include EGF, PDGF, FGF, HGF, IGF, NGF, VEGF and M-CSF receptors. Immediately following activation of RTKs, these receptors are rapidly translocated from cell surface into the endosomal compartment. Then, these are sorted into lysosomes for degradation.Recently, evidence is accumulating that numerous adaptor proteins are involved in RTKs downregulation by internalization and endocytosis. For example, we have been interested in the role of Cbl (Casitas B-lineage lymphoma) that is a multi-adaptor protein with E3 ubiquitin ligase activity and mediate ubiquitylation of active RTK. We have identified that polyubiquitylation of EGF receptor by Cbl ligase is essential for its internalization and degradation. In this symposium, recent progress on the new mechanisms and adaptor molecules regarding regulation of RTK endocytosis will be introduced by researchers from five leading laboratories in this field.

S7 (1S-03C2)
Endocytosis of vascular endothelial growth factor receptor-1, Flt-1.

Maru, Yoshiro (*Dept.Pharmacol. Tokyo Women 'S Medical Univ. Tokyo, Japan*)

Vascular endothelial growth factor (VEGF) has two receptors with catalytic activity: Flt-1 and KDR. The uniqueness of Flt-1 is that it is also expressed in macrophages and plays an essential role in atherosclerosis. Given that oxidized LDL and proteasome inhibitors appeared to downregulate the Flt-1 expression, regulation of Flt-1 on the cell surface as for example a VEGF-trapping molecule for KDR may have a close linkage with initiation of atherosclerotic plaque formation. We have shown that Flt-1 is endocytosed upon binding to VEGF as in the case of other tyrosine kinase growth factor receptors. The minor autophosphorylation site Y 1333 seems to be utilized for recruiting the c-Cbl/CD2AP complex to Flt-1. c-Cbl is an E3 ligase that ubiquitinates Flt-1 with subsequent degradation in proteasomes. Although CD2AP overexression changes endosomal morphology and therefore it appears to be involved in vesicle formation, the precise mechanisms and biological roles of endocytosis still remain to be elucidated. Inhibitors for heat shock protein (Hsp) 90, which is assumed to stabilize the degrading molecule, induced a seemingly VEGF-independent degradation of Flt-1. In this paper, we hopefully discuss possible biological significance and molecular mechanisms of Flt-1 disappearance from the cell surface from the standpoint of atherosclerosis.

S8 (1S-03C3)
Receptor endocytosis triggers production of PtdIns(3,4,5)P3 at endomembranes

Sato, Moritoshi[1,2]; Umezawa, Yoshio[1] ([1]*Sch. Sci. Univ. Tokyo, Tokyo, Japan;* [2]*PRESTO, JST, Saitama, Japan*)

Phosphatidylinositol-3,4,5-trisphosphate (PtdIns(3,4,5)P_3) regulates diverse cellular functions including cell proliferation and apoptosis, and is related to diabetes, cancer, etc.; however, little is known about exactly when, where and how PtdIns(3,4,5)P_3 is produced. We have developed fluorescent indicators for PtdIns(3,4,5)P_3 to reveal spatio-temporal regulations of PtdIns(3,4,5)P_3 production in single living cells. After ligand stimulations, PtdIns(3,4,5)P_3 levels increased to a larger extent at the endomembranes, i.e. the ER and Golgi, than at the plasma membrane. This PtdIns(3,4,5)P_3 increase at the endomembranes was found to originate from its in situ production at the endomembranes, a process stimulated directly by receptor tyrosine kinases endocytosed from the plasma membrane to the endomembranes. The demonstration of PtdIns(3,4,5)P_3 production through receptor endocytosis addresses a long-lasting question about how downstream signaling pathways including Akt are activated at intracellular compartments remote from the plasma membrane.

S9 (1S-03C4)

Regulation of growth factor receptor downregulation by deubiquitination

Komada, Masayuki (*Grad. Sch. Biosci. Biotech., Tokyo Tech, Yokohama, Japan*)

Ligand-activated receptor tyrosine kinases (RTKs) undergo endocytosis and are transported via endosomes to lysosomes for degradation. This process, known as receptor downregulation, is crucial to terminate the cell proliferation signals produced by activated RTKs. During the process, ubiquitination of RTKs serves as a sorting signal for their trafficking from endosomes to lysosomes. The sorting of RTKs is executed by a complex of two ubiquitin-binding proteins, Hrs and STAM, which localizes on the early endosomal membrane. STAM has been shown to interact with a deubiquitinating enzyme UBPY, also known as USP8. Here we studied the role of UBPY in the downregulation of epidermal growth factor receptor (EGFR).

Immunopurified UBPY deubiquitinated EGFR in vitro. Overexpression of UBPY in EGF-stimulated cells reduced the ubiquitination level of activated EGFR and delayed its degradation. Conversely, depletion of UBPY by RNA interference resulted in elevated ubiquitination and accelerated degradation of EGF-activated EGFR. Stimulation of cells with EGF induced the association of UBPY with endocytosed EGFR on Hrs-positive early endosomes, and this association required the interaction of UBPY with the Hrs-STAM complex. On the other hand, the endosomal localization of UBPY did not depend on the Hrs-STAM complex. Together, we conclude that UBPY deubiquitinates activated EGFR which is sorted by the Hrs-STAM complex on early endosomes, thereby removing its sorting signal and regulating its downregulation negatively.

SYMPOSIA
Regulation of plasma membrane localization of membrane transport proteins [YFI (Young Foreign Investigator) Symposium]

S10 (1S-03C5)

Regulation of cell signalling by ubiquitylation

Dikic, Ivan; Hoeller, Daniela; Bienko, Magda; Crosetto, Nicola; Zapart, Gregorz; Haglund, Kaisa (*Institute of Biochemistry II, Goethe University, Frankfurt, Federal Republic of Germany*)

The attachment of a single ubiquitin, monoUb, to a substrate serves as an important regulatory modification implicated in receptor endocytosis, virus budding, gene transcription, DNA repair and replication, etc. The discovery of Ub-binding domains (UBDs), such as UBA, UIM, CUE and others, has indicated how monoUb can regulate such distinct cellular functions. We have cloned two new Ub-binding domains named UBM (Ub binding motif) and UBZ (Ub binding Zn finger) found in numerous cellular proteins. Their functional and biophysical characterization will be presented. In addition to binding Ub, several UBDs promote the monoubiquitylation of host proteins. We have recently shown that monoubiquitylation of the endocytic proteins Sts1, Sts2, Eps15 and Hrs facilitates intramolecular interactions with the UBDs, thus preventing them from binding to ubiquitylated cargoes. We mapped the in vivo monoubiquitylation site in Sts2 and demonstrated its functional importance for EGF receptor endocytosis. We propose that monoubiquitylation of Ub-binding proteins represent a general regulatory mechanism that inhibits their capacity to bind to and control functions of ubiquitylated targets in vivo.

S11 (1S-04D1)

Regulation of organic anion transporters by PDZ proteins

Anzai, Naohiko; Kanai, Yoshikatsu (*Kyorin Univ. Sch. Med., Mitaka, Tokyo, Japan*)

Organic Anion Transporters (OATs), belonging to SLC22 family, are mainly localized in the renal proximal tubules and play important roles in the detoxification and the secretion of xenobiotics such as drugs and toxins and in the reabsorption of endogenous organic anions such as urate and estrone sulfate. Recently, it has been reported that the transporters at the apical membrane of the proximal tubules that have PDZ motif at their extreme C-terminus bind to the NHERF family proteins via PDZ interaction. They exist and function on the network consisted of such proteins beneath the plasma membrane (Anzai et al., *Curr Opin Nephrol Hypertens*, 2005). Our yeast two-hybrid assays revealed that urate transporter URAT1 and organic anion transporter OAT4, localized at the apical membrane of the proximal tubules, interact with PDZ proteins. The interaction of PDZ proteins enhanced their transport activity in the overexpressed mammalian cells through the increased surface protein expression (Anzai et al., *J Biol Chem*, 2004: Miyazaki et al., *J Am Soc Nephrol*, 2005). To date, other transporters related to the renal organic anion handling have also been found to interact with PDZ proteins. These results indicate that the organic anion transport molecular complex (organic anion transportsome) is formed through apical PDZ network and it may contribute to the renal organic anion handling as a functional unit.

S12 (1S-04D2)

Regulation of membrane localization of renal sodium-dependent phosphate transporter

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Phosphate (Pi) homeostasis is mainly regulated by control of Pi reabsorption in the kidney. Sodium-dependent Pi transport system localized in brush border membrane of renal tubular cells is a responsible for the Pi reabsorption. Until now, three isoform of the sodium-dependent phosphate transporter (NaPi-I, NaPi-IIa, NaPi-IIc) have been identified in the brush border membrane of the renal proximal tubular cells. Among them, NaPi-IIa is the most regulatable transporter by various hormone and environmental changes. PTH is a potent inhibitor of NaPi-IIa and is rapidly involved in the translocation of NaPi-IIa from plasma membrane to intracellular compartments. Recent studies demonstrated that NaPi-IIa can predominantly localize in the membrane microdomains (such as lipid rafts or caveolae) of apical membrane of renal proximal tubular cells, and NaPi-IIa can bind to actin cytoskeleton via NHERF-1/EBP50 and ezrin. Formation of the complex has thought to be important to determine the subcellular localization and hormonal regulation of the NaPi-IIa. We identified ezrin is a target molecule for PTH signal. Repression of ezrin function inhibited both membrane targeting and PTH-dependent endocytosis of NaPi-IIa. These findings suggest that ezrin would be a key molecule for both subcellular localization and hormonal regulation of the NaPi-IIa.

S13 (1S-04D3)

Molecular basis of the channels responsible for K^+ and water transport and their specific localization in brain astrocytes

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The brain astrocytes transport excess extracelluar K^+ yielded by synaptic activation to regions of low K^+ uni-directionally. This called "K^+ buffering" is accompanied with water flux. Physiological coupling of these fluxes is essential for proper brain function. Inwardly rectifying K^+ (Kir) channels are assumed to be crucial for K^+ buffering. We found two types of Kir channels, homomeric Kir4.1 and heteromeric Kir4.1/5.1, distributed on astrocytic membranes and were involved in K^+ buffering. Perivascular processes harbor the heteromer, which would secrete K^+, and perisynaptic processes differentially express either channel in a region specific manner, which may play a distinct role in K^+ uptake. Because activity of Kir4.1/5.1 is dynamically regulated by intracellular pH (pH_i) change in a physiological range, K^+ outflow and part of K^+ influx may be finely controlled by pH_i. We further found that the two Kir channels occurred together with AQP4, only one water channel in astrocytes, at the same membrane surface of the processes. Dystrophin associated protein complex could specifically target the Kir and water channels to the perivascular processes. Moreover, we have identified that "lipid raft" microdomain selectively gathers not only apparatuses responsible for water and K^+ transport such as Kir4.1, Kir5.1 and AQP4 but also other molecules including astroglical Cl^- channel ClC-2 and glutamate transporter GLT-1. Accordingly, lipid rafts may serve as a functional microplatform synchronizing salt, water, and glutamate transports in astrocytes.

S14 (1S-04D4)

Translocation of biliary transporters under diseased or drug treatment condition

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ATP-binding cassette (ABC) transporters expressed on the bile canalicular membrane of the hepatocytes are rapidly internalized from and re-inserted to the plasma membrane by a variety of stimulus including oxidative stress, osmolarity change, drug treatment and so on. Such re-localization of the transporter molecules sometimes leads to cholestasis or choleresis, although precise intracellular signaling pathway and final molecular determinants involved in the specific transporter internalization is not elucidated. ABCC2/MRP2 and ABCB11/BSEP are both biliary transporters involved in bile flow, by excreting organic anions (glutathione conjugates, glucuronide conjugates, reduced glutathine) and bile salts, respectively, into bile. We are studying the intracellular signaling pathway triggered by GSH depletion and finally leading to Mrp2-specific internalization using isolated rat hepatocytes coulplets as a experimental model. As a result, GSH depletion induced by ethacrynic acid treatment produces nitric oxide (NO) followed by novel protein kinase C (nPKC) activation. Molecular mechanism regulating Mrp2-specific internalization are discussed in relation to canalicular scaffold proteins and other canalicular ABC transporter molecules.

S15 (1S-04D5)

Analysis of disease-causing mutants of renal channels and their regulators.

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In kidney, various types of channels and transporters present in the specific nephron segments are functioning to maintain body fluid balance. Mutations of these membrane proteins have been found to cause some human genetic diseases, such as Bartter syndrome and nephrogenic diabetes insipidus (NDI). These naturally occurring mutations sometimes tell us important functional domains within proteins, especially in terms of protein sorting. Recently, we found three frame-shift mutations in the AQP2 gene in patients having autosomal dominant (AD)-type NDI. Previously, we have demonstrated that the AQP2 (763-772 del), a 10 nucleotide-deletion mutant, was mis-localized to the basolateral membrane in MDCK cells. To analyze this sorting abnormality in vivo, we created AQP2 (763-772 del) knock-in mice, which showed severely impaired urine concentrating ability. Using this mouse model, we will discuss the molecular pathogenesis of AD-NDI in vivo, and also the usefulness of knock-in mouse models in the study of transportsome.

SYMPOSIA
TRP channels: Regulatory aspects and physiology

S16 (1S-05E1)
Regulation of the TRPV2 channel in macrophage

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The TRPV2 channel is expressed in various tissues including neurons, neuroendocrine cells and blood cells including macrophages. We examined the regulation of the TRPV2 channel in macrophages. In serum-free condition, immunoreactivity of TRPV2 was detected largely in cytoplasm. Addition of a chemotactic peptide fMLP induced translocation of the TRPV2 to the plasma membrane. In accordance with this, fMLP increased the Cs^+ current, which was inhibited by ruthenium red and the transfection of the dominant-negative mutant of TRPV2. fMLP-induced translocation of the TRPV2 was blocked by PI 3-kinase inhibitors and pretreatment with pertussis toxin. When cytoplasmic calcium concentration ($[Ca^{2+}]_c$) was monitored by using fura-2, fMLP induced a rapid and sustained elevation of $[Ca^{2+}]_c$, the latter of which was abolished by removal of extracellular calcium. Addition of ruthenium red or transfection of the dominant-negative mutant of TRPV2 did not affect the initial rise but blocked the sustained phase of fMLP-induced $[Ca^{2+}]_c$ response. In stimulated macrophages, TRPV2 localized in the podosome, a microdomain involved in adhesion and migration, and colocalized with Rho family small G proteins. Transfection of the dominant-negative Rac inhibited translocation of TRPV2. Finally, addition of ruthenium red or transfection of dominant-negative mutant of TRPV2 inhibited chemotaxis of macrophage induced by fMLP. These results indicate that fMLP induces translocation of TRPV2 by a PI 3-kinase dependent mechanism and this translocation is important for sustained elevation of $[Ca^{2+}]_c$ in macrophage.

S17 (1S-05E2)
Single particle analysis of TRPC3

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TRPC3 plays important roles in neuronal differentiation and immune cell maturation by mediating the cationic current in response to phospholipase C activation, Ca^{2+} depletion, and diacylglycerol stimulation. In collaboration with Dr, Yasuo Mori (Kyoto Univ.), we purified the TRPC3 channel as a glycosylated tetramer and observed the structure using electron microscopy for single particle analysis[1)]. Negatively stained specimens demonstrate homogeneous protein particles containing an internal cavity-like structure. These particle images were selected by automated pick-up programs[2)], aligned, and classified by the growing neural gas network method[3)]. Similarly oriented projections were averaged to decrease the signal-to-noise ratio. The averaged images progress from the top view to the side views, which are representative of their raw images. The top view confirmed the hypothesis of a four-domain structure, and the side view demonstrates a large cytoplasmic domain with a capped structure at the bottom, which is near a predicted locus of ion release. The total image of the protein is a blunt-edged trapezoid: both width and height of the molecule are over 200 angstrom. This large dimension of TRPC3 is also supported by the Stokes radius (92 angstrom) obtained from gel filtration chromatography.

1) Mio, K., Ogura, T., Hara, Y., Mori, Y. & Sato, C. The non-selective cation-permeable channel TRPC3 is a tetrahedron with a cap on the large cytoplasmic end. *Biochem. Biophys. Res. Commun.* **333**, 768-777 (2005).

2) Ogura, T. & Sato, C. *J Struct Biol* **146**, 344-58 (2004).

3) Ogura, T., Iwasaki, K. & Sato, C. *J Struct Biol* **143**, 185-200 (2003).

S18 (1S-05E3)
Physiological significance of diacylglycerol-acivated TRPC3 channels in B cell receptor signalling

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In B lymphocytes, B cell receptor (BCR)-activated Ca^{2+} signaling comprises initial transient responses followed by a Ca^{2+} entry-dependent sustained and/or oscillatory phase. BCR stimulation induces phospholipase C γ2 activation and simulates Ca^{2+} influx across the plasma membrane through multiple mechanisms, such as store-operated Ca^{2+} channels via IP_3-induced Ca^{2+} store depletion and cation/ Ca^{2+} channels directly activated by diacylglycerol (DAG). Previously, we have revealed requirement of store-operated Ca^{2+} channels for the generation of BCR-induced Ca^{2+} oscillations and subsequent gene expression. However, the importance of DAG-activated channels is largely unknown in BCR signalling. Canonical transient receptor potential (TRPC) 3 is known as cation/ Ca^{2+} channels coupled with PLC γ2 and activated by DAG. In this study, we have disrupted *TRPC3* gene in DT40 B lymphocytes by targeting method to study its impact on BCR signalling. Endogenous TRPC3 formed DAG-activated Ca^{2+} channels in DT40 B lymphocytes. BCR-induced Ca^{2+} oscillation and NF-AT activation were suppressed in TRPC3-deficient cells. Furthermore, extracellular signal-regulated kinase (ERK), one of the mitogen activated protein kinases (MAPK), activation was reduced in TRPC3-deficient cells. This was attributable to suppressed plasma membrane translocation of PKC βII, a signalling components upstream of the ERK pathway. In conclusion, DAG-dependent activation of TRPC3 plays an important role in BCR-mediated Ca^{2+} and MAPK signalling.

S19 (1S-05E4)

Molecular mechanisms of thermosensituve TRP channel activity

Tominaga, Makoto (*Sec. Cell Signaling, Okazaki Inst. Integrative Biosci.*)

Among the huge TRP super family of ion channels, some have been proven to be involved in thermosensation. Insight into the molecular nature of temperature-gated ion channels came with the cloning of the capsaicin receptor, TRPV1 and the recognition that this ion channel protein could be activated by elevated temperatures with a threshold near 43°C. Three other TRPV channels, TRPV2, TRPV3 and TRPV4, have been cloned and characterized as thermosensors. The threshold temperatures for activation of these channels range from relatively warm (TRPV3 and TRPV4) to extremely hot (TRPV2). In contrast to the four heat-sensitive TRPV channels, TRPM8 and TRPA1, have been found to be activated by cold stimuli. Most of the mammalian thermosensitive TRP channels (thermoTRPs) identified to date can alternatively be activated by chemical stimuli, such as capsaicin for TRPV1. We found that temperature acts as a co-activator of thermoTRPs. In addition, thermoTRPs have various mechanisms for their functional regulation such as TRPV1 regulation through phosphorylation by PKC. Furthermore, we found that TRPM2, phylogenetically close to TRPM8, is a new thermoTRP activated by warm temperatures. We also found that TRPV4 needs other cytosolic proteins for its functional regulation. I summarize the recent progress in thermoTRPs research, especially about molecular mechanisms of their functional regulation by focusing on our own results.

SYMPOSIA
The effect of steroid hormone and related compounds on cellular proliferation: Studies using breast cancer cells

S20 (1S-05E5)

Novel regulatory mechanisms for the vascular receptor-operated Ca^{2+} entry channel TRPC6

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TRPC6 (a canonical subfamily member of transient receptor potential protein) is a predominant isoform expressed in vascular smooth muscle and likely serves as an integrative non-voltage-gated Ca^{2+} entry channel regulating the vascular tone and remodeling. Activation of this channel occurs polymodally by stimulation of PLC-linked, G-protein-coupled and tyrosine kinase receptors and mechanical forces. Although store depletion and diacylglycerol have been proposed to be important activating signals, we have recently found that two novel mechanisms, i.e., Ca^{2+}/calmodulin (CaM)-dependent phosphorylation and generation of an arachidonic acid (AA) metabolite, 20-hydroxyeicosatetraenoic acid (20-HETE), strongly affect TRPC6 channel activities. Assuming a similar membrane topology to TRPC1, the former may involve the phosphorylation of T487 on the II-III intracellular loop of TRPC6 channel presumably via CaM-dependent kinases bound to its C-terminus, which leads to priming of the channel for opening in response to receptor stimulation. In contrast, the latter requires the preceding activation of TRPC6 channel by receptor stimulation, which appears to render the channel mechanosensitive through mechanical activation of phosphopliase A2 and subsequent metabolization of AA into 20-HETE via vascular smooth muscle specific cytochrome P450 enzymes having ω-hydroxylase activities. The both mechanisms seem to contribute to maintaining the vascular tone.

S21 (1S-06F1)

Observation of hormone-responsive growth using three-dimensional cultures

Enami, Jumpei (*Research Laboratory, Zenyaku Kogyo Co., Ltd.*)

The use of *in vitro* culture methods which faithfully reproduce the *in vivo* behavior of cells is expected to add further information for prediction of effectiveness of drugs used for treatment of mammary cancer. The conventional monolayer culture method, however, has not always been satisfactory in this respect. For example, only limited degrees of growth and differentiation of mouse mammary epithelical cells have been observed *in vitro* in response to mammogenic and lactogenic hormones. Analysis of physicochemical microenvironment surrounding the mammary epithelial cells suggested that extracellular matrix as well as chemical mediators produced by the stromal cells of the mammary gland may be the key factors for the hormone-responsiveness. Our studies led us to hypothesize that hepatocyte growth factor (HGF) may be the mammary stroma-produced environmental factor which mediates the growth of adjacent epithelial cells. Use of three-dimensional collagen gel matrix culture method further enabled us to observe the hormone-responsive growth as well as branching morphogenesis of mammary epithelial cells. In the absence of homornes in the culture medium, the cells underwent apoptosis. Similarly to the observations on mammary epithelial cells, ventral prostate epithelial cells of the mouse grew in response to androgens under the three-dimensional collagen gel culture conditions in the presence of HGF. These observations strongly support the hypothesis that three-dimensional culture conditions allow the cells cultured *in vitro* to behave as those *in vivo*.

S22 (1S-06F2)

Age- and parity-dependent change in biological characteristics of rat mammary stem cells (clonogens)

Shimada, Yoshiya; Nishimura, Mayumi (*Natl. Inst. Radiol. Sci., Chiba, Japan*)

It is hypothesized that stem cells are the targets for carcinogenesis. If cancer arises from stem cells, cancer risk would depend on population size and susceptibility to carcinogens of stem cells . Study on A-bomb survivors shows clear age-related decline in the susceptibility to radiation-induced breast cancer. It is also known that women who undergo full-term pregnancy have a reduced lifetime risk of breast cancer. These results suggest that protection results from intrinsic effect of aging and parity on breast tissues. We here examined change in the biological characteristics of rat mammary stem cells (clonogens) with aging and parity. The results are as follows. (1) Total numbers of clonogens increased exponentially with a population doubling time of 4 days during pre-pubertal period. After puberty, it lengthened to 30 days. The total number of clonogens in abdominal and inguinal mammary glands of 2 week-old rats was 200, while that in 8 week-old and thereafter was more than 5,000. (2) The number of mammary clonogens in rats which underwent pregnancy was less than 500, while that of nulliparous rats was 6,000. (3) Prolactin treatment increased clonogen number by 8 folds in 8 week-old rats whereas it increased by just 2 folds in one year-old rats. (4) The surviving fraction of clonogens before puberty after 5 Gy was 0.1, while it was 0.3 after puberty. These results suggest that population size, response to prolactin and radiobiological characteristics of clonogens, which change in age- and parity-dependent fashion, is associated with susceptibility to radiation-induced mammary tumors.

S23 (1S-06F3)

Analysis of Estrogen-Responsive Genes Involve in Growth and Progression of Breast Cancer

Hayashi, Shin-ichi (*Dept. Med. Technology, Sch. Med., Tohoku Univ., Sendai, Japan*)

Since estrogen plays an important role in the growth and progression of human breast cancer, understanding the whole picture of estrogen signaling is a very important goal towards clarifying the biology of this disease. So far, we have studied the molecular mechanisms of estrogen-dependent breast carcinogenesis, specifically from the viewpoints of estrogen receptor (ER) gene expression and functional modulation of ER in breast cancer. Recent several years, we are focusing the development of new tools such as estrogen-responsive microarray and ERE-GFP reporter cells, which enable to characterize the estrogen-responsive genes in breast cancer cells and estrogen signal-sensitivity in individual breast cancer. We first identified estrogen-responsive genes by the comprehensive expression profiling in ER-positive breast cancer cells, and produced a custom-made estrogen-responsive microarray of narrowed-down subset. Using this microarray, we studied several basic researches for estrogen signaling such as the effect of estrogen-antagonists and endocrine-disruptors on estrogen-responsive gene expression profile. In this study, we found that transcription factor EGR3 is the bona fide target gene for ERa and involved in the estrogen-signaling pathway in breast cancer cells. Furthermore, the expression of another new estrogen-responsive gene HDAC6 significantly correlated with survival of breast cancer patients. In vitro study revealed that the HDAC6 caused the deacetylation of a-tubulin in cytosol and induced cell motility in ER-positive breast cancer cells.

S24 (1S-06F4)

Effectors of estrogen and tamoxifen actions in breast cancer cells.

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Estrogen receptor (ER) belongs to the nuclear receptor super family, and is a key regulator of proliferation and differentiation in normal mammary gland and breast cancer cells. It has been reported that steroid and xenobiotic receptor (SXR), a new member of nuclear receptors, is expressed in breast cancer cells. We investigated the role of SXR and found a series of novel actions. (I) tamoxifen (TAM) activated SXR-mediated transcription of cytochrome P-450 3A4 (CYP3A4) and multidrug resistance-1 (MDR-1) genes, which are involved in TAM metabolism. Thus, SXR may be involved in TAM resistance by decreasing its local concentration. (II) ER-mediated transcription was potentiated by SXR in a receptor-concentration dependent manner in MCF-7 cells. We then further investigated the mechanism of SXR action. SXR did not bind with ER or estrogen response element, and did not alter ER-coactivator binding. On the other hand, the binding between ER and silencing mediator of retinoid and thyroid receptors (SMRT) was decreased by SXR in a dose dependent manner. These results suggest that (III) SXR augmented the ER-mediated transcription, by squelching limiting amount of SMRT.

These series of studies have shown that SXR expression in breast cancer may alter the sensitivity to estrogen and its related compounds. SXR may stimulate the development by potentiating estrogen action through ER. It may decrease the effect of TAM by facilitating its metabolism.

Taken together, SXR may be an exacerbation factor of breast cancer.

SYMPOSIA
Organized spontaneous activities in the brain network—mechanism and significance

S25 (1S-07G1)
Structures and functions of spontaneous activity in the cortex

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The brain is continuously active. Spontaneous neuronal activity is prevalent in vivo and in vitro and could reflect intrinsic functional properties of the microcircuit, so its dynamics may help reveal the basic logic of network operations. However, it is largely unknown how such naturally generated spikes are organized or how they can affect individual synaptic efficacy. We reconstructed spike patterns of many cortical neurons in vitro and found that sequences of activity were reactivated in the same spatiotemporal order. Spontaneous activity drifts with time, recruiting different sets of cells, and thereby, sequences are replaced with novel patterns. Patterns of spontaneous activity were predictable by training a feedback neural network model with a past period of dataset. We also sought to determine whether spontaneous activity alters synaptic strength. When hippocampal slices were exposed to ACSF that mimicked the extracellular ionic compositions in vivo, cells started to exhibit slow wave oscillations with rhythmic action potentials. After wash-out, postsynaptic currents were altered at CA3 synapses. The direction of synaptic plasticity was determined by the frequency of UP-DOWN state alternations. When the modified ACSF was repetitively applied, identical cells generated different oscillation rhythms, and thus, changes in synaptic efficacy varied from trial to trial. Therefore, spontaneous self-excitation of cortical networks is non-randomly structured and can modify synaptic weights. Our talk provides a novel regimen of cortical operations, i.e., self-rewritable microcircuitry with ongoing plasticity.

S26 (1S-07G2)
Neocortex-hippocampus interactions through slow and fast network oscillations

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The neocortex and the hippocampus are connected by way of the entorhinal cortex and the subiculum. To examine the ongoing network interactions among these cortical areas during neocortical slow (<1 Hz) oscillations and hippocampal fast (80-250 Hz) oscillations, we recorded intracellular potentials in single neocortical, entorhinal, subicular, and hippocampal neurons, together with hippocampal field potentials and multi-unit activity in adult rats, anesthetized with urethane and ketamine. We have found that 1) most entorhinal and subicular neurons displayed slow oscillations, including bimodal depolarizing (up) and hyperpolarizing (down) states, in synchrony with neocortical slow oscillations, 2) no bimodal up-down distribution of the membrane potential was present in hippocampal CA3 and CA1 neurons, 3) while hippocampal granule cells were directly driven by the up state (by way of the entorhinal input), CA3 and CA1 neurons discharged during both up and down states, 4) gamma (30-80 Hz) and fast (ripple) oscillations were observed in the hippocampal CA1 area irrespective of the up-down transition, 5) hippocampal ripples and neocortical slow oscillations correlated only weakly and at a long (sec) time scale. These observations suggest that entorhinal and subicular regions are "neocortex-like" and distinct from hippoocampal circuits that lack the necessary mechanisms for the maintenance of slow oscillations; hippocampal networks can generate self-organized gamma and ripple activities independent of the neocortical/entorhinal slow oscillations.

S27 (1S-07G3)
Spontaneous activity, plasticity in cultured cortical networks

Jimbo, Yasuhiko (*Grad. Sch. Engng. Univ. Tokyo, Tokyo, Japan*)

Activity-dependent plasticity probably plays an important role in learning and memory as well as proper network formation during development. Though synaptic plasticity has been widely and extensively investigated, little is known about its consequences in network activity. We have applied micro-electrode arrays (MEAs) for neuronal ensemble recording. The MEA is a dish for cell culture, on the surface of which multiple micro-electrodes are embedded. Cortical neurons were taken from E18 Wistar rat embryos and cultured on the MEAs. Spontaneous activity started at about 3 days *in vitro* (DIV). Relatively long-lasting activity with loose network coupling was observed. Then the activity grew up to periodic synchronized bursts with tight coupling. In about one month, it reached a steady state. The steady-state activity was composed of synchronized bursts at approximately 1 Hz, and some asynchronous components. The spatial propagation patterns were not unique, but could be classified into a few groups. The substrate-embedded electrodes could also be used for stimulation. Several evoked activities were recorded and the effects of focal high frequency stimulation were evaluated. After the high frequency stimulation, some of the signal propagation pathways were strengthened. The same high frequency stimulation weakened some of the pathways. The correlation analysis revealed that the pathways that were tightly correlated with the repeatedly activated pathway were selectively potentiated. The next step will be to study how spontaneous activity during development affects the network and single cell properties.

S28 (1S-07G4)

Involvement of endogenously released ATP in the generation of network-driven spontaneous oscillation in the neonatal hippocampus

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The earliest organized intrinsic activity in the hippocampal network appears during the perinatal period, which is characterized by slow synchronized bursting of the pyramidal neurons and interneurons in forms of giant depolarizing potentials (GDPs) in the slices and "sharp waves" in vivo. These activities are proposed to be involved in the maturation of the interneuronal networks (Ben-Ari et al., 2004). Since the generation of GDP requires depolarisation of pyramidal neurons by GABA released from interneurons and since exogenous ATP excites inhibitory interneurons in the CA3 through activation of $P2Y_1$ receptors (Kawamura et al., J Neurosci, 2004), we examined whether activation of interneuronal purinoceptors by endogenous ATP and subsequent excitation of interneurons underlie the GDP generation. The spontaneous GDP activities were recorded by patch-clamp recording from CA3 pyramidal neurons and by imaging of $[Ca^{2+}]_i$ with fluo-4 in the coronal hippocampal slice of the rats (P4-8). The responses of GDPs to pharmacological manipulations of ATP and adenosine receptors and their metabolism suggest a possible involvement of activation of interneuronal purinoceptors by endogenous extracellular ATP in the maintenance of GDP, which might provide an important mechanism linking on-going metabolic condition and large-scale network activities in the early development.

S29 (1S-08H1)

Functional analysis of clock genes: from clock gene expression to physiology

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Circadian clocks constitute a global regulatory system found in most eukaryotes. The center of the circadian rhythm is located in the suprachiasmatic nuclei (SCN) and informs the peripheral organs of the timing via neuronal and hormonal pathways. It is driven by complexes of the transcription factors CLOCK and BMAL1, while CRY and PER oppose CLOCK/BMAL1 activity, closing a negative feedback loop that results in an approximately 24-hour rhythm. CLOCK/BMAL1 bind the E-box in the promoter region of not only clock components, but also so-called output genes of the circadian clock. Core clocks are located all over the body and control the circadian expression of genes that regulate fat metabolism, the cell cycle, neural activity, and so on. In this symposium, we will discuss the core clock mechanism and clock gene functions from the cellular level to the level of the human body.

SYMPOSIA
The circadian timing system: From clock gene expression to physiology

S30 (1S-08H2)

Neural functions and clock genes

Takumi, Toru (*Osaka Bioscience Inst. (OBI), Suita, Japan*)

Several non-clock functions of clock genes have been discovered. In mammals, the circadian system and stress systems, both centers of which are located in the hypothalamus, are involved in an adaptation to predictable and unpredictable environmental stimuli, respectively. Although the interaction and relationship between these 2 systems are intriguing and have been studied in different ways since the "pre-clock-gene" era, the molecular interaction between them largely remains unknown. I show by systematic molecular biological analysis that acute physical stress elevated only Period1 (Per1) mRNA expression in mouse peripheral organs. Although behavioral rhythms in vivo and peripheral molecular clocks are rather stable against acute restraint stress, the results of a series of promoter analyses, including chromatin immunoprecipitation (ChIP) assays, indicate that a glucocorticoid responsive element (GRE) in the Per1 promoter is indispensable for induction of this mRNA both in vitro and in vivo. These results suggest that Per1 can be a potential stress marker and that there may exist a third pathway of Per1 transcriptional control in addition to the clock-regulated BMAL1/CLOCK-E-box and light-responsive CREB-CRE pathways.

S31 (1S-08H3)

Clock genes responsible for the circadian rhythms in cytosolic calcium concentrations in mice and Drosophila

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We have shown that cytosolic Ca^{2+} concentration oscillates in circadian fashion in cultured mouse suprachiasmatic nucleus (SCN) neurons and in the *in vivo* Drosophila ventral lateral neurons (LN_vs). Since these neurons play a critical role for the generation of behavioral rhythms, the circadian Ca^{2+} oscillations may mediate cellular output processes in circadian pacemaker neurons in diverse systems. To further analyze the function of clock genes for the circadian Ca^{2+} rhythm generations, the present study examined over-expression of Bmal1 genes or of its dominant-negative genes in cultured SCN neurons. Also, Per01 mutant flies carrying pdf-gal4/UAS-cameleon were generated to investigate the function of clock genes in the Ca^{2+} dynamics of Drosophila LN_vs. All of these genetic manipulations caused reduction of circadian Ca^{2+} rhythms. Therefore, we concluded that temporal patterns of cytosolic Ca^{2+} concentrations are regulated by the above clock gene families in circadian pacemaker neurons.

S32 (1S-08H4)

Clock gene and obesity

Oishi, Katsutaka; Ishida, Norio (*Clock Cell Biol. Res. Group, IBRF, AIST, Tsukuba, Japan*)

Recent studies of clock genes have revealed that an autoregulatory transcriptional feedback loop forms the core circadian rhythm generating mechanism in mammals. *Clock* is the first clock gene identified in vertebrates by forward mutagenesis using *N*-ethyl-*N*-nitrosourea in a behavioral screening, and encodes a basic helix-loop-helix (bHLH)-PAS transcription factor. Previously, we identified putative CLOCK target genes in the mouse liver using microarray analyses and found that in addition to being a core component of the circadian oscillator, CLOCK is involved in various physiological functions. We show here that serum levels of triglyceride and free fatty acid were significantly lower in circadian *Clock* mutant ICR than in wild-type control mice, whereas total cholesterol and glucose levels did not differ. Moreover, an increase in body weight induced by a high-fat diet was attenuated in homozygous *Clock* mutant mice. We also found that dietary fat absorption was extremely impaired in *Clock* mutant mice. Circadian expressions of cholecystokinin-A (CCK-A) receptor and lipase mRNAs were damped in the pancreas of *Clock* mutant mice. We therefore showed that a *Clock* mutation attenuates obesity induced by a high-fat diet in mice with an ICR background through impaired dietary fat absorption. I will also talk about our recent findings that CLOCK is involved in the diabetes- and obesity-induced cardiovascular diseases by increasing the expression of plasminogen activator inhibitor-1 (PAI-1).

S33 (1S-08H5)

Sleep-wake cycle and clock genes

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Some people cannot adjust their sleep-wake cycle to socially-desired time schedule, called circadian rhythm sleep disorders (CRSD). Recent studies revealed that functional variations in human clock genes confer susceptibility to CRDS, such as delayed sleep phase syndrome (DSPS), advanced sleep phase syndrome (ASPS), and non-24-hour sleep-wake syndrome (N-24). Missense variations in *Period2* (*Per2*) gene and *Casein kinase1 delta* (*CK1*δ) gene, each of which reduces phosphorylation of PER protein, reportedly cause familial ASPS. We have already reported that a missense variation in *Per3* gene, which presumably affect phosphorylation of PER3 protein, increases the risk for DSPS and that a missense variation in *CK1*ε gene, which increases the kinase activity, plays a protective role in the development of DSPS. It is intriguing that all of the CRDS-susceptibility variations found so far, as described above, seem to alter the phosphorylatrion of PER proteins.Functional clock gene variations are also observed in apparently normal subjects and likely to induce interindividual differences in circadian period. Comprehensive genetic analysis for variations of human circadian rhythmicity will make it possible to fully understand the characteristics of each indivisual's internal clock, leading to alleviation of health injury and economic loss induced by sleep disorders or maladaptation to socially-desired time schedule, with which a large number of people are afflicted in the modern society.

S34 (1S-08H6)

Consequences of a mutation in the murine Per2 gene on physiological parameters

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Living on earth has made us use the sun as reference and the 24-hour succession of light and darkness is probably the most pervasive epigenetic influence in the evolution from a single cell organism to man. This periodic succession of light and darkness provided the base for relative timing of biological processes over the 24 hours of a day. Because energy supply is the limiting parameter for survival, a system for optimal timing of energy expenditure and uptake developed. The mechanism of this system took the shape of a cycle reflecting the recurrence of sunrise and sunset, and is termed a " circadian clock" - a clock with a period of about one day (latin: circa diem). The internalization of environmental time within the organism not only allows organization of biological processes along the 24-hour time scale but also prediction of recurring events, such as availability of food and emergence of predators. Therefore it is not surprising that alterations in the genetic machinery of the circadian clock leads to alterations in biochemical and physiological processes.Data illustrating the influence of the Per2 gene on adaptation to changing lighting conditions, addiction, the aging process and food anticipation will be presented.

SYMPOSIA
Molecular and cellular physiology of the metabolic control [Korea–Japan Joint Symposium]

S35 (1S-09B1)
Role of the orphan nuclear receptor SHP on the control of metabolic homeostasis

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Small heterodimer partner (SHP; NR0B2) is a member of the large nuclear receptor family of transcriptional factors that lacks a conventional DNA binding domain. Various studies have reported SHP to be a repressor of transcriptional activities of a number of nuclear receptors, including glucocorticoid receptor, estrogen receptor, androgen receptor, thyroid hormone receptor, retinoic acid receptor, retinoid X receptor, constitutive androstane receptor, pregnane X receptor, HNF4α, liver receptor homologue 1, estrogen-related receptor-γ, Nur77 and liver X receptor (LXR). The very broad range of receptors sensitive to inhibition by SHP suggests a central role for SHP in modulation of nuclear receptor signaling pathways. SHP is expressed in a wide variety of tissues, including heart, brain, liver, spleen, adrenal gland, small intestine, and pancreas. Moreover, human *SHP* gene is located on chromosome 1p36.1 and consists of two exons separated by an intron. *SHP* gene transcription is regulated by several members of the nuclear receptor superfamily including the bile acid receptor farnesoid X receptor, steroidogenic factor-1, HNF4α, liver receptor homologue 1, estrogen receptor and estrogen-related receptor-γ. Recent progresses on the elucidation of molecular mechnism of SHP gene expression and function will give us a chance to develop new drug therapies treating a variety of human diseases including diabetes, obesity and disorder of lipid and cholesterol metabolism.

S36 (1S-09B2)
Implication of Myosin Light Chain Kinase in the Insulin-Stimulated GLUT4 translocation in Adipocytes

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In adipocytes, insulin stimulates glucose transport principally by promoting translocation of glucose transporter GLUT4 from an intracellular compartment to the plasma membrane. Requirements for Ca^{2+}/calmodulin during insulin-stimulated GLUT4 translocation have been demonstrated; however, the mechanism of action of Ca^{2+} in this process is unknown. Recently, myosin II, whose function in non-muscle cells is primarily regulated by phosphorylation of its regulatory light chain (RLC) by the Ca^{2+}/calmodulin-dependent myosin light chain kinase (MLCK), was implicated in insulin-stimulated GLUT4 translocation. We have investigated, using 3T3-L1 and 3T3-F442A adipocytes, the possibility that MLCK may be involved in the insulin-stimulated translocation of GLUT4. Insulin significantly increases phosphorylation of the myosin II RLC in a Ca^{2+}-dependent manner. ML-7, a selective inhibitor of MLCK, as well as inhibitors of myosin II, such as blebbistatin and 2,3-butanedione monoxime, block insulin-stimulated GLUT4 translocation and subsequent glucose transport. In addition, suppression of MLCK expression via stably expressing antisense-MLCK decreases insulin-stimulated glucose transport. Our studies strongly suggest that MLCK may be a regulatory target of Ca^{2+}/calmodulin and may play an important role in insulin-stimulated GLUT4 translocation in adipocytes.

S37 (1S-09B3)
Roles of Diet in Muscle Insulin Resistance

Kim, Jong-Yeon (*Department of Physiology, Yeungnam University College of Medicine, Daegu, Republic of Korea*)

Dietary factors have been implicated in the development of hepatic and peripheral insulin resistance. Differently composed diets can induce insulin resistance in different ways, but the mechanisms underlying these phenomena are not yet clear. This study was conducted to evaluate whether dietary composition change affects insulin resistance in the skeletal muscles of rats fed high-carbohydrate diet or high-fat diet. We assessed glucose transport in the skeletal muscles of rats in vitro. Diets given were rat chow, high-starch (HT), high-sucrose (HS), high-fat high-starch (HFHT), high-fat high-sucrose (HFHS, HF), HF with fish oil (HF+FO), and HF with linseed oil (HF+LO). Both of HS diet and HT diet with or without high-fat depressed insulin-stimulated glucose transport compared with chow diet, but there were no significant difference between groups. HF diet markedly decreased the insulin-stimulated glucose transport, and fish oil improved this partially, but linseed oil did not significantly. Percent visceral fat pad mass, plasma insulin and triglyceride in high carbohydrate or high-fat diet groups that developed muscle insulin resistance were much higher compared with chow diet group. Fish oil and linseed oil decreased percent visceral fat pad mass, and fish oil decreased plasma insulin and triglyceride. The composition of fat diet was more important factor than that of carbohydrate diet to induce muscle insulin resistance assessed by glucose transport in vitro. Plasma triglyceride and insulin concentrations seemed to be important factors to induce muscle insulin resistance in rats.

S38 (1S-09B4)

Transcriptional Control of the Differentiation of Pancreatic β Cells

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The main role of pancreatic β cells is to secrete insulin in response to an increase of the blood glucose level. To accomplish this, β cells express numerous genes essential for glucose-responsive insulin secretion. To allow the expression of such strictly selected multiple sets of genes, various differentiation steps are required during pancreatic development. As is the case for other types of cells, recent studies have identified several transcription factors that control the activation and repression of a large number of genes during pancreatic development and how these factors function. Accumulation of such knowledge has revealed that transcription factors orchestrate the intricate pathways of cellular growth, death, and differentiation by direct regulation of gene expression. Amongst the transcription factors in this well-organized cascade, neurogenin 3 (Ngn3) plays a key role in determining the fate of cells in the endocrine pancreas. We recently found how signals from adjacent cells regulate the expression of Ngn3 in pancreatic precursor cell. In addition, we found that Ngn3 regulates the expression of Pax4 and Nkx2.2 cooperated with HNF factors, thus induces β cell differentiation.

S39 (1S-09B5)

Ubc9 Regulates Insulin Sensitivity by Promotion of GLUT4 Targeting to the Insulin-Sensitive Storage Compartment

Shibata, Hiroshi (*IMCR, Gunma University, Maebashi, Japan*)

In muscle and adipose cells, insulin stimulates glucose uptake more than several folds by recruiting the insulin-regulated glucose transporter, GLUT4 from intracellular compartments to the plasma membrane. While such large insulin stimulation of glucose transport coincides with the expression of GLUT4 during differentiation of these cells, GLUT4 expression does not necessarily confer insulin sensitivity to glucose transport in other types of cells. Previous studies have shown that in muscle and adipose cells, GLUT4 is targeted to a unique GLUT4 storage compartment (GSC) sequestered from the constitutive recycling pathway, whereas the significance and the mechanism of GLUT4 targeting to GSC have remained obscure. We have recently found that Ubc9, the SUMO conjugating enzyme, may be an important regulatory protein in subcellular targeting and turnover of GLUT4. Adenovirus vector-mediated overexpression of Ubc9 in 3T3-L1 adipocytes substantially increased GLUT4, which was accompanied by promoted targeting of GLUT4 to GSC, consequently leading to enhanced insulin responsiveness of glucose transport. On the other hand, siRNA-mediated depletion of Ubc9 caused a marked down-regulation of GLUT4, with a selective loss of GLUT4 in GSC, and significantly attenuated the insulin effect on glucose transport. Interestingly, the turnover of GLUT4 was significantly retarded by targeting to GSC, but was accelerated by residing in the recycling pathway. Thus, Ubc9 plays an indispensable role in acquisition and maintenance of the insulin sensitivity of glucose transport in adipocytes.

S40 (1S-09B6)

Transcription factors that regulate insulin sensitivity in the liver and metabolic syndrome

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Liver plays a central role in energy metabolism depending upon nutritional states and hormones. Since long-term regulation of carbohydrate and lipid metabolisms is controlled at the transcriptional level, hepatic nutritional transcription factors play a pivotal role in energy metabolism. SREBP-1c has been established as a transcription factor that controls synthesis of fatty acids and triglycerides based upon our data from SREBP-1c transgenic and knockout mice. SREBP-1c also regulates insulin sensitivity via direct regulation of IRS-2, a key insulin-signaling molecule in the liver. Nutritional induction of hepatic SREBP-1c by dietary carbohydrates and saturated fatty acids well explains how liver shifts metabolism from glycogen synthesis to lipogenesis in the feeding cycle. To excess, SREBP-1c activation contributes to components of metabolic syndrome such as dyslipidemia, diabetes, fatty liver, and insulin resistance, and finally leading to atherosclerosis as observed in our murine models. Meanwhile, we have identified TFE3 as a strong activator of insulin signaling. TFE3 transcriptionally activates IRS2 and diversely participates insulin signaling and markedly ameliorates diabetes in different models. TFE3 and FOXOs synergistically activate, and SREBP-1c competitively suppresses IRS-2 promoter. Collectively, these energy transcripton factors regulate carbohydrate-lipid metabolism, insulin signaling, and might be involved in metabolic syndrome and diabetes. Thus, these factors could be future therapeutic targets.

SYMPOSIA
Mechanisms for regulation of exocytosis and its physiological significance

S41 (1S-10C1)
Timing of synaphin/complexin action in neurotransmitter release

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The release of neurotransmitters at synapses requires the temporally-ordered trafficking of synaptic vesicles. More than 20 proteins are involved in this process, including the SNARE proteins that participate in membrane fusion during exocytosis. To discern the specific role that each protein plays, it is necessary to sort out the temporal order in which they interact. Here we consider the timing of action of synaphin (also named as complexin), a SNARE-binding protein that plays an important role in calcium-triggered neurotransmitter release. A light-activated binding-site peptide was used to perturb the interaction of synaphin with SNARE proteins at precise time intervals before neurotransmitter release. We find that this peptide inhibits neurotransmitter release within 180 milliseconds before synaptic vesicles fuse with the presynaptic plasma membrane. These results indicate that synaphin binds to SNARE complex after synaptic vesicles dock but well before the fraction of a millisecond required for calcium ions to trigger synaptic vesicle fusion. These results are compatible with a role for synaphin in preparing trans-SNARE complexes for membrane fusion.

S42 (1S-10C2)
Multiple types of Calcium channels and their distribution in the presynaptic nerve terminal

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After exocytosis the synaptic vesicle (SV) membrane is recycled by endocytosis. Exocytosis requires external Ca and so does endocytosis, which is supplied through voltage-gated Ca channels in the presynaptic membrane. Multiple types of calcium channels in the presynaptic terminal are participating in these processes. Ca channels designated for exocytosis are highly localized at the release site. Other types of Ca channels are probably diffusely distributed and may contribute to endocytosis. Two types of endocytosis have been demonstrated in EM at the Drosophila neuromuscular junction, namely, active-zone endocytosis that occurs at the presynaptic active zone and non-active-zone endocytosis that operates at the area away from the active zone. Recently we found that two separate types of Ca channels support these two types of endocytosis. Non-active-zone endocytosis is blocked by low concentrations of La, while active-zone endocytosis is inhibited by a spider toxin, PLTXII. Yet another type of Ca channel encoded by the gene, cacophony, is specifically designated for exocytosis. This type of channel, cac-Ca channel, is highly localized at the presynaptic active zone. The distribution of other types of Ca channels is difficult to demonstrate, but physiological findings indicate that PLTXII-sensitive channels are located close to or within the active zone, while the La-sensitive channels reside away from it. Ca is ubiquitously used a messenger. Its temporal and spatial characteristics mold its specific role.

S43 (1S-10C3)
Bidirectional regulation of synaptic efficacy through AMPA-receptor trafficking in cerebellar Purkinje cell

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Regulation of synaptic expression of AMPA type glutamate receptors (AMPA-Rs) through a receptor-trafficking is one of the underlying mechanism for synaptic plasticity in hippocampal and cerebellar neurons. In cerebellar Purkinje cell, induction of the long-term depression (LTD) of parallel fiber (PF)-EPSC requires clathrin-mediated endocytosis, however, relation between the constitutive rapid trafficking and LTD-induction was unclear. Suppressing exocytosis or endocytosis, we addressed whether regulation of the rapid constitutive trafficking of AMPA-Rs was underlying synaptic plasticity in cerebellar Purkinje cell. Effects of intracellular infusion of tetanus toxin (TeTx), a blocker of VAMP2, on PF-EPSC were analyzed in cerebellar slice using whole-cell patch-methods. Infusion of TeTx reduced amplitude of PF-EPSCs to 60% within 20 min. Since infusion of botulinum neurotoxin C (a blocker of syntaxin) reduced PF-EPSC amplitude to similar extent, contribution of TeTx-insensitive receptor-trafficking was suggested to be negligible. As a counterpart of constitutive elimination of synaptic AMPA-R, constitutive insertion of AMPA-Rs into PF-synapse was demonstrated by blocking of dynamin. As for relation between constitutive trafficking of AMPA-Rs and synaptic plasticity, LTD did not occlude with constitutive elimination of AMPA-Rs at PF-synapse, suggesting that internalization of synaptic AMPA-Rs during LTD did not belong to constitutively recycling pool of AMPA-Rs.

S44 (1S-10C4)

Analysis of presynaptic exocytosis and its plasticity using mice genetically expressing synaptopHluorin in the hippocampus

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The synaptic transmission is potentiated by the activation of adenylyl cyclase (AC) and protein kinase A (PKA) at hippocampal mossy fiber-CA3 synapses. Although the AC/PKA activation was suggested to facilitate the transmitter release from MF presynaptic terminals, the most of these classical evidences were indirect. In this study the presynaptic exocytosis was directly investigated in the hippocampal slice of a synaptopHluorin (SpH) transgenic mouse (TV-42 line) which expresses SpH specifically at the mossy fiber terminals of hippocampus (Araki et al. 2005). The repetitive stimulation (10 Hz for 1 s) of mossy fiber bundle transiently increased the SpH fluorescence in the presynaptic terminal. The SpH fluorescence was sampled before and after application of forskolin (50 μM) and IBMX (100 μM), a combination which activates AC/PKA. The AC/PKA activation increased the activity-dependent increment of SpH (ΔSpH) by 2.42 ± 0.49 (mean ± SEM, n = 29) on average ($p<0.001$). However, both the rising and falling time course of δSpH was not changed. The ΔSpH was also largely facilitated in the presynaptic terminal of which it was null at baseline. It is suggested that the AC/PKA activation facilitates the presynaptic exocytosis and that it turns some presynaptically silent synapses into active.

Ref: Araki R. et al. (2005) *genesis* 42, 53-60.

S45 (1S-11D1)

Discharge properties of neurons in the rat medial vestibular nucleus

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The vestibular nucleus (VN) is a center for stabilizing gaze and posture in response to the head rotation and tilt. VN neurons are physiologically classified into regularly and irregularly discharging neurons on the basis of the regularity of spacing of action potentials. The segregation of VN neurons is involved in different response properties to head movements. Although the discharge regularity has been considered to be attributed to afterhyperpolarization (AHP), the relationships between the discharge regularity and profiles of AHP are still unclear. In this study, we investigated discharge patterns of VN neurons using whole-cell patch clamp technique not only in vitro slice preparations but in vivo preparations obtained from young rats. Previously, AHPs were classified into AHP without a slow component [AHP(s-)], AHP with a slow component [AHP(s+)], and AHP with a slow component and an afterdepolarization [AHP(s+) with ADP]. Both in vitro and in vivo, neurons exhibiting AHP(s+) fired more regularly than the other types of neurons. Application of 100 μM apamin to block Ca^{2+}-dependent K^+ channels abolished the slow component of AHP(s+) and made regular discharges of neurons exhibiting AHP(s+) to be irregular. These suggest that neurons exhibiting AHP(s+) are regularly discharging neurons, whereas neurons exhibiting AHP(s-) and AHP(s+) with ADP are irregularly discharging neurons. The regular firings of neurons exhibiting AHP(s+) are attributed to activation of apamin-sensitive Ca^{2+}-dependent K^+ channels.

SYMPOSIA
Integrative approaches to neural circuit function [YFI (Young Foreign Investigator) Workshop]

S46 (1S-11D2)

Mechanisms for submillisecond coincidence detection in the chick auditory brainstem

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Localizing sound sources requires discriminating differences of sound arrival time of a microsecond order between the two ears (interaural time difference, ITD). In nucleus laminaris (NL) of birds, neurons calculate ITDs by detecting the coincidence of binaural synaptic inputs. We utilized slice-patch recordings, immunohistochemistry and computer simulations to explore the acuity and cellular mechanisms of coincidence detection in NL neurons of the chick. At 40 °C, the avian body temperature, the acuity of coincidence detection was high enough to account for the animal behavior. This acuity was achieved by the acceleration of EPSP time course due to the activation of Kv1.2-mediated low-threshold K^+ conductance. In NL, neurons are tuned to a specific frequency of sound (characteristic frequency, CF), and are arranged so that the CF decreases from rostro-medial (high-CF) to caudo-lateral (low-CF) direction. Along this tonotopic axis, NL neurons were specialized morphologically and functionally depending on their CF. In the high- and middle-CF neurons, dendrites were short and expression of Kv1.2 channels was strong, which made the EPSP time course rapid and improved the coincidence detection. In the mid-high CF neurons, the process of generating spikes was also specialized; the axon initial segment was myelinated and Nav channels were clustered at some distance from the soma (20-50 μm) in the axon. Theoretical model predicted that this unique distribution of Nav channels in the axon is essential for making the high-frequency generation of action potentials and enhancing the ITD detection.

S47 (1S-11D3)

Surround inhibition of nociceptive transmission in the superficial spinal dorsal horn through activation of tactile C afferent fiber. -*In vivo* patch-clamp analysis of modality dependent synaptic responses-

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Disinhibition such as a loss of inhibitory interneurons or a shift in the transmembrane anion gradient especially in the substantia gelatinosa (SG) of the spinal dorsal horn is thought to be a crucial etiology for chronic pain syndromes. However, there is little direct evidence to elucidate the natural inhibitory mechanism for nociceptive transmission because of difficulties in recording inhibitory synaptic responses from small size SG neurons in vivo. In this study, whole-cell recordings were obtained from SG neurons *in vivo* and in slice preparations to analyze how inhibitory synaptic inputs modulate noxious transmission and the underlying neuronal circuits. SG neurons *in vivo* responded to cutaneous pinch accompanied with a barrage of EPSCs. On the other hand, touch evoked a barrage of IPSCs during the stimulation and the receptive fields were larger than those of pinch-evoked EPSCs. After cessation of a brief touch, a burst of IPSCs lasted for about 10 s in some cells. The number of action potentials generated by pinch was decreased by the simultaneous stimulation of touch applied to the surrounding area. In slice experiments, activation of C fiber was required to elicit the burst of inhibitory response and large islet cells known as an inhibitory interneuron received C fiber inputs. The results suggest that innocuous stimulation even brief touch sufficiently suppresses noxious sensation in the SG through activation of C fibers.

SYMPOSIA
Physiological studies on environmental health—Vulnerability of biological functions to xenobiotic chemicals

S48 (1S-11D4)

Transgenic approach to cerebellar cortical network

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In the cerebellar circuit, Golgi cells receive inputs from granule cells and in turn terminate their axons on granule cell dendrites. Since Golgi cells are the only element that controls the activity of granule cells, Golgi cells are thought to play an important role in information processing via feedback mechanisms.First we investigated the role of Golgi cells by selective ablation using the immunotoxin-mediated cell targeting technique. The elimination of Golgi cells caused severe acute motor disorders. These mice gradually recovered but retained a continuing difficulty in performing fine movements. Electrophysiological analyses indicated that disruption of Golgi cells not only eliminates GABA-mediated inhibition but also attenuates functional NMDA receptors in granule cells. These results demonstrate that synaptic integration involving GABA inhibition and NMDA receptor activation is essential for motor coordination. Next we investigated the synaptic mechanisms of postsynaptic metabotropic glutamate receptor subtype 2 (mGluR2) on Golgi cell dendrites, using whole-cell patch-clamp recording of green fluorescent protein-positive Golgi cells of wild-type and mGluR2-deficient mice. Postsynaptic mGluR2 was activated by glutamate released from granule cells and hyperpolarized Golgi cells via G protein-coupled inwardly rectifying K channels. This hyperpolarization induced long-lasting silencing of Golgi cells, the duration and extents of which were dependent on stimulus strengths. Postsynaptic mGluR2 thus senses inputs from granule cells and plays a pivotal role in spatiotemporal modulation of mossy fiber-granule cell transmission.

S49 (1S-12E1)

Indoor air quality and sick house syndrome

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Sick house syndrome is a disorder of nerve function, mainly affecting the central nervous system/autonomic nervous system, caused by a sensitivity reaction induced by exposure to trace amount of deleterious chemical substances present in the living environment. Diagnosis is not easy because pathophysiological understanding of the syndrome is not sufficiently complete.

In this syndrome, functional assessment of nerve function is especially relevant. For example, the electronic iriscorder is useful as one of the tests of autonomic nerve functioning in this syndrome. There are many cases of this syndrome in which some abnormality and/or instability of pupillary light reaction, that is primarily caused by functional abnormality of autonomic nerve function, has been observed. Furthermore, evaluation of eye movement by Electro-Oculograph is also very useful, as many patients have some disorder of smooth pursuit movement. Modulation Transfer Function, which evaluates the higher optical center (visual cortex) is also useful, and a decrease in Visual Contrast Sensitivity is often observed.

Genetic polymorphism testing of drug metabolizing enzymes, such as CYP, GST, NST, and PON1 are useful in evaluating an inherited sensitivity to chemical substances in the patient. From our latest investigation, differences such as absence, decrease in concentration, and delay in induction of these enzymes are observed in some of the patient groups which are obviously different from healthy people. This is important knowledge leading to possible specification of the genes active in expression of this syndrome.

S50 (1S-12E2)

Modulation of functional development of brain by polychlorinated biphenyls

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Polychlorinated biphenyl (PCB) is an environmental chemical that may cause adverse health effects. Previous studies have shown that developing central nervous system is one of the most vulnerable organs against its exposure. However, the molecular mechanisms of PCB action have not yet been fully understood. Since PCB exposure induces abnormal brain development similar to those seen in perinatal hypothyroid animal, we have been studied the effect of PCB/dioxin on thyroid hormone (TH) receptor (TR)-mediated transcription. We have previously identified that PCB may not competitively bind to TR ligand binding domain. Instead, it partially dissociated TR from TH-response element located on the promoter region of target gene. The mechanisms of such dissociation in not well known. However, our recent findings have indicated that PCB may bind to DNA binding domain of TR, which may alter the structural conformation of TR protein.In addition to PCB action on TR, we have also studies the effects of PCB on several other nuclear receptors. It may affect to estrogen receptor (ER)-, and steroid and xenobiotic receptor (SXR)-mediated transcription, but not to glucocorticoid or progesterone receptor action. Further, PCB may also act to neuronal membrane to induce an increase in intracellular calcium concentration, which then stimulate the expression of calcium-induced transcription factors such as c-Jun.These results indicate that PCB may act at multiple systems to alter the gene expression profile of neuronal cells that may affect the normal brain development.

S51 (1S-12E3)

Central neurotoxicity induced by 1-bromopropane, a substitute for specific chlorofluorocarbons

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1-Bromopropane ($CH_3CH_2CH_2Br$ 1-BP) is a newly introduced substitute for specific chlorofluorocarbons whose production was prohibited because of depletion of ozone layers, and is mainly used for degreasing agents and spray adhesives. Although case studies in the USA and China have demonstrated that 1-BP could adversely affect the human nervous system, the underlying mechanism for the effects of 1-BP inhalation exposure on the CNS has not been understood. We investigated the effects of 1-BP exposure on the CNS using different models of exposure. 1-BP potentiated GABA but inhibited ACh responses in *Xenopus* oocytes expressing $GABA_A$ and nicotinic ACh receptors, respectively, and enhanced recurrent inhibition in the rat hippocampus. On the other hand, hippocampal disinhibition was observed in a concentration-dependent manner (200 - 1500 ppm) after chronic inhalation of 1-BP in the rats and, at the highest concentration of 1-BP inhalation, epileptic potentials were evoked in the dentate gyrus. Moreover, prenatal exposure to 1-BP vapor resulted in enhanced stimulation/response (S/R) curve of population spikes in the CA1 area of PND 11-15 rats and reduced S/R curve of field excitatory postsynaptic potentials in the CA1 area in adults (6-8w). These results suggest that 1-BP inhalation exposure disrupts neuronal excitability of the hippocampal formation.

S52 (1S-12E4)

Effects of environmental chemicals on emotional behaivor and the brain

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The effects of environmental chemicals on sexual differentiation of exploratory behavior and emotional behaviors and brain were investigated. We exposed bisphenol A (BPA, 0.05-5 ppm) to mother rats pre-, peri- or postnatal period or 1-bromopropane (1-BP, 700 ppm) for 6 h/day during prenatal period. In the open field test, control females explored more frequently than males. This sex difference was not affected by neonatal BPA treatment but was abolished by pre- and perinatal treatment or 1-BP. The time spent in open arms in the elevated plus maze test decreased by neonatal or prenatal BPA treatment although sex difference was not clearly affected. In the forced swimming test, both prenatal and neonatal BPA exposures increased immobility time, an index of depressive behavior, in male rats and reduced immobility latency in both sexes. The duration of immobility decreased in the forced swimming test and the sex difference were disappeared by 1-BP. These findings suggest that prenatal BPA or 1-BP exposure is more effective to impair sexual differentiation of exploratory behavior than neonatal BPA exposure but neonatal period is also important for development of emotional behavior

SYMPOSIA
Trafficking and localization of the NMDA receptor

S53 (1S-13F1)
Regulation of molecular organization and morphology of postsynapses by Cupidin/ Homer

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Homer is a postsynaptic scaffold protein with the N-terminal target binding and C-terminal self-assembly domains. Homer multimers likely link their targets, including proteins related to the Glu receptor and Ca^{2+} signaling (mGluR1a/5, Shank, IP3R) and to the actin cytoskeleton (Drebrin and Cdc42), at postsynaptic density (PSD). The Homer family consists of three long-form Homers H1b/c, Cupidin/H2, and H3. A natural dominant-negative, short-form H1a with only the N-terminal domain is also activity-dependently expressed. In hippocampus, H1b/c and Cupidin/H2 predominate in CA1 region, whereas H3 is largely localized in CA2-CA3 region. In cultured hippocampal cells, dendritic clustering and synaptic targeting of long Homers coincide with those of NMDAR and PSD-95 throughout development. Overexpression of long Homers increases mature-shape spines, whereas that of H1a alters PSD target contents and spine morphology. In cerebellum, H1b/c and Cupidin/H2 are concentrated in PSDs of granule cells. In cultured granule cells, their clustered distribution is changed by NMDAR-mediated Ca^{2+} influx, and H1a has a neuroprotective action against excess Glu exposure probably by interfering a target linkage via long Homers, which inhibits NMDAR activity. On the other hand, H3 is exclusively localized in Purkinje cells. mGluR1a binding and dendritic localization of H3 is controlled by its Ca^{2+}-dependent phosphorylation states. Thus, Homer is involved in synapse formation and function by regulating molecular organization of PSD and spine morphology.

S54 (1S-13F2)
Down-regulation of drebrin A expression suppresses homeostatic synaptic targeting of NMDA receptors

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Drebrin is a major F-actin binding protein in the brain. We have recently demonstrated that the expression of drebrin A (neuron specific isoform) is rapidly upregulated in parallel with synapse formation, and that it governs the targeting of postsynaptic density (PSD) protein PSD-95 to synapses. Immunoelectron microscopy demonstrated that drebrin A is first appeared at the submembranous regions of developing excitatory postsynaptic sites at the initial stage ofsynapse formation. To determine the role of drebrin A on excitatory synapse formation, we analyzed whether the suppression of drebrin A expression affects filopodia-spine morphology and synaptic targeting of NMDA receptors in cultured hippocampal neurons. Suppression of developmentally programmed upregulation of drebrin A by antisense treatment significantly decreased the density and width of filopodia-spines. Immunocytochemistry showed that the antisense treatment did not attenuate synaptic clustering of NMDA receptors under condition that permitted spontaneous activities, but inhibited the accelerated targeting of NMDA receptors into synapses by its antagonist AP5. These results indicate that drebrin A upregulation play a pivotal role in spine morphogenesis and activity-dependent synaptic targeting of NMDA receptors.

S55 (1S-13F3)
Synaptic localization and left-right asymmetrical allocation of NMDA receptors

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NMDA receptors play a key role in synaptic plasticity in the hippocampal CA1 pyramidal cells. Two subunits of NMDA receptors NR2A and NR2B have distinct expression patterns in development and may contribute differently to induction of long-term potentiation and depression. We discovered input-dependent left-right asymmetry of NR2B subunit allocation in Schaffer collateral (Sch)- and commissural fiber- pyramidal cell synapses. However, it has not been known if NR2B has such asymmetrical distribution in Sch-interneuron synapses, and if NR2A has also asymmetry to neutralize the NR2B asymmetry in pyramidal cell synapses. Here, we investigated distribution of NR2A and NR2B in single synapses by postembedding immunogold and SDS-digested freeze-fracture replica labeling methods. To facilitate the detection of NR2B density difference, we utilized NR2A knockout mice, which have a simplified NMDA receptor subunit composition.The labeling density for NR2B but not NR1 in Sch-CA1 pyramidal cell synapses was significantly different between the left and right hippocampus with opposite directions in strata oriens and radiatum. No significant difference in NR2B density, however, was detected in CA1 stratum radiatum between the left and right Sch-interneuron synapses. Immunoblot analysis of PSD fractions from CA1 radiatum confirmed significant difference in protein amount for NR2B but not for NR1 and NR2A between left and right hippocampus. These results indicate that the asymmetry of NR2B distribution is target-cell specific and unique to this subunit.

S56 (1S-13F4)

Roles of NMDA receptor phosphorylation in the amygdala

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While NMDA-type ionotropic glutamate receptor (NMDAR) is widely accepted as a key regulator for certain forms of memory and learning and LTP induction, not much is known about how NMDAR function is regulated at physiological circumstances. The NR2B subunit of the NMDAR is tyrosine phosphorylated in brain, with Tyr-1472 as a major phosphorylation site. Phosphorylation of neural proteins is one of the main mechanisms underlying dynamic changes in neural functions, so we investigated the physiological significance of NR2B phosphorylation in neuronal plasticity and learning behavior. Mice with a knockin mutation of the Tyr-1472 site to phenylalanine (Y1472F) showed impaired induction of amygdaloid long-term potentiation and fear-related learning. Basic properties of synaptic transmission were normal in YF/YF mice, suggesting that impaired LTP in YF/YF mice is not caused by direct modification of NMDAR current properties but is associated with some intracellular signaling downstream from NMDAR activation. In fact, CaM kinase II, a key regulator for synaptic plasticity was undetectable in NMDAR complex of YF/YF mice. Electron microscopic analyses revealed that NMDAR localization at synapses was impaired in YF/YF mice, presumably resulting in altered NMDAR complex of YF/YF mice. These results strongly argue that phosphorylation of Tyr-1472 regulates NMDAR localization at synapses leading to modulating synaptic plasticity and fear-related learning.

S57 (1S-14G1)

Critical role of cross modal integration of sensory cues for the development of peer relationship

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Human is a social animal and unique to communicate with language. We want to understand the neurobiological basis of this feature in the light of comparative neuroethology. Social animals, like primates and birds, could communicate with conspecific mates by vocalization. We have established the developmental model of social communication with domestic chick which is a precocial bird and can grow itself without parent care. This allows us to investigate the development of peer relationship without considering the effect of parent-infant relationship. We reared chicks under grouped or individual condition, and compared socialization between two conditions. We measured the association and calling behavior as indicating socialization. The chicks reared as a group for 8-14 days after hatching showed socialization. In contrast, the chicks reared under socially deprived condition showed strong fear response in the novel environment and could not develop socialization. We, next, tested uni-modal social deprivation, that is, either visual or vocal cue was presented during individually rearing. Under these conditions, the fear response was suppressed when the behavior was tested on 8-14th day. Socialization, however, was different between the sensory social cues presented. Only vocally communicated chick could develop socialization. These results suggest that social interaction during infant is critical to develop peer relationship and mutual vocalization is important. Now, we are localizing the neuronal substrate for the development of peer relationship

SYMPOSIA
Environmental factors during development affect behavioral patterns: Critical period and molecular mechanisms

S58 (1S-14G2)

Olfactory imprinting: Molecular mechanism of olfactory learning in pups

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Young rats prior to eye opening depend on somatosensory and olfactory function for survival, as they can learn their dam's odor and approach her without visual information. In order to establish olfactory learning, the pairing of odor and tactile stimulation is crucial. Noradrenergic activation through the locus coeruleus by a somatosensory stimulus is implicated in olfactory learning. Within the olfactory bulb (OB), the noradrenergic innervation modulates the efficacy of dendrodendritic synapses between the mitral and granule cells. At the dendrodendritic reciprocal synapses, mitral cell activity is inhibited by GABA released from the granule cells. It is noteworthy that disinhibition of the mitral cells is a crucial step in the formation of an olfactory memory. We previously showed that intrabulbar infusion of the GABA receptor antagonist, bicuculline facilitated olfactory learning. These results implicate the OB as a critical site for olfactory learning. Since the transcription factor, CREB is well known to be involved in plasticity, we examined whether CREB is involved in olfactory learning. Behavioral pharmacology shows that only long-term olfactory memory was prevented by CREB antisense infusion, but short-term memory was intact. Western blot analyses reveal that P-MAPK/ERK was increased for 1 hour after odor exposure paired with shock, followed by increase of P-CREB lasting for 6 hours. These may be evidence suggesting that synaptic plasticity in the OB underlies aversive olfactory learning.

S59 (1S-14G3)

Factors affecting rhythm entrainment and stress responsiveness by periodic maternal deprivation

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The life of newborn rats totally depends on their maternal care. Although the first several days of their life are characterized by stress-hyporesponsiveness, absence of mother acts as a strong stressor which overrides it and results in long-lasting stress-vulnerability. Periodic maternal deprivation (MD) for the first few days act as a strong non-photic time cue and entrains pups' circadian clock. However, little is known as to the mechanisms how the MD affects on the pups' circadian clock and stress-responsiveness in the adulthood. We imposed newborn rats to MD of various durations at different time in the light phase, at different period in the postnatal life, with or without keeping pups warm. In addition, by restricting food access of mother rats to 2h (RF), behavioral rhythms of mothers were modified without depriving them from pups. We measured clock gene expression rhythms in the suprachiasmatic nucleus of the fetes and neonates, and behavioral rhythms after weaning. At 8 weeks of age, stress responsiveness was examined by measuring plasma corticosterone levels after exposing to mild stress of cage exchange. 12 h MD during the light phase in day1-6 completely reversed the circadian rhythms of clock gene expression. The behavioral rhythms after weaning were also shifted depending on the phase of MD. Rats exposed to MD exhibited hyper responsiveness to the novelty stimuli, which was abolished by MD with warming. These results suggest that the maternal care is important to entrain pups' clock, while heat loss due to isolation is critical for the stress hyper-responsiveness in adulthood.

SYMPOSIA
Research frontiers in cardiovascular physiology—New original concepts

S60 (1S-14G4)

Molecular mechanism of stress vulnerability and resilience induced by early environment

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Although an early adversity is a major risk factor for the vulnerability to stress later in life, the mechanism of the stress vulnerability remains to be unknown. It is known that while neonatal isolation (NI) induces stress vulnerability in adult rats, environmental enrichment (EE) following NI leads to resilience. We examined whether NI induced the susceptibility to learned helplessness (LH) (animal model of depression) and EE ameliorated this susceptibility. Pups were individually isolated from postnatal day 2 to 9. After weaning, EE was administrated until the beginning of LH session. In adulthood, we measured the number of escape failures and escape latency 24 hours after exposure to inescapable shock session. Behavioral analyses revealed that whereas the population of LH in NI rats was significantly higher than that in sham rat, EE markedly decreased the population of LH in NI rats. We tried to identify genes involved in the molecular mechanism underlying the susceptibility to LH using a cDNA array, and real-time PCR. The comparison of hippocampal gene expression between NI-LH and sham-nonLH rats revealed the significant decrease in LIMK1 mRNA in NI-LH. EE prevented the decrease in the expression of LIMK mRNA in the hippocampus of NI rats. These findings suggest that LIMK may play an important role in stress vulnerability developed by an early environment.

S61 (1S-15H1)

Blood Pressure Control -Two significances and Two mechanisms-

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It is well established that blood pressure (BP) is controlled by feedback mechanisms, e.g. via baroreceptor reflex. The feedback mechanism supports blood pressure homeostasis to save life at sudden orthostasis or massive hemorrhage. We have shown that sinoaortic denervation abolished BP resetting to a higher level at daily physical activity. This indicates that the baroafferent signal supports active resetting of BP during exercise, suggesting that BP should be controlled not only homeostatically by feedback but also homeodynamically by feedforward mechanisms. The above mechanisms are involved in the short-term control of BP. Resting level of BP is thought to be relatively constant for life-long in health subjects. Lesion of the area postrema, which is a site for neurohumoral interaction with vasopressin and angiotensin II, did not show any abnormality in day-to-day control of BP. In animal-models with salt-sensitive hypertension, vascular endothelial functions or renal functions are impaired but central sympathetic control is preserved, although the central nNOS systems are upregulated to inhibit sympathetic activation. These data lead to the possibility that main causes for abnormality in long-term control of BP might be present in the effector organs but not in the central control system, rather that the central sympathetic system functions to compensate hypertension. These data suggests that BP should be regulated by feedback and local mechanisms homeostatically for saving life and by feedforward mechanisms homeodynamically for helping organ-functions.

S62 (1S-15H2)

Resetting of Cardiovascular Regulation by Emotional Stimuli

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The word "homeostasis" implies a regulatory mechanism that stabilizes the biological parameters around an operating point during the resting condition. During exercise or stressful condition, however, a new operating point will be set. This operating point is different from that under the resting condition and should be suitable for the bodily demand during the new condition. To explore neural mechanisms of such resetting of the operating point, we have recently focused on the stress-induced defense response because stressor induces not only cognitive, emotional and behavioral changes but also autonomic changes. These changes include increases in blood pressure, heart rate, muscular blood flow, respiratory frequency, and tidal volume and suppression of the baroreceptor reflex and pain sensitivity. Although research on the neural circuits underlying such autonomic changes has implicated the hypothalamus in the defense response against stressors, neurotransmitters in this multifacet and coordinated response have not been revealed. In my talk, I will summarize our recent discovery of possible contribution of orexin as a master switch to elicit multiple efferent pathways in the defense response and discuss future directions.

S63 (1S-15H3)

Mechanisms of generation for sympathetic spontaneous discharge on the cardiovascular center in the medulla oblongata; the study by an in situ arterially perfused preparation

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A major source of excitatory drive to the pre-ganglionic sympathetic neurons originates from the rostral ventrolateral medulla (RVLM). RVLM sympathetic premotor neurons (RVLM neurons) have spontaneous activity *in vivo*. For the generation of RVLM neuron activity, "Network theory" and "Pacemaker theory" have been suggested by *in vivo* and *in vitro* studies. However, it is still unclear which one is right. We studied how RVLM sympathetic premotor neurons generate their activity using an *in situ* arterially perfused preparation (the working heart-brainstem preparation). We recorded activities of the thoracic sympathetic chain and RVLM neurons. The firing response of RVLM neurons during hypoxic-induced gasping is heterogeneous with some inhibited and others persisting. The finding that some RVLM neurons continue to fire after blockade of fast synaptic transmission is consistent with the hypothesis that they may have intrinsic pacemaker activity. Since some RVLM neurons remained responsive to hypoxia after blockade of fast excitatory and inhibitory synaptic transmission, they may be directly sensitive to hypoxia. Some RVLM neurons may be central oxygen sensors and exhibit pacemaker activity during hypoxia to ensure high levels of sympathetic activity and hence maintenance of arterial pressure.

S64 (1S-15H4)

Role of the intracranial vasodilative system that regulates cerebral parenchymal microvessels.

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Cerebral blood flow is vital for the survival and function of the brain. In particular, the hippocampus and cerebral cortex are very sensitive to transient ischemia. The hippocampus and cerebral cortex receive cholinergic vasodilative fibers that originate in the medial septum and the nucleus basalis of Meynert (NBM), respectively, of the basal forebrain (see a review by Sato et al., 1995, Alzheimer Dis. Assoc. Disord. 9: 28). Recently, we showed that increases in blood flow in the hippocampus and cerebral cortex in rats during activation of the vasodilative system, either by pharmacological (i.v. nicotine) or physiological (electrical stimulation of the NBM) methods, can prevent delayed death of hippocampal and cortical neurons following transient ischemia. Stimulation of the NBM increased the diameter of cortical parenchymal microvessels during stimulation. In addition, after the end of stimulation, an increase in the concentration of brain-derived neurotrophic factor (BDNF) in the cortical extracellular fluid was observed. From these findings, we suggest that activation of the intracranial vasodilative system provides protection against ischemia-induced delayed neuronal death by inducing increases in both the diameter of parenchymal microvessels and the release of an endogenous neuroprotective factor, BDNF. We also showed that activation of the vasodilative system occurred during passive somatosensory stimuli and active movements such as walking. Thus, this intracranial vasodilative system may contribute to the beneficial effect of physical activity on cognitive brain functions.

S65 (1S-15H5)

Regulation of cardiac function by cAMP/Ca

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Catecholamine signal is a major mechanism of regulating cardiac function. Norepinephrine released from the synaptic terminal binds to beta adrenergic receptors, leading to the activation of the stimulatory G protein and thus adenylyl cyclase. Cyclic AMP generated by adenylyl cyclase activates protein kinase A, which initiates multiple phosphorylation reactions within cardiac myocytes. A major impact of catecholamine stimulation is the enhancement of Ca cycling within myocytes. In the past decade, multiple molecules have been identified that are involved in Ca cycling. Transgenic studies using mouse models have elucidated the function of such molecules. Indeed, a growing body of evidence has shown that Ca cycling and Ca-dependent signaling pathways play a pivotal role in cardiac hypertrophy and heart failure. In addition, recent studies identified that mutations of the genes encoding sarcoplasmic reticulum proteins cause human cardiomyopathies and lethal ventricular arrhythmias. The regulation of Ca homeostasis via the SR proteins may have potential therapeutic value for heart diseases such as cardiomyopathy, heart failure and arrhythmias. Similarly, molecular mechanisms of catecholamine signal have been elucidated and the diversity of cAMP signal within the heart has been demonstrated. For example, it is now well known that the heart expresses multiple isoforms of adenylyl cyclase. The role of each adenylyl cyclase isoform is different in regulating cardiac function and the viability of cardiac myocytes under normal and pathological conditions. We will summarize our recent progresses in the study of this pathway in the heart.

SYMPOSIA
New stream of system biology by novel bioactive substances and hormones [Science Council of Japan Symposium]

S66 (2S-16A1)
Regulatory role of leptin-AMP kinase system in body energy metabolism

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Leptin is an adipocyte-secreted hormone that regulates body energy metabolism. We have recently shown that leptin stimulates fatty acid oxidation in skeletal muscle by activating α2 AMP-activated protein kinase (AMPK). Leptin exerts this effect directly at the level of muscle and through the hypothalamic-sympathetic nervous system. In contrast, hypothalamic α2 AMPK activity is inhibited by anorexigenic hormones (leptin and insulin), a melanocortin (MC) receptor agonist (anorexigen), high glucose and refeeding. AGRP (orexigenic neuropeptide), fasting and MC4 receptor-KO obese mouse increase hypothalamic AMPK activity. Expression of dominant-negative (DN) and constitutively active (CA) AMPK in the hypothalamus is sufficient to change food intake, body weight and expression of orexigenic neuropeptides such as NPY, AGRP and MCH. CA-AMPK blocks leptin-induced suppression of food intake.

We recently examined the signaling pathway of leptin's effects on AMPK, using muscle and neuronal cell lines that express leptin receptor Ob-Rb. Leptin activates α2 but not α1 AMPK in muscle cells through activation of ataxia telangiectasia mutated and calcium/calmodulin-dependent protein kinase kinase β. Furthermore, cellular localization of α2 AMPK is changed in response to leptin. In contrast, leptin suppresses α2 AMPK activity and NPY expression in neuronal cells.

Thus, leptin reciprocally regulates AMPK activity in neuronal and muscle cells. Our data indicate that leptin-AMPK system plays a critical role in peripheral and central regulation of body energy metabolism.

S67 (2S-16A2)
Physiological roles of adiponectin and adiponectin receptors

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Adiponectin/Acrp30 is a hormone secreted by adipocytes that acts as an antidiabetic and anti-atherogenic adipokine. We reported that AdipoR1/R2 serve as receptors for adiponectin and mediate increased fatty-acid oxidation and glucose uptake by adiponectin. Moreover, obesity was associated with decreased plasma adiponectin levels as well as decreased expression levels of AdipoR1/R2, the latter reduced adiponectin sensitivity, both of which finally lead to insulin resistance.

In this study, to clarify the physiological and pathophysiological roles of AdipoRs in vivo, we studied the effects of adenovirus-mediated upregualtion of AdipoRs in the mice liver. Here we show that adenovirus-mediated expression of AdipoR1 in the liver of db/db mice increased adiponectin effect such as increased activation of AMP kinase by adiponectin, decreased molecules involved in gluconeogenesis and increased fatty-acid oxidation, thereby ameliorating diabetes. Moreover, adenovirus-mediated expression of AdipoR2 in the liver of db/db mice increased adiponectin effect such as increased PPARalpha target genes including molecules involved in fatty acid oxidation and energy dissipation, thereby ameliorating diabetes. These data raised the possibility that AdipoR1 may be more tightly linked to activation of AMP kinase pathway, while AdipoR2 may be more tightly linked to activation of PPARalpha pathway.

Adiponectin receptor agonists and adiponectin sensitizers should serve as versatile treatment strategies for obesity-linked diseases such as diabetes and metabolic syndrome.

S68 (2S-16A3)
Novel functions of appetite-regulating peptides

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Feeding is a basic behavior that is necessary for life. Long-term lack of food results in death. It is well accepted that appetite is controlled by the brain and that feeding behavior is regulated by complex mechanisms in the central nervous system, in particular the hypothalamus. However, recent identifications of novel neuropeptides and peptide hormones develop a paradigm in appetite regulatory mechanisms in the central nervous system. In this presentation, I will discuss the two appetite-regulating peptides, ghrelin and neuromedin U. Ghrelin is a growth-hormone releasing and appetite-stimulating hormone secreted mainly from stomach. On the other hand, neuromedin U (NMU) is a potent appetite-suppressing peptide. Moreover, we recently revealed that ghrelin directly acts on osteoblast cells to regulate bone formation, and NMU is involved in the regulation of peripheral inflammation. Thus, both ghrelin and NMU are more than appetite regulators, but have multifaceted roles in, for example bone formation and inflammation.

S69 (2S-16A4)

Regulation and dysregulation of metabolism by new bioactive factors derived from muscle and fat

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Skeletal muscle and fat tissue are involved in the homeostasis of glucose metabolism. Here, we introduce new bioactive factors derived from skeletal muscle and fat tissue.

Musclin was identified via signal sequence trap of mouse skeletal muscle cDNAs. Musclin protein contained a region homologous to nariuretic peptide family. Its mRNA was expressed almost exclusively in skeletal muscle of mice, and regulated by nutritional changes. Recombinant musclin protein significantly attenuated insulin-stimulated glucose uptake and glycogen synthesis in myocytes.

A newly identified adipocytokine, visfatin, is highly enriched in the visceral fat of both human and mice and whose expression level in plasma increases during the development of obesity. Visfatin exerted insulin- mimetic effects in cultured cells and lowered plasma glucose levels in mice. Heterozygous knockout mice of visfatin had modestly higher levels of plasma glucose relative to wild type littermates. Surprisingly, visfatin binds to and activates the insulin receptor.

Production of ROS increased selectively in fat tissue of obese mice. In cultured fat cells, oxidative stress caused dysregulated production of adipocytokines, including adiponectin, PAI-1, IL-6, and MCP-1. In obese mice, treatment with NADPH oxidase inhibitor reduced ROS production in fat tissue, attenuated the dysregulation of adipocytokines, and improved diabetes.

Further study on the physiological role of these factors may lead to new

SYMPOSIA
Diverse functions of the gap junctions and their molecular mechanisms

S70 (2S-16A5)

Disecting behaviors from orphan GPCRs

Yanagisawa, Masashi (*Univ. of Texas Southwestern Med. Ctr., Howard Hughes Med. Inst., Dallas, TX, USA*)

To be filled in...

S71 (2S-17C1)

Interaction between gap and tight junctions

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It is thought that gap junctions may be closely associated with tight junctions. However, the mechanisms are still undefined. We found that Cx32 but not Cx26 was closely related to tight junctional proteins in primary cultured rat hepatocytes (Exp. Cell Res. 263, 193-201, 2001) and that Cx32 formation and/or Cx32-mediated intercellular communication could induce expression and function of tight junctions in a mouse hepatic cell line (Exp. Cell Res. 276, 40-51, 2002). When we performed cDNA microarray analysis of Cx32-transfectants, compared to parental cells derived from Cx32-deficient hepatocytes, an increase in expression of membrane-associated guanylate kinase with inverted orientation -1 (MAGI-1), which is known to be localized at adherens and tight junction regions, was observed (Cell Tissue Res. 319, 341-347, 2005). More recently, we performed to express short interfering RNA (siRNA) for Cx32 in primary cultured rat hepatocytes which highly expressed Cx32 and tight junction proteins and examined changes in expression of tight junction proteins and activated MAP-kinase. Down-regulation of Cx32 was associated with a decrease of claudin-1 and an increase of claudin-2. Furthermore, up-regulation of phosphorylated MAP-kinase was observed by the siRNA. Cx32 expression may in part regulate expression of tight junctions through the signal transduction pathway such as MAP-kinase.

S72 (2S-17C2)

Chemical gating of gap junction channels: role of carboxyl terminal of connexin43 as a regulatory domain.

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It has been known that intracellular acidification leads to gap junction channel closure. This phenomenon is called "chemical gating", which may be one of the causes of lethal arrhythmia during cardiac ischemia. Chemical regulation of Cx43 follows a ball-and-chain model, in which the carboxyl terminal (CT) domain acts as a gating particle that binds to a receptor affiliated with the pore. However, the location of the "receptor" for the CT has been unknown.Electrophysiological analysis shows that Cx43 channels reside in three states; closed (C), open (O) or residual (R). Since the R state is eliminated by truncation of the CT, it is hypothesized that the R state results from the interaction of the CT with the receptor. Recently, we showed in vitro that there is an intramolecular interaction of the CT with a region in the cytoplasmic loop of Cx43 (amino acids 119-144; dubbed "L2"). To determine the function of the L2, Cx43 channels were recorded in the presence of a peptide corresponding to the L2 region, delivered via the patch pipette. This manipulation eliminated the R state in a manner similar to that observed after truncation of the CT, indicating that L2 peptide competitively inhibits the interaction between the CT and the native L2 region. Thus, we propose that the L2 acts as a "receptor" that interacts with the CT during channel gating.

S73 (2S-17C3)

Degradation of connexin 43 induced by phosphorylation and dephosphorylation

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In cardiac muscle, the gap junction greatly contributes to intercellular impulse propagation. The remodeling of the gap junction is induced in pathological myocardium and influences the cardiac function. We investigated remodeling of connexin 43 (Cx43) which is dominant in the ventricular muscle cells, in reference to phosphorylation and dephosphorylation of the protein, using methods of electrophysiology, immuno blot and immunohistochemistry in adult guinea-pig and rat hearts.An activation of PKA promoted the PKA-mediated phosphorylation of Cx43 in association with an increase in the electrical intercelluar coupling and in expression of Cx43. In hypoxia, intracellular Ca-overload or acidosis, the PKA-mediated phosphorylation of Cx43 was inhibited in association with a suppression of the intercellular coupling and of expression of Cx43. These deteriorated changes of Cx43 were alleviated by PKA-activators. In the diabetic or PMA-treated heart, the PKC-mediated phosphorylation of Cx43 was augmented in association with an inhibition of the intercellular coupling and of expression of Cx43. These effects of an activation of PKC were ameliorated by a treatment of PKC-inhibitors, proteasome inhibitors or lysosome inhibitors. These results indicate that Cx43 hyperphosphorylated by PKC is highly susceptible to proteolytic degradation. It is concluded that Cx43 is up-regulated by PKA and down-regulated by PKC, and the remodeling of Cx43 is essentially induced by an excess activation of PKC.

S74 (2S-17C4)

Gap junctions and cell death: Changes in connexin localization during apoptosis in cells expressing Cx43-GFP

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Gap junctions are considered to play an important role in moderating cell death including apoptosis. However, the basic phenomena underlying when and where the alterations of gap junctions occur during apoptosis have not been well documented. In this study, To answer these questions, we analyzed the spatiotemporal changes of Cx during UV light-induced apoptosis using Cx43-EGFP-expressing HeLa cells, and compared them with those of mitochondrial membrane potential (MMP) using tetramethylrhodamine ethyl ester (TMRE) and nuclear morphological observation using Hoechst 33342. At 2 hr post-UV-irradiation, a third of the cells became TMRE-negative, i.e., they showed the loss of MMP, but with slight nuclear fragmentation, and high percentages of linear Cx43-EGFP plaques were found among both TMRE-positive and TMRE-negative cells. At 4 hr post-UV-irradiation, the percentage of these linear plaques was decreased, and both punctate and diffuse localization of Cx43-EGFP were noted in the cytoplasm of TMRE-negative cells without nuclear fragmentation. At 8 hr post-irradiation, punctate cytoplasmic localization of Cx43-EGFP was noted in TMRE-negative cells with nuclear fragmentation. Treatment with the caspase inhibitor Z-VAD-FMK blocked nuclear fragmentation and partially preserved both gap junctional plaques and MMP. These results indicate that, during apoptosis, Cx mobilization into the cytoplasm occurs after MMP depolarization but before nuclear fragmentation and that this alteration partly depends on caspase.

S75 (2S-17C5)

EDHF responses and Gap Junction

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Connexins are expressed in vascular endothelial and smooth muscle cells. However, the roles of connexins in the regulation of arterial tone are unclear. In this symposium, we would like to introduce recent evidence that connexins mediate endothelium dependent arterial relaxation caused by endothelium-derived hyperpolarizing factor (EDHF). The molecular identity of EDHF is not convincing. We assessed that whether NO, PGI_2, K^+, anandamide, H_2O_2 or EET act as EDHF. However, none of that act as EDHF in rat mesenteric artery. Recent studies suggest that gap junctional communication between endothelium and smooth muscle may account for EDHF responses. In rat mesenteric artery, endothelium-dependent relaxation and hyperpolarization by EDHF were inhibited by gap junction inhibitors. RT-PCR experiment showed that connexin 37, 40, 43 & 45 were expressed in the artery. In immunohistochemistory, connexin37, 40 & 43 were expressed in endothelium and connexin 43 was expressed in smooth muscle cells. EDHF-mediate hyperpolarization and relaxation were correlated with serum estrogen level. The expressional levels of connexin40 & 43 were also dependent on estrogen level. These results suggest that EDHF is not a molecule and its responses are mediated by gap junctional communications. Connexin may play a pivotal role in the regulation arterial tone in physiological and pathophysiological states in especially small arteries.

S76 (2S-17C6)

Connexins in human endometrium and correlation to carcinogenesis

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There are several lines of evidence suggesting that connexin expression is suppressed and/or aberrantly localized in pre-cancerous lesions in several organs and many, if not all, tumor-promoting agents have been shown to inhibit gap junctional intercellular communication (GJIC) of cultured cells as well as those in vivo, suggesting that the loss of GJIC enhances clonal dispersion, causing loss of the growth-suppression signals from the surrounding cells. For endometrial carcinogenesis, it may be concluded that the loss of GJIC caused by the suppressed expression and the aberrant localization of connexin support the clonal evolution of endometrial cancer cells originating in the hyperplasia cells. In the present study, GJIC of IK-ER1, which overexpresses ER-alpha was markedly reduced in the estradiol-containing medium and the reduction was found to be inhibited by ICI182.780, a pure anti-estrogen substrate, as demonstrated by Lucifer-Yellow dye-transfer assay. Western blot analysis indicated that the expression of both Cx26 and Cx32 also decreased in E(+) and the reduction was inhibited by adding ICI182.780. These results supported the result of the dye-transfer assay. Thus, estrogen, which suppresses connexin expression of endometrial epithelium and causes cell proliferation, may act as a tumor-promoting agent for endometrium.

S77 (2S-18D1)

Simultaneous observation of pre- and postsynaptic morphological changes in hippocampal slice culture

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Dendritic spines are highly motile structures, but how spines move while keeping their contacts with presynaptic varicosities is not clear. To analyze movements of these synaptic structures simultaneously, we labeled CA1 pyramidal cells with green fluorescent protein and CA3 pyramidal cells with rhodamine-dextran in hippocampal slice cultures. We obtained stable expression of GFP in a limited population of CA1 pyramidal neurons by using transgenic mice with a Cre-loxP recombination system. CA3 pyramidal neurons of the slice cultures were loaded with rhodamine-dextran by electroporation. Labeled varicosities of CA3 pyramidal cells and spines of CA1 pyramidal cells were visualized using two-photon microscopy to detect close association of the two components. Time-lapse imaging revealed that they performed rapid morphological changes without losing their contacts. The extent of overall structural changes between varicosities and spines was correlated, while the direction of short-term volume changes was regulated independently. Furthermore, dendritic morphological changes induced by electrical stimulation had little effect on their association. These results suggest that synaptic junctions provide stable attachment sites functioning to correlate presynaptic and postsynaptic motility.

SYMPOSIA
Mechanisms of synapse development, maintenance and plasticity [YFI (Young Foreign Investigator) Symposium]

S78 (2S-18D2)

Neuronal phenotype and chronic network activity both influence the susceptibility of hippocampal neurons to NMDA-induced F-actin reorganization at synapses

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Actin cytoskeletal organization in dendrites and dendritic spines are believed to form a molecular basis for the morphological plasticity at brain synapses. We here demonstrate that actin cytoskeleton of hippocampal neurons is rapidly rearranged by N-methyl-D-aspartate (NMDA) receptor activation in both neuronal phenotype and developmental activity-dependent manner. In rat hippocampus primary cultures, a certain population (20-30%) of neurons showed drastic redistribution of its dendritic filamentous (F-) actin after a stressful NMDA stimulation (50μM for 30 s). The NMDA-induced actin rearrangement correlated with changes of spine morphology and disruption of several postsynaptic components like Homer1b/c, GluR1 and NMDAR2A, even though the synaptic contacts seemed to stay preserved. Immunohistochemical characterization showed that NMDA-susceptible cells did not express calbindin-D_{28}k. Reduction of network activity by chronic tetrodotoxin application resulted in an increased number of calbindin-D_{28}k -negative and NMDA susceptible cells. Exogeneous calbindin expression in these neurons could recover their resistance to NMDA induced F-actin redistribution. These data indicate that F-actin organization is diverse among different populations of neurons, which are selectively sensitive to hyper excitatory input. Such neuronal type-specific heterogeneity also points toward specific molecular mechanisms that contribute to cytoskeletal regulation in dendrites.

S79 (2S-18D3)

Role of drebrin in dendritic spine morphogenesis and dual regulation of drebrin dynamics by AMPA and NMDA receptors

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Dendritic spines represent the developmentally-regulated and activity-dependent pleomorphism based on actin cytoskeleton. However, molecular mechanisms governing the pleomorphism are unclear. First, we find that during development, synaptic drebrin clustering in dendritic filopodia is required for spine morphogenesis. Drebrin clustering with actin filaments occurs at postsynaptic sites of axon-filopodia contact. The drebrin clustering precedes and governs synaptic PSD95 clustering and spine morphogenesis. Second, using fluorescence recovery after photobleaching (FRAP) technique and immunocytochemistry, we find that activities of AMPA receptors (AMPA-Rs) and NMDA-Rs orchestrate drebrin dynamics for synaptic clustering of drebrin and PSD95. AMPA-R blockade reduces binding capacity of drebrin within spines, observed as a reduction of unrecoverable fraction. Consequently, Chronic AMPA-R blockade inhibits synaptic clustering of drebrin and PSD95. NMDA-R blockade facilitates transport of drebrin into spines, observed as a reduction of time constant. Further, chronic NMDAR blockade promotes synaptic targeting of NMDA-Rs but inhibits that of PSD95. Finally, we find that drebrin is involved in activity-dependent synaptic NMDA-R targeting. Drebrin-A knockdown inhibits the accelerated targeting of NMDA-Rs into synapses by NMDA-R blockade despite no effect on NMDA-R localization under conditions of spontaneous activities. In conclusion, activity-regulated actin-cytoskeletal system based on drebrin is critical for the diversity of spine structure.

S80 (2S-18D4)

Role of phospholipase Cβ as a coincidence detector for retrograde endocannabinoid signaling

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Endocannabinoids (eCB) mediate retrograde signal at various brain regions. Postsynaptic release of eCB can suppress neurotransmitter release through activating presynaptic CB1 receptor and cause short-term or long-term synaptic plasticity. The eCB release is induced by strong increase in postsynaptic $[Ca^{2+}]_i$ or activation of $G_{q/11}$-coupled receptors. Furthermore, coincidence of $[Ca^{2+}]_i$ elevation and receptor activation markedly enhances eCB release. Phospholipase C (PLC) is involved in biosynthesis of the major eCB 2-arachidonoylglycerol. To determine the role of PLC in eCB release, we used cultured hippocampal neurons and monitored the eCB release by measuring CB-sensitive synaptic currents. We found that the receptor-driven eCB release was absent in PLCβ1-knockout mice. This PLCβ1-mediated eCB release was dependent on physiological levels of $[Ca^{2+}]_i$. We measured PLCβ1 activity in intact neurons by using exogenous TRPC6 channel as a biosensor for the PLC product diacylglycerol. The receptor-driven TRPC6 currents were absent in PLCβ1-knockout mice and showed a similar $[Ca^{2+}]_i$ dependence to that of receptor-driven eCB release. These results indicate that PLCβ1 serves as a coincidence detector for triggering eCB release in the hippocampus. PLCβ contributes to various neuronal signaling. Therefore, Ca^{2+} dependency of PLCβ may play an important role in various synaptic modulations and plasticity.

S81 (2S-18D5)

Synaptotrophin, a novel neurotrophin required for synaptic integrity and information processing

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Learning and memory formation requires continuous synaptic plasticity at both the functional and structural level. The stability of synapse is maintained by bidirectional signals between pre- and post-synaptic molecules in response to synaptic activity. However, very little is known about molecules that are involved in such a transsynaptic action. Synaptotrophins (Sptn1-Sptn4) structurally belong to C1q/tumor necrosis factor (TNF) family. Recently, Sptn1 has been revealed to be glycoprotein secreted from cerebellar granule cell, and to regulate synaptic plasticity and synaptic integrity between parallel fiber-Purkinje cell (PF-PC) synapse. sptn1-null mice are ataxic, and exhibit molphological abnormalities of PF-PC synapse [e.g., naked spine, mismatched Postsynaptic density (PSD)]. Whereas sptn1 mRNA is predominantly expressed in the cerebellum, other members of synaptotrophin family, of which structure are highly similar to that of Sptn1, are expressed in not only cerebellum but also other brain regions. We hypothesized that the transsynaptic action of the secreted synaptotrophins plays a critical role in structural remodeling of synapses in various central nervous systems. Here we present the expression and the biochemical characteristic of Synaptotrophin family.

SYMPOSIA
Application of physiological study to various branches: Physiological approach to music and dance [Associates of Young Researchers Symposium]

S82 (2S-19E1)
I do dance, I do science.

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DANCE AND SCIENCE

Observation, invention, experiment, control, speculation, discussion and presentation...these are processes that a dance work is born. Movements come from nature both inside and outside me. To pick up and compose such movements, what I need to do everyday is sharpen my senses and hone my skill. It is same for doing science, isn't it?

PHYSIOLOGY FOR ART

Sharpen senses, make imagination from what we sense and express the internal image as a work...these are the processes of artistic expression. These processes are composed of every physiological functions beginning from the sensory inputs. When I do dance, I need to act on natural physiological limits such as joint angle, muscle mobility and so on. In other words, these limitations are the origins of the characteristic dance movement. Such limitation is the very source of beauty and confidence for movements. Audible sound, visible color, actable movement...these physiological limitations have important meanings for both artistic expression and impression. With these physiological limitations, we can converge and decide the artwork and can share impressions over the cultures and age universally.

Many people believe that art is too subjective to be a scientific object. Indeed,individual experience of art is hard to be described in objective way. However, I believe it still should have a great importance to study about art in physiological paradigm, because art is the work of the human beings limited physiologically.

S83 (2S-19E2)
"We are alive." at the Rave Party.

Seino, Eiichi (*SEINO Eiichi Office, Balearic Sunrise Org., Tokyo, Japan*)

Rave is the outside dance music party, started in 1988 in Britain, and has spread all over the world.

Now it's one of the biggest youth culture.

And before all, it's a strong and personal experience of sound, dance and ecstasy.

I was in the scene from 80's, and wrote some books about the rave culture, dance music and trance, dancing high and ecstasy, the party as a temporary autonomous zone etc.

What Rave gave the youth is the shout & feeling of "We are alive."

But what's that?

And for me, it's a "big somewhere" related to the writing.

Where is there?

Is it the place religion calls "a holy", or Timothy Leary's LSD revolution, or alcoholic junkie's blue devil?

Nobody explain yet, but Rave clearly gives us.

"We are alive."

S84 (2S-19E3)
How can physiology approach music?: A case study on the hypersonic effect

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In the Western modern framework, which had Cartesian dualism as one of its vital origins, music, which induces beauty and pleasure in human mind, and physiology, which illuminates physical mechanism of human body, were considered to belong to exclusively independent domains. Physiological approaches to music, therefore, are basically challenges against the paradigm dating back to Descartes and inevitably involve essential difficulties in practice.

As a successful instance of physiological approach to music, I will examine the discovery of the "hypersonic effect" in this presentation. The hypersonic effect is the phenomenon that imperceptible high-frequency component of air vibration above human audible range activates neural circuit of beauty and pleasure, and makes the sound more comfortable to hear. Regarding the phenomenon, there had long been a serious disagreement between artists and researchers. It may be easy to recognize that the critical factor for the discovery of the hypersonic effect, beyond this historical conflict, was the fact that the discoverer, Tsutomu OOHASHI, was a distinguished artist, Shoji YAMASHIRO, at the same time. Sharing scientific ability and artistic sensibility in one single personality, however, was just a necessary condition but not a sufficient condition for this discovery. This paper will introduce "Eiffel-Tower, Pyramid, and volcanic-islands models of human activity" that realized the physiological approach to music.

SYMPOSIA
The function of neocortical inhibitory interneurons

S85 (2S-20F1)
Nonpyramidal cells and their wiring in cortical microcircuit

Kubota, Yoshiyuki; Kawaguchi, Yasuo (*Div. Cerebral Circuitry, NIPS*)

Activities of cortical pyramidal cells are regulated by GABAergic nonpyramidal cells with temporally and spatially differentiated inhibitotry wiring. Cortical inhibitory synapses were believed to make synaptic contacts mainly on soma and/or proximal dendrites of pyramidal cells, however the latest our finding shows approximately 1/3 of axon terminals of cortical nonpyramidal cells, such as double bouquet cell, Martinotti cell and neurogliaform cell, make synaptic contact on spine head, which also receive an asymmetrical input, called double innervated (DI) spine. From morphological point of view, the inhibitory synapse on DI spine probably has a vetoed function to the asymmetrical excitatory input. We studied morphological properties of the double innervated spines using vesicular glutamate transporters (VGLUTs) positive terminals, which show complementary distribution in cortex, VGLUT1 positive terminals were mostly originated from cortical pyramidal cells and VGLUT2 positive terminals mostly from thalamic projection neurons. This complementary localizations permitted to study an origin of the excitatory terminals on the DI spines. We observed 291 VGLUT1 innervated and 442 VGLUT2 innervated spine heads and found the target of these inhibitory synapses were almost exclusively DI spines received VGLUT2 positive excitatory synapse. Forty four (9.6%) out of 442 spine heads innervated by VGLUT2 positive synapses received symmetrical synaptic input and only 2 (0.7%) out of 291 VGLUT1 innervated DI spines were found. These results indicated that part of thalamo-cortical efferent fibers were vetoed by inhibitory synapse selectively at spine head.

S86 (2S-20F2)
Regulation of inhibitory synaptic transmission by presynaptic glutamate receptors in visual cortex

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We studied the roles of presynaptic glutamate receptors in synaptic transmission and plasticity in cortical inhibitory connections. Whole-cell recording was conducted from layer 2/3 pyramidal cells in visual cortical slices of young mice. Inhibitory postsynaptic currents (IPSCs) were recorded at the reversal potential of excitatory synaptic transmission. The frequency of miniature IPSCs (mIPSCs) recorded in the presence of tetrodotoxin was increased by bath application of glutamate, AMPA and NMDA, while it was decreased by the AMPA receptor antagonist NBQX and the NMDA receptor antagonist APV. These compounds did not change mIPSC amplitude, suggesting that AMPA and NMDA receptors are present at the presynaptic terminal of inhibitory synapses and their activation facilitates inhibitory synaptic transmission. Indeed, application of either APV or NBQX decreased the amplitude of evoked IPSCs considerably with accompanying increases in both paired-pulse ratio and coefficient of variation of IPSC, consistent with presynaptic action of these antagonists. High-frequency stimulation produced long-term potentiation (LTP) of IPSCs in normal solution. The magnitude of LTP decreased in the presence of NBQX and LTP did not occur in the presence of NBQX and APV, suggesting that AMPA and NMDA receptors both contribute to the facilitation of LTP production. These results indicate that both AMPA and NMDA receptors are present at the presynaptic terminal of inhibitory synapses in layer 2/3 pyramidal cells and that their activation facilitates inhibitory synaptic transmission and LTP.

S87 (2S-20F3)
Thalamocortical innervation to inhibitory neurons in the cortex: relevance to cholinergic control of cortical network

Kimura, Fumitaka (*Div Neurophysiol. Osaka Univ. Grad. Sch. Med., Suita, Japan*)

Mammalian cortex receives dense cholinergic innervation from basal forebrain cholinergic neurons, but how acetylcholine (ACh) regulates cortical circuits is still unclear. Recent experiments favor the hypothesis that instead of producing simple facilitation or inhibition, ACh serves to shift the cortical circuits into a condition where cortical neurons are influenced predominantly by afferent inputs from thalamus rather than other cortical inputs. This is achieved by muscarinic suppression of intracortical connections and nicotinic facilitation of thalamocortical inputs, both presynaptically. Indeed, excitatory postsynaptic potentials (EPSPs) in layer 4 neurons elicited by thalamic stimulation were enhanced or in some cases "unsilenced" in the presence of a nicotinic agonist. Optical recordings further supported facilitatory effect of ACh, but it also revealed that this facilitation was followed by suppression only in layer 4. By comparing the sensitivity to a nicotinic agonist between excitatory and inhibitory cells, we found that inhibitory neurons were more susceptible to nicotine. Incidentally, we also found that thalamic activation of GABAergic neurons preceded that of excitatory neurons in a given barrel, which effectively works to produce a feedforward inhibition on excitatory relay cells. Thus, exploiting such intrinsic network property, ACh not only facilitate thalamic input to cortex, but also restrict the excitation of postsynaptic cells to a narrow window of time by selectively enhancing thalamic innervation to inhibitory neurons in the cortex.

S88 (2S-20F4)

GABA uptake determines critical period onset in mouse visual cortex

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Strengthening GABA-A receptor α1 subunit-mediated inhibition with diazepam triggers ocular dominance (OD) plasticity prematurely (Fagiolini et al., 2004). Yet, the endogenous determinant of critical period (CP) induction remains unknown. Several biochemical analyses intriguingly reveal an elevated GABA uptake with a peak before the CP onset. The main GABA transporter (GAT1) localized to inhibitory axon terminals may be responsible for this transient activity based on its developmental expression profile. Here, we tested directly whether loss of GAT1 function regulates CP onset. In GAT1 knockout (KO) mice, neuronal response strength in visual cortex was normal, but prolonged discharge reflecting weak intracortical inhibition was lost earlier, suggesting an accelerated maturation of GABA function in vivo. Brief monocular deprivation (MD) revealed that OD plasticity of GAT1 KO mice was prematurely activated and rapidly eliminated. When CP onset is delayed by genetic disruption of GABA synthesis, infusion of a GAT1 inhibitor into visual cortex concomitant with MD restored robust OD shifts. Similarly, natural CP delay by dark-rearing is counteracted by diazepam treatment (Iwai et al., 2003) and was also prevented by loss of GAT1. Interestingly, western blot analysis showed that 2d diazepam treatment in the dark triggers a reduction of GAT1 expression, similar to the normal developmental decline in the light. Indeed, GAT1 heterozygous mice mimic such a downregulation and also exhibited an accelerated CP. These findings indicate that GAT1 expression acts as a "brake" to delay CP onset.

SYMPOSIA
Dysfunction of intracellular trafficking and neuropathophysiology

S89 (2S-20F5)

Inhibitory neuronal circuitry underlying visual object recognition in area TE

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Neurons in area TE of the monkey inferior temporal cortex respond selectively to images of particular objects and are considered to be a neural basis for visual object recognition. The mechanism of generation of the stimulus selectivity, however, is largely unknown. We addressed the role of inhibitory TE neurons in this process by examining their visual response properties and interactions with adjacent target neurons. We applied cross-correlation analysis to spike trains simultaneously recorded from pairs of adjacent neurons in anesthetized macaques (Macaca fuscata). Neurons whose activity preceded a decrease in activity from their partner were presumed to be inhibitory neurons. Most inhibitory neurons responded to a variety of visual stimuli in our stimulus set, which consisted of several dozen geometrical figures and photographs of objects, with a clear stimulus preference. On average, 10% of the stimuli increased firing rates of the inhibitory neurons. Degree of stimulus selectivity of inhibitory neurons was similar to that of excitatory neurons. Although inhibitory neurons occasionally shared the most preferred stimuli with their target neurons, overall stimulus preferences were less similar between adjacent neurons with inhibitory linkages than adjacent neurons with common inputs and/or excitatory linkages. These results suggest that inhibitory neurons in area TE are activated selectively and exert stimulus-specific inhibition on adjacent neurons, contributing to shaping of stimulus selectivity of TE neurons.

S90 (2S-21G1)

Vesicular transport of Alzheimer's disease related proteins and neurodegeneration

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Dysfunctions of vesicular transport are linked to neurodegenerative disease, including Alzheimer disease (AD). Amyloid β-prrotein precursor (APP) has been implicated in the development and progression of AD. Recent reports suggest that APP functions as cargo receptor for kinesin I. APP interacts with kinesin light chain (KLC) indirectly *via* JNK-interacting protein 1b (JIP1b). We have reported that APP associates with Alcadein, a novel type I membrane protein, in neuron through their cytoplasmic interaction with X11-like (X11L) protein. We also found that Alcadein associates with KLC directly, thus Alcadein and APP/JIP1b competed for KLC. Alteration in APP- and Alcadein-transport system in neuron impairs the vesicle trafficking in axon, suggesting that inappropriate assignment of these cargos leads to neuronal malfunction and the degeneration in future.

S91 (2S-21G2)

Functions of Presenilins in Mediating Protein Trafficking

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Alzheimer's disease (AD), the most common form of senile dementia, is characterized by excessive production and accumulation of neurotoxic β-amyloid (Aβ) peptides which are proteolytically derived from β-amyloid precursor protein (APP) via β- and γ-secretase cleavages. Experimental evidence from several groups including our own has demonstrated that the production of Aβ occurs largely in the trans-Golgi network (TGN) where APP molecules predominantly reside. Mutations in presenilins genes are associated with the majority of familial AD likely through a mechanism of increase Aβ42 production. Presenilins (PS, PS1 and PS2) along with their associated proteins including nicastrin (Nct), PEN2 and APH1 are essential for the γ-secretase activity. The precise functions of Nct, APH-1 and PEN-2 have not been fully elucidated. Recent studies including ours suggest that PEN-2 mediates endoproteolysis of PS1, while APH-1 and Nct play regulatory roles in maintaining the stability of PS1 and the complex. PS1 knockout mice exhibit pre-neonatal lethality and PS1 has also been shown to affect numerous physiological functions including calcium homeostasis, skeletal development, neurite outgrowth, apoptosis, synaptic plasticity, tumorigenesis. These data strongly indicate critical physiological roles of PS1 addition to its essential role in γ-secretase activity. We and others have reported that PS1 plays an important role in intracellular trafficking (especially from the TGN to the plasma membrane) of select membrane proteins including APP, PEN2 and nicastrin. The detailed cell biological mechanism for PS-mediated protein trafficking will be discussed.

S92 (2S-21G3)

Aβ generation and APP trafficking in Alzheimer's disease

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Alzheimer's disease is a slowly progressive neurodegenerative disorder which causes severe dementia. Amyloid-beta peptide (Aβ) deposition in senile plaques is one of the pathological hallmarks in Alzheimer's disease. Aβ peptide is derived from the amyloid precursor protein (APP) by proteolytic processing by beta-secretase which cleaves APP at the N-terminus of Ab, and by gamma-secretase which cleaves at the C-terminus of Aβ. In spite of extensive research, the precise subcellular localization of Ab generation has not been identified yet. Using the recently developed fluorescence resonance energy transfer (FRET) approach and pulse-chase ELISA, we examined the subcellular localization of interactions between APP and beta-secretase. Our data showed a close APP-BACE interaction in early endosomes, and highlight the cell surface as an additional potential site of APP-BACE interaction. Furthermore, we identified a novel interaction between LRP, an endocytic receptor for APP, and beta-secretase, in the early endosomes and on the cell surface. The interaction between LRP and beta-secretase was not detected when cholesterol was depeleted, suggesting that LRP encounters beta-secretase in the lipid raft of the membranes. Taken together, we propose that APP interacts with beta-secretase in the lipid rafts of the cell membrane and in early endosomes, and that LRP may be a scaffold protein which links APP and BACE upon endocytosis. We believe that investigation of the interaction between APP and its secretases helps us understand the mechanisms of Aβ generation and pathogenesis of Alzheimer's disease.

S93 (2S-21G4)

Cdk5 is a membrane-associated protein kinase whose mislocalization induces neuronal cell death

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Cyclin-dependent kinase 5 (Cdk5) is a multifunctional Ser/Thr protein kinase activated by binding to its activator p35 or p39, which is expressed predominantly in neurons. Cdk5 is shown to be involved in neuronal migration during brain development, synaptic activity in matured neurons, and neuronal cell death in aged brains. However, exact roles of Cdk5 in those neuronal activities have not been answered yet. Cellular localization would be critical to understand the detailed functions of Cdk5/p35 or Cdk5/p39. p35 or p39 activator controls the cellular localization as well as the kinase activity. The Cdk5/p35 and Cdk5/p39 complexes bind to plasma membranes and Golgi apparatus via myristoylation at the N-terminal Gly of p35 or p39 that may compartmentalize the active Cdk5 complexes in the cytoplasm. When Gly is mutated to Ala, Cdk5/p35 and Cdk5/p39 become soluble in the cytoplasm and then is translocated into nucleus. This mislocalization is observed at the time of neuronal cell death. For example, endoplasmic reticulum (ER) stress deregulates the Cdk5 activity by cleavage of p35 to p25 with calpain. The cleavage of p35 changes the cellular distribution of active Cdk5, stabilizes the p25/Cdk5 complex, and stimulates the kinase activity of Cdk5, thereby allowing potentially aberrant phosphorylation of neuronal proteins, which would adversely affect the survival of neurons. We would also like to discuss on its localization in relation to membrane trafficking in living neurons.

S94 (2S-21G5)

Critical role of calpain-dependent cleavages of amphiphysin I in regulation of synaptic vesicle trafficking during hyperexcitation

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Clathrin-mediated endocytosis plays a key role in the recycling of synaptic vesicles in nerve terminals and amphiphysin I is one of the components of the molecular machinery involved in this process. Amphiphysin I mediates invagination and fission of synaptic vesicles in cooperation with dynamin. We found that amphiphysin I was cleaved to three fragments by treatment with high KCl (80 mM) and by high-frequency electrical stimulation in the mouse hippocampal slices. The cleavage sites were localized in the CLAP domain. The cleaved amphiphysin I was unable to interact with dynamin and disrupted the co-polymerization into a ring formation with dynamin and liposome in a cell-free system. The calpain-dependent cleavages inhibited clathrin-mediated endocytosis. Finally the amphiphysin I cleavages were found in the hippocampus of kainate-treated FVB/N mice and the cleavages inhibited the neural hyperexcitation of the mice. I will review these findings and discuss the role of calpain-dependent cleavages of amphiphysin I in protecting neurons against excitotoxicity and hyperexcitation.

SYMPOSIA
Kickoff symposium of the Journal of Physiological Sciences in succession of the Japanese Journal of Physiology

S95 (2S-23B1)
SODIUM-CALCIUM EXCHANGER: INFLUENCE OF METABOLIC REGULATION ON ION CARRIER INTERACTIONS.

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The Na-Ca exchangers family of membrane transporters is widely distributed in cells and tissues of the animal kingdom and constitutes one of the most important mechanisms for extruding calcium from the cell. Two basic properties characterize them: 1-Their activity is not predicted by thermodynamic parameters of classical electrogenic counter-transporters (dependence on ionic gradients and membrane potential), but is markedly regulated by transported (Na and Ca) and non-transported ionic species (protons and other monovalent cations). These modulations take place at specific sites in the exchanger protein located at extra, intra and trans-membrane protein domains. 2- Exchange activity is also regulated by the metabolic state of the cell. The mammalian and invertebrate preparations share MgATP in that role; the squid has an additional compound, phosphoarginine. This presentation emphasizes the interrelations between ionic and metabolic modulations of Na-Ca exchange, focusing mainly in two preparations where most of the studies have been carried out: the mammalian heart and the squid giant axon. A surprising fact that emerges when comparing the MgATP related pathways in these two systems is that although they are different (PIP2 in the cardiac and a soluble cytosolic regulatory protein in the squid), their final target effects are essentially similar: sodium-calcium-proton interactions with the exchanger. A model integrating both ionic and metabolic interactions in the regulation of the exchanger will be discussed.

S96 (2S-23B2)
Novel roles of anion channels in physiology and pathophysiology

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Anion channels play a stabilizing role in excitability in muscle and neuronal cells and a Cl^- transporting role in epithelial cells. Recent investigations have revealed their more general functions including cell volume regulation and cell proliferation. Here, we present additional roles that have been found in our laboratory. First, the volume-sensitive outwardly rectifying (VSOR) Cl^- channel, which is ordinary activated by cell swelling, plays an inductive role of apoptotic cell death. An apoptotic inducer rapidly activated the VSOR current without cell swelling and thereby induced apoptotic volume decrease (AVD) in epithelial and cardiac cells. Second, the same channel is involved in excitotoxic neuronal cell death. Stimulation with NMDA induced activation of the VSOR Cl^- channel, varicosity formation, somatic swelling and eventually necrotic death in cortical neurons. Third, the maxi-anion channel with a single-channel conductance of around 400 pS serves as the release pathway of ATP, which is an extracellular signal for cell-to-cell communication, in mammary cells, kidney macula densa cells, cardiomyocytes and astrocytes activated by a variety of stimuli. Forth, the maxi-anion channel also mediates glutamate release from cortical astrocytes under ischemic conditions. Molecular understanding of physiological or pathoplysiogical functions of these anion channels will progress after identification of their molecules.

S97 (2S-23B3)
Cardiovascular modules in the cerebellum

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The cerebellum is involved in the control of not only motor but also autonomic functions. I will summarize roles of the cerebellum in cardiovascular control. I propose that the cerebellum contains five distinct modules (cerebellar corticonuclear microcomplexes) dedicated to cardiovascular control. First, a discrete rostral portion of the fastigial nucleus and the overlying medial portion of the anterior vermis (Lobules I, II and III) conjointly form a module that controls the baroreflex. Second, anterior vermis also forms a microcomplex with the parabrachial nucleus. Third, a discrete caudal portion of the fastigial nucleus and the overlying medail portion of the posterior vermis (lobules VII and VIII) form another module controlling the vestibulosympathetic reflex. Forth, the medial portion of the uvula may form a module with the nucleus tractus solitarius and parabrachial nucleus. Fifth, the lateral edge of the nodulus and the uvula, together with the parabrachial nucleus and vestibular nuclei, forms a cardiovascular microcomplex that control the magnitude and /or timing of sympathetic nerve responses and stability of the mean arterial blood pressure during changes of head position and body posture. Another region of the flocculus, which has recently been found to be related to cardiovascular control, will be also discussed (Nisimaru and Ito, 2005).

S98 (2S-23B4)

Inherited cardiomyopathies as a troponin disease

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Troponin, one of the sarcomeric proteins, plays a central role in the Ca^{2+} regulation of contraction in vertebrate skeletal and cardiac muscles. More than two hundred of mutations in the cardiac sarcomeric proteins, including myosin heavy/light chains, actin, troponin, tropomyosin, myosin-binding protein-C, and titin/connectin, have been found to cause various types of cardiomyopathy in human since 1990, and over sixty mutations in cardiac troponin subunits have been identified in the hypertrophic (HCM), dilated (DCM) and restrictive (RCM) cardiomyopathies. To explore molecular mechanisms for the pathogenesis of these cardiomyopathies, recombinant mutants of human cardiac troponin subunits were exchanged into permeabilized rabbit cardiac muscle fibers and their effects on the Ca^{2+}-dependent force generation in cardiac muscle were examined. Most mutations in cardiac troponin subunits associated with HCM had Ca^{2+}-sensitizing effects. In contrast, DCM-linked mutations in cardiac troponin T decreased the Ca^{2+} concentrations required for force generation, strongly suggesting that changes in the Ca^{2+} sensitivity of force generation in cardiac muscle in opposite directions, i.e. Ca^{2+}-sensitization and desensitization, play important roles in the pathogenesis of these two distinct forms of cardiomyopathy. RCM-linked mutations in cardiac troponin I had much greater Ca^{2+}-sensitizing effects on force generation than HCM-linked mutations, suggesting that HCM and RCM-linked mutations in troponin subunits share a common feature of increased Ca^{2+} sensitivity of cardiac myofilament, but more severe change in Ca^{2+} sensitivity is associated with the clinical phenotype of RCM.

S99 (2S-25D1)

Synaptic connections and rhythmic activity of Renshaw cells in GAD67-EGFP knock-in mouse

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One of the first functionally identified groups of inhibitory neurons in the mammalian central nervous system are Renshaw cells (RCs, Renshaw 1946). RCs are excited by axon collaterals from motor neurons (MNs), and provide recurrent inhibition of MNs (Eccles et al. 1954). It has been shown by studies using cat spinal cord since then that, 1) excitatory synaptic inputs from MNs are mediated by acetylcholine (Curtis and Ryall 1966) and 2) these inputs are the main driving force for RC activity during locomotion (Noga et al 1987). We examined the physiological nature of RCs in detail using visually guided whole cell recording in isolated spinal cord preparations from glutamic acid decarboxylase (GAD) 67-EGFP knock-in mouse neonates. Among the GFP-positive cells in the lumbar ventral horn, RCs were uniquely identified by electrically stimulating the adjacent ventral root to evoke a short latency EPSC and by filling the cell with alexa-dyes to confirm its expression of calbindin-28k after recording. The short latency EPSCs that were reduced to 20-40% of control in amplitude by nicotinic receptor blockers and further reduced by blocking ionotropic glutamate receptors indicating that glutamate is also mediating synaptic inputs to RCs from MNs. During the locomotor-like rhythmic activity evoked by bath-application of 5-HT and NMDA, RCs fired rhythmically and modulated not only by excitatory synaptic inputs but also inhibitory ones. These results show that such technique is a powerful tool to reveal the neuronal mechanisms of motor control.

S100 (2S-25D2)

In vitro and in vivo analyses of the neuronal network involved in the regulation of sleep/wakefulness using transgenic mice

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Orexin A and B are a pair of neuropeptides implicated in the regulation of sleep/wakefulness and energy homeostasis. The regulatory mechanism of orexin neurons is poorly understood since the small number of orexin neurons is sparsely distributed in the lateral hypothalamus. We made the following transgenic (Tg) mice to study the physiological role in the regulation of sleep/wakefulness.Orexin/cameleon Tg mice, in which orexin neurons specifically express calcium sensing protein, were used for calcium imaging to screen what kind of neurotransmitter affects the activity of orexin neurons.Orexin/EGFP Tg mice, in which orexin neurons express EGFP, were used for electrophysiological studies to reveal intracellular mechanisms involved in the activation or inhibition response. Orexin/GFP::TTC Tg mice, in which orexin neurons express a retrograde tracer, were used for immunohistochemical studies to reveal which neurons directly innervate orexin neurons. These studies using Tg mice revealed how orexin neurons are regulated by afferent neurons.

SYMPOSIA

Transgenic approach to mammalian neurophysiology—Functional dissection with genetic and electrophysiological tools [YFI (Young Foreign Investigator) Workshop]

S101 (2S-25D3)

Sodium-sensing mechanism in the brain: from molecular to behavior

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Dehydration causes an increase in the sodium (Na) concentration and osmolarity of body fluid. For Na homeostasis of the body, controls of Na/water-intake and -excretion are of prime importance. However, the system for sensing the Na level within the brain that is responsible for the control of Na/water-intake behavior remains to be elucidated. Using physiological and behavioral techniques in combination with genetic manipulations, we have demonstrated that a sodium channel Na_x is indispensable for the Na-level-sensing in the brain. We previously showed that Na_x channel is preferentially expressed in glial cells in the circumventricular organs (CVOs) and that Na_x-knockout mice ingest saline in excess under dehydrated conditions. Subsequently, we demonstrated that Na_x is a Na-level-sensitive Na channel. Recently, we confirmed the physiological role of the Na_x *in vivo* by infusion of hypertonic Na solution to the cerebral ventricle. The infusion induced prompt intake of water and aversion to salt in wild-type mice. In contrast, such aversive behavior was not observed in the knockout mice. When Na_x cDNA was introduced into the brain of the knockout mice with an adenoviral expression vector, only animals with a transduction of the Na_x gene into the subfornical organ (SFO) among the CVOs recovered salt-avoiding behavior under dehydrated conditions. These results clearly indicate that the SFO is the center of the control of salt-intake behavior in the brain, where the Na-level-sensitive Na_x channel is involved in sensing the physiological increase in the Na level of body fluids.

SYMPOSIA
Physiological approaches to lombic and hypothalamic circuits for emotion, learning and behavior

S102 (2S-25D4)

Synaptic transmission in the basal ganglia in the process of neuronal repair with grafted neuroepithelial stem cells

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The fate of grafted neuroepithelial stem cells in the mature brain environment was assessed to confirm their feasibility in the functional repair of damaged neural circuitry. The neuroepithelial stem cells were harvested from the mesencephalic neural plate of enhanced-GFP-carrying rat embryos, and implanted into the striatum of normal adult rat or Parkinson's disease model rat. The differentiation pattern of donor-derived cells was monitored immunohistochemically. The functional abilities of the donor-derived cells and communication between them and the host were investigated using host-rat brain slices incorporating the graft with whole-cell patch-clamp recording. Vigorous differentiation of the neuroepithelial stem cells into mostly neurons was noted in the short-term with positive staining for tyrosine hydroxylase, suggesting that the donor-derived cells were following their genetically programmed fate. In the long-term, the large number of donor-derived neurons was sustained, but the staining pattern showed appearance of medium spiny or cholinergic neurons, suggesting that some neurons were following environmental cues. Some donor-derived astrocytes were also seen in the graft. Firing pattern and membrane properties suggest the presence of both dopaminergic and non-dopaminergic neurons in the donor-derived neurons. Glutamatergic and GABAergic post-synaptic currents could be evoked by electrical stimulation applied in the host region. Neuroepithelial stem cells are therefore an attractive candidate as a source of donor material for intracerebral grafting in functional repair.

S103 (2S-26E1)

Implications of abnormal temporolimbic and prefrontal morphology in development of schizophrenia

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In order to clarify the implications of morphological brain changes in development of schizophrenia, we have made extensive comparisons of brain morphology using MRI between established schizophrenia and schizotypal disorder, a schizophrenia-spectrum disorder without overt and sustained psychotic episode. Compared with controls, bilateral volumes of the amygdala, hippocampus and posterior superior temporal gyrus were reduced comparably in both schizotypal and schizophrenia patients. Total prefrontal grey matter was smaller bilaterally in schizophrenia patients than in controls, whereas schizotypal patients had larger right prefrontal grey matter than controls. In schizophrenia patients, the bilateral superior frontal, inferior frontal and straight gyri, and the left middle frontal gyrus were smaller than those in controls, while schizotypal patients had larger bilateral middle frontal gyri and smaller right straight gyrus. In white matter, decreased volume of the anterior limb of the internal capsule, a fiber bundle connecting the frontal cortex and thalamus, was found bilaterally in schizophrenia but only on the right in schizotypal disorder. These findings suggest that volume reductions in the medial and postero-lateral temporal regions are the common morphological substrates for the schizophrenia-spectrum which presumably represent the vulnerability. Additional widespread involvement of the prefrontal cortex might lead to the loss of inhibitory control in other brain regions and play a critical role in the manifestation of overt psychosis.

S104 (2S-26E2)

Social cognition related neural responses in the monkey amygdala

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The previous neuropsychological studies demonstrated that the human amygdala (AM) increased its response to emotional facial expressions and gaze direction toward to the subject, and suggested that the AM is essential in social cognition. In the present study, the monkey AM neuronal activity was recorded during performance of a delayed non-matching to sample task using human photos with various facial expressions and gaze direction. Some neurons were further tested with various human actions such as approaching toward the monkey. Autonomic activity (pupil radius) of the monkey, which reflected emotional expression, was simultaneously recorded. The results indicated that the AM neurons differentially responded to various emotional expressions and/or gaze directions. These facial expression-differential neurons were most sensitive to those of the familiar persons to the monkeys. These results suggest that social cognition might develop based on learning through social interaction, and the AM is involved in such learning. Activity of other AM neurons increased when the experimenter approached toward the monkey, or when the experimenter moved its arm or leg. Pupil radius also increased during this approaching. These results suggest that the AM is essential in primate social cognition as well as emotional expression.

S105 (2S-26E3)

Regulation of the hippocampal function by the supramammillary nucleus of the hypothalamus.

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It is a continuing question how emotions enhance memory formation. To answer the question, we study the connection between the hippocampus and the hypothalamus, which is involved in Papez circuit. We have hypothesized that the hippocampal activity is enhanced by direct inputs from the supramammillary nucleus (SuM) of the hypothalamus to the dentate gyrus (DG) and the CA2 region. Immunocytochemistry of Fos positive neurons (FN) demonstrated that the SuM-hippocampal pathway was activated when animals were exploring a novel environment. Number of FN in SuM and the hippocampus increased when rats were placed in an open field. SuM lesions significantly suppressed the increase of FN in the entire hippocampus. Small lesions in the lateral SuM significantly suppressed the increase of FN in the ipsilateral CA2 compared with the control side, although there was no difference in the number of FN between both sides of DG. These data suggest that CA2 neurons is specifically activated by ipsilateral inputs from SuM, while DG is activated by bilateral inputs from SuM. Since SuM is related to anxiety, anxiety enhances the neuronal activity of the dentate granule cell and CA2 neurons and results in the enhancement of memory formation.

S106 (2S-26E4)

The role of the primate amygdala in processing emotional facial expressions.

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The primate amygdala plays an important role in differentiating between facial expressions, yet the neural properties underlying this process are largely unknown. We recorded from the monkey amygdala neural responses to images of monkey faces, human faces, and objects. Most neurons differentiated between these image categories, yet monkey faces, human faces, and objects were equally likely to elicit stimulus-selective responses. In certain animals threatening faces appeared to elicit increased firing rates compared to neutral or appeasing faces suggesting a processing bias in favor of stimuli that signals potential danger. Neural responses to monkey faces were further examined to determine whether the observed changes in firing rate can be best accounted for by face identity or facial expression. The majority of neurons responded to unique combinations of identity and expression suggesting that in the amygdala identity and facial expressions are merged into a single representation. This representation might carry information about the emotional and social significance of facial expressions encountered during social interactions. The amygdala is also involved in orchestrating overt behavioral and autonomic responses to images with emotional value. We recorded skin conductance response, heart rate, and facial muscular activity in conjunction with neural responses in the amygdala and found correlations between stimulus-selective neural activity and peripheral autonomic responses.

SYMPOSIA
Current status of Japanese Guideline and Regulation on Animal Experimentation [Symposium Organized by Physiological Society of Japan: Symposium on animal ethics and animal supply (held in Japanese)]

S107 (2S-27F1)
Current status of Japanese Guideline and Regulation on Animal Experimentation

Tamaoki, Norikazu (*Central Institute for Experimental Animals Kawasaki, kanagawa, Japan*)

Scientific institution in Japan established an institutional animal care and use committee for reviewing animal experimentation protocols and advise the institutional director to improve the welfare of laboratory animals according to the administrative guidance "Notification Concerning Animal Experimentation Conducted by Universities etc."(1987). In 2004, Science Council of Japan proposed a new regulation rule of animal experimentation to promote the public understanding of ethical and scientific animal experimentation. Major points of revision are as follows:1. Establishment of a guideline for animal experimentation commonly applicable to the all scientific institutions in Japan.2. Establishment of an objective evaluation system on the institutional self regulation for animal experimentation. Establishment of new regulation system is now on going. On the other hand, Amended Law for the Humane Treatment and Management of Animals (2005) stipulated the 3Rs principle in animal experimentation.For the development of health research for humans as well as animals, balance between science and animal welfare is indispensable.(Former chair of Committee on Laboratory Animal Science, Science Council of Japan)

S108 (2S-27F2)
The Notification System for the Importation of Animals

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The Ministry of Health, Labour, and Welfare introduced the Notification System for the Importation of Animals, which is effective from 1st September 2005, to prevent the outbreaks of human infectious diseases derived from imported animals. Any importers of animals, including experimental animals or transgenic animals, are required to submit a written declaration giving the specified information on the animals, such as their species name and quantity, to the quarantine station of the Ministry of Health, Labour, and Welfare. This declaration has to be accompanied by a health certificate issued by the government authorities of the exporting country certifying that the animals are free from the infectious diseases specified for each species. Procedure to issue the health certificate by the government authorities varies among the exporting countries. For example, in the United States the animal facility that stores the exporting animals publishes the heath certificate corresponding to the regulation proposed by the Ministry of Health, Labour, and Welfare, and sends the certificate to the USDA office located in each state, where the official veterinarians endorse the certificate. In addition, the Ministry of Health, Labour, and Welfare is asking to register the health certificate form for the government authorities in other countries that have not yet responded to the notifying system for importation of animals. Researchers who are planning to import the experimental animals need to understand the current situation of the importation and correspond to this new system to ensure the smooth importation of animals into Japan.

SYMPOSIA
Gene manipulation for research of cardiovascular system

S109 (2S-28G1)

Lentiviral vector-mediated SERCA2 gene transfer improves the heart failure and left ventricular remodeling induced by myocardial infarction in rat

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Introduction Reduced gene expression of SERCA2 impairs calcium handling and represents a hallmark of heart failure. Unlike adenovirus- or adenoassociated vectors, lentivirus can stably integrate into host genome of terminally differentiated cardiac myoctes and induces permanent expression. We developed lentivirus-based SERCA2 gene transfer system and examined its feasibility as a therapy for heart failure. **Results** The therapeutic effect of Lenti-SERCA2 vector ($1x10^{11}$ IU/300g BW) was compared with the Lenti-β-Gal control vector in the failing heart induced by myocardial infarction (MI) in rats. Echocardiography revealed that Lenti-SERCA2 introduction prevented an increase in left ventricular diameter and a decrease of fractional shortening by 10-15% compared with Lenti-β-Gal group rats from days 30 to 180. Pressure-volume analysis demonstrated that Lenti-SERCA2 introduction improved systolic (dP/dt max, 7677 vs 3028 mmHg/sec; Emax, 0.68 vs 0.37) and diastolic function (tau, 18.4 vs 22.6). Northern and Western blot analyses revealed that SERCA2 mRNA and protein were elevated and the BNP mRNA was significantly decreased in Lenti-SERCA2 group. Finally, SERCA2 gene transfer prevented the expansion of MI region and decreased the mortality rate. **Conclusion** Our study showed that the SERCA2 gene was successfully integrated into hearts and supports the premise that a lentivirus-based SERCA2 gene therapy improves heart failure.

S110 (2S-28G2)

The involvement of Fyn tyrosine kinase and membrane rafts in the signal transduction of abnormal vascular smooth muscle contraction

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Rho-kinase (ROK)-mediated Ca^{2+} sensitization plays a pivotal role in abnormal vascular smooth muscle (VSM) contraction such as vasospasm. Previously we identified sphingosylphosphorylcholine (SPC) and Src family tyrosine kinase (Src-TK) as an upstream signaling molecule of ROK-mediated Ca^{2+} sensitization. Since VSM contains both Fyn and c-Src among Src-TK, we analyzed which Src-TK was truly important for the Ca^{2+} sensitization mediated by SPC/ROK pathway. Immunofluorescent study showed that SPC induced the translocation of Fyn, but not c-Src, to plasma membrane in cultured VSM cells and eicosapentaenoic acid, a specific inhibitor of SPC-induced VSM contraction, blocked the translocation of Fyn. The siRNA which specifically knockdown Fyn diminished SPC-induced contraction remarkably in cultured VSM cells. In β-escin permeabilized VSM strips, constitutively-active Fyn, which was expressed by baculovirus system, induced Ca^{2+} sensitization and dominant-negative Fyn blocked Ca^{2+} sensitization. In confocal study, SPC induced the translocation of Fyn to plasma membrane where it colocalized with caveolin-1, a membrane-raft-associated protein. A functional proteomics approach identified p160 and its phosphorylation site as a possible target of Fyn. Those findings suggested that membrane rafts and its associated Fyn played an essential role in ROK-mediated Ca^{2+}-sensitization of VSM contraction.

S111 (2S-28G3)

Identification of phosphoinositide 3-kinase C2α as an essential signaling molecule in Ca^{2+}-dependent Rho activation and myosin phosphatase inhibition by using RNA interference-mediated gene silencing

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Excitatory receptor agonists such as noradrenaline stimulate the activity of the small G protein Rho and inhibit myosin phosphatase (MP) through mechanisms involving Rho kinase-dependent phosphorylation of the MP regulatory subunit MYPT1 in VSM. We have recently demonstrated that a novel, Ca^{2+}-dependent mechanism for Rho activation and myosin phosphatase (MP) inhibition is operating in receptor agonist- and membrane depolarization-induced vascular smooth muscle (VSM) contraction. We found that phosphoinositide 3-kinase (PI3K) was required for Ca^{2+}-dependent Rho activation; the PI3K inhibitors wortmannin and LY294002 inhibited all of the Ca^{2+}-dependent Rho activation, MYPT1 phosphorylation, MP inhibition, MLC phosphorylation and contraction. We tried to identify a PI3K isoform by adopting RNA interference and cultured VSM cells. The selective down-regulation of the expression of class II alfa isoform (PI3K-C2α), but not the class I p110α, by a specific siRNA markedly inhibited Rho kinase-dependent MYPT1 phosphorylation, MLC phosphorylation and contraction in differentiated VSM cultures. Noradrenaline as well as membrane depolarization stimulated the activity of PI3K-C2α, but not p110α, in a Ca^{2+}-dependent manner. Thus, these observations unveiled a novel role of the PI3K-C2α as an upstream regulator of Rho and consequently MP and contraction.

S112 (2S-28G4)

A innovative anti-inflammatory therapeutic strategy for regeneration of atherosclerosis with biodegradable gene eluting stent

egashira, kensuke (*Cardiovasc Med. Kyushu University*)

Recent evidence suggests that stent-associated inflammation is a prominent feature in animals and humans, and thus can be a promising next-generation target for prevention of restenosis. We have shown great benefit of anti-monocyte chemoattractant protein-1 (MCP-1) therapy by systemic transfer of an N-terminus deletion mutants of human MCP-1 (called 7ND) gene for prevention of restenotic changes in animals. Therefore, to translate our achievement on MCP-1 pathobiology to clinic, we tested the hypothesis that stent-based local delivery of 7ND gene reduces in-stent neointimal formation. Bare, polymer-coated, and 7ND plasmid-coated stents were implanted in iliac arteries of hypercholesterolemic rabbits (n=8-10 each) and cynomolgus monkeys (n=7-10 each). 7ND gene-eluting stents attenuated stent-associated monocyte infiltration/activation and neointimal formation (about 30% reduction) in rabbits. In monkeys, significant reduction of neointimal formation was noted 1, 3, and 6 month after stenting, indicating long-term benefits of 7ND gene ES in monkeys. No evidence of incomplete healing process was noted in 7ND-eluting stent sites. In conclusion, anti-MCP-1 strategy with 7ND gene-eluting stents was strikingly effective in reducing experimental restenosis in rabbits and monkeys. Our finding in nonhuman primates has significant clinical significance, implying that this anti-inflammation strategy targeting MCP-1 might be a promising therapy against human restenosis.

S113 (2S-28G5)

Novel Signaling Mechanism of Angiotensin II Type 2 Receptor in the Cardiovascular System.

Senbonmatsu, Takaaki (*Saitama Medical School, Saitma, Japan*)

The role of Angiotensin II (Ang II) in the regulation of the cardiovascular system under normal and pathologic conditions have been well documented. A variety of angiotensin converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs) are selected as first choice medicine for hypertension or heart failure. Although two major subtypes Ang II receptors, exist type1 (AT_1) and type2 (AT_2), most studies and treatments have focused on AT_1 coupled events. Previous reports indicated that AT_2 plays a role in essentially growth suppression such as through the tyrosine phoaphatase SHP-1 and MKP-1 activation. However, a detailed signaling mechanisms of these responses still remain unclear. Interestingly, an increasing number of recent reports indicate that AT_2 plays a role in growth promoting similar to the AT_1 function. We reported that AT_2 gene-deleted mice lose the ability to develop cardiac hypertrophy in response to pressure overload or to chronic Ang II stimulation, and also found a novel signaling mechanism of AT_2 mediated by the transcription factor promyelocytic leukemia zinc finger (PLZF) leading to cardiac hypertrophy. PLZF is selectively expressed in the heart, but not in the kidney or aorta. Upon Ang II stimulation, AT_2 and PLZF are internalized, and PLZF translocates into the nucleus, whereby nuclear PLZF activates phosphoinositide 3-kinase (PI3K) regulatory subunit 85α leading to cardiac hypertrophy. However, in the absence of PLZF, Ang II evoked SHP-1 activation leading to growth suppression via AT_2. These results suggest that AT_2 may have dual switching functions mediated by PLZF.

S114 (2S-28G6)

Primary role of inositol 1,4,5-trisphosphate receptor type 1 in agonist-induced aortic contraction in mice

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Contraction of vascular smooth muscles is under the regulation of sympathetic activity and vasoactive hormones. It is known that release of Ca^{2+} from inositol 1,4,5-trisphosphate (IP_3)-sensitive stores causes the initial phase of agonist-induced vasocontraction. In the present study, phenylephrine (PE)-induced contraction was measured in thoracic aortas isolated from the wild-type (WT) and IP_3 receptor type 1 knockout (IP3R1-KO) mice, in order to specify the IP_3 receptor subtype responsible for the agonist-induced contraction. PE (10^{-8} - 10^{-6} M)-induced aortic contraction in the IP3R1-KO mice was greatly diminished, compared to that in WT mice, and lacked the steep contraction which was invariably seen in WT aortas immediately after PE application at 10^{-6} M. But, high K^+-induced contraction was indistinguishable between WT and IP3R1-KO aortas. Immunoblotting analysis demonstrated the presence of three IP_3 receptor subtypes (IP3R1, IP3R2 and IP3R3) in WT mouse thoracic aorta; however, abundance of each subtype was in the order of IP3R1 > IP3R3 >> IP3R2. These results indicate that IP3R1 constitutes the Ca^{2+} release channels critical to vasocontraction regulated by sympathetic activity and vasoactive hormones.

SYMPOSIA
Motor control mechanism by the cerebellum

S115 (2S-29H1)
Identification of loci involved in the memory of chronic motor learning of the vertical vestibuloocular reflex in squirrel monkeys

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The vestibuloocular reflex (VOR) stabilizes vision during head turn by counter-rotating the eyes in the orbit. Its gain (eye velocity/head velocity) can be modified by visual-vestibular mismatch, but following cerebellar inactivation, the gain cannot be further modified. Thus, the VOR has been a model system to study potential cerebellar roles in motor learning. The cerebellum may have different roles in acute versus chronic VOR motor learning, because cerebellar inactivation entirely eliminates any acutely learned component, but it only partially eliminates the memory of long-term gain change, suggesting multiple loci for the chronic memory. To pinpoint these, a series of experiments in which activities of cerebellar Purkinje cells (PCs) and their target neurons in dorsal Y group (YNs) were recorded before and after chronic VOR motor learning. The sensitivities of PCs to both vestibular (V) and efference copy (E) signals changed with learning, YNs changed their sensitivities to V modalities, and these changes are asymmetric for gain increase and decrease. Computational modeling revealed significant changes in 1) V pathway to cerebellar flocculus (FL), 2) direct V pathway to YNs after gain increase, and in 3) E pathway to FL, 4) direct V pathway to YNs, 5) pathway from PCs to YNs, and 6) V pathway excluding those through FL and YNs after gain decrease. The results suggest involvement of several loci in chronic learning and different neuronal mechanisms for gain increase and decrease.

S116 (2S-29H2)
Role of the cerebellum in the acquisition and consolidation of motor memory revealed by long-term adaptation of ocular reflex paradigm

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Adaptation of ocular reflexes is a prototype of cerebellum-dependent motor learning. Two different views are proposed for its neural mechanisms: one that the memory of adaptation is formed within the cerebellar flocculus through the cerebellar long-term depression (LTD) of parallel fiber-Purkinje cell synapses, and the other that the memory is formed within the vestibular nuclear neurons using signals mediating through the flocculus. We developed a long-term adaptation paradigm adaptation of mouse eye movements. We revealed that the memory trace of motor learning induced by short-term (day-long) training is located within the cerebellar cortex, while that induced by long-term (week-long) training in the cerebellar or vestibular nuclei, by evaluating the effects of reversible pharmacological shutdown of the cerebellar cortex. These results suggest that the memory trace of motor learning is initially formed in the cerebellar cortex, and later shifts transsynaptically to cerebellar/vestibular nuclei for consolidation. We further revealed that LTD plays a critical role in both the acquisition and consolidation of memory by pharmacological and gene-knockout mouse experiments.

S117 (2S-29H3)
Feed-forward associative motor learning by the cerebellum

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There are some unresolved problems in motor learning theory. One is determining the source of a learning signal, sometimes called a motor error signal. Another one is the credit assignment problem of the motor error, since the erroneous performance perceived by a subject is due to the actions of many elementary motor units. The feed-forward associative learning theory attributes the source to the movement system itself. When a subject performs a corrective movement after his primary movement, the proposed neural learning device learns to associate the primary motor command with the corrective motor command by using a place-coding system. In the subsequent trials, the primary movement will involve a correction due to the participation of this mechanism, thus resulting in better performance. The device consists of many adaptive units each of which is specialized for a particular elementary motor unit, and naturally resolves the assignment problem. The theory assumes three conditions, namely, that a motor center and the learning device share the same place-encoded motor information; the motor center issues a command and a learning signal simultaneously from the same unit; and a learning signal issued with a corrective command has a heterosynaptic interaction with the previous primary command. The cerebellum is a reasonable candidate for the device satisfying these conditions. The reaction time of a corrective movement, usually 100-300 ms, almost satisfies the coincidence condition for long-term depression of the granule-to-Purkinje synapses. As an application, this theory is demonstrated to account for behavioral results regarding saccadic adaptation.

S118 (2S-29H4)

Mechanism of cerebellar function studied using mutant mice

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The cerebellum plays critical roles in motor control and learning. Relative simplicity of the cerebellar cortical circuit has prompted the study on how it works, which has made the cerebellum one of the best characterized structure of central nervous system. However, respective role of each component or its function such as a particular type of synapse, neuron or synaptic regulation has been elusive. We have been addressing these issues using several types of mutant mice, and here I present our recent data on the GluRδ2 (glutamate receptor δ2 subunit) knockout mice (δ2-/-). GluRδ2 is a molecule related to ionotropic glutamate receptor, which is specifically expressed at parallel fiber (PF) - Purkinje neuron (PN) synapses. The δ2-/- show impairment in the long-term depression, synaptic stabilization of PF-PN synapses and elimination of surplus climbing fiber (CF) inputs resulting in the multiple innervation to a PN, and also show motor discoordination and motor learning failure. We studied the eye movements and found that δ2-/- show involuntary spontaneous eye movements and large phase delay in the optokinetic response (OKR). We have been analyzing the mechanism of these abnormal motor regulations by simultaneous recording of eye movements and PN activity. Our results suggest that the enhanced CF activity in δ2-/- largely disturbed the normal pattern of PN activity regulating eye movement, that highlights the importance of synaptic inputs balance on a PN in motor regulation.

SYMPOSIA
Functional coupling between neuron and glia

S119 (3S-30B1)

Introduction: Importance of glia-neuron functional coupling in higher order brain functoin

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The concept for glial cells as non-excitable- supporting elements has been accepted until almost the end of 20th century without doubt. However, total number of glial cells in human brain was found to be far larger than that of neuronal cells, and the ratio of glial cells to neurons in the brain was found to be higher in the highly developed animals than that in primitive animals. Those evidences suggested that the cells may be required for establishing higher order brain function. The dynamic feature of the cells has been revealed by the Ca^{2+} imaging techniques. Since then astrocytes have been recognized as dynamic cells and are regarded as intimate collaborators with neuronal cells. The concept of"tri-partite synapse" has been put forward to explain the possible participation of astrocytes into the synaptic transmission and information processing in the brain. However, the interaction among neuronal cells and astrocytes may not be such small scale. The transmission of the Ca^{2+} waves from an astrocyte to the other has been found to be performed through gap-junctions and also specific transmitters and receptors system. Thus astrocytes themselves form a wide network among them, which may be woven into the neuronal networks and construct large and highly organized information processing system. Neuronal networks as main system in the brain information processing may be controlled slowly and widely by astrocytes networks. This "glia-neuron functional coupling" will establish a higher order brain function and its deficit will cause brain dysfunctions.

S120 (3S-30B2)

Identification of peri-interneuronal glial cells and its modulatory effects on neurons in the hippocampus of rat

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Recent studies have demonstrated the existence of direct interactions between neurons and glial cells. To evaluate these interactions, we focused on interneuron/peri-interneuronal glial cell pairs in the hippocampal CA1 region, because of the close proximity of these two cells. Based on the electrophysiological, morphological and immunohistochemical studies, the peri-interneuronal glial cells were classified into astrocytes and oligodendrocytes, and we worked with the peri-interneuronal astrocytes (PNAC) in this study. Excitatory postsynaptic currents (EPSCs) recorded in an adjacent interneuron were suppressed by the depolarizing current injection into the PNAC. These suppression of EPSCs accompanied the increase of paired-pulse ratio and were blocked by the application of adenosine A_1 receptor antagonist, indicating the involvement of presynaptic adenosine A_1 receptors. Moreover, PNAC depolarization modified the directly induced firing of the interneuron. These results demonstrate directly modulatory effects of the PNAC on neuronal activities.

S121 (3S-30B3)

Regulation of excitatory synaptic transmission by glial glutamate transporters

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Glial glutamate transporters, GLAST and GLT-1, are co-localized in processes of Bergmann glia (BG) wrapping excitatory synapses on Purkinje cells (PCs). Although GLAST is expressed 6-fold more abundantly than GLT-1, no change is detected in the kinetics of climbing fiber (CF)-mediated excitatory postsynaptic currents (CF-EPSCs) in PCs in GLAST(-/-) mice compared to the wild-type mice (WT). The prolongation of the decay kinetics of CF-EPSCs in GLAST(-/-) mice is found only in the presence of cyclothiazide (CTZ), which attenuates the desensitization of AMPA receptors. We attempted to clarify the mechanism(s) underlying this unexpected finding using a selective GLT-1 blocker, dihydrokainate (DHK), and a novel antagonist of glial glutamate transporters, (2S,3S)-3-[3-(4-methoxybenzoylamino)benzyloxy]aspartate (PMB-TBOA). In the presence of CTZ, DHK prolonged the decay time constant (τ_w) of CF-EPSCs in WT, indicating that GLT-1 plays a partial role in the removal of glutamate. The application of 100 nM PMB-TBOA, which inhibited CF-mediated transporter currents in BG by ~80%, caused no change in τ_w in WT in the absence of CTZ, whereas it prolonged τ_w in the presence of CTZ. This prolonged value of τ_w was similar to that in GLAST(-/-) mice in the presence of CTZ. These results indicate that glial glutamate transporters can apparently retain the fast decay kinetics of CF-EPSCs if a small proportion (~20%) of functional transporters is preserved, and that GLT-1 alone in GLAST(-/-) mice is sufficient to keep the fast kinetics of EPSCs in the absence of CTZ.

S122 (3S-30B4)

The role of neural-glial communication in dynamic remodeling of the extracellular space

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Neural-glial communication has been assumed to be mediated by spillover of transmitter from the synaptic cleft. In the cerebellum, Bergmann glia cell (BG) processes encase synapses between presynaptic climbing fiber (CF) and parallel fiber elements and postsynaptic Purkinje cell (PC) spines and glutamate released from these fibers can activate Ca^{2+}-permeable AMPA receptors on BGs. Quantal responses recorded from BGs were not coincident with quantal responses recorded in adjacent PCs sharing the same CF input. By combining electrophysiological recordings and quantitative immunogold electron microscopic analysis, high-concentration (1.5 mM) rapid-transients (0.5 ms) of glutamate were estimated to underlie BG quantal events. We propose that exocytosis can occur from ectopic release sites located directly across from BG membranes. Ectopic release may be necessary to activate low affinity AMPA receptors on BGs, which may provide a geographical cue to guide BG membrane to surround active synapses and ensure efficient glutamate uptake. We have recently started to employ two-photon microscopy to study the result of such neural-glial communication. Morphological refinement of BG processes occurs within a few days in early development and rapid motility and spontaneous remodeling of extracellular space by BG protrusions were observed in minutes. Synaptic activation leads to Ca^{2+} influx at the tip of the protrusions via Ca^{2+}-permeable AMPA receptors. We are currently probing the mechanisms that manipulate the motility and refinement of BG processes and their effect on synaptic transmission.

S123 (3S-30B5)

Alexander disease model mice

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Alexander disease is caused by heterozygous mutation in glial fibrillary acidic protein (GFAP). The pathological hallmark is the presence of astrocytic GFAP aggregation called Rosenthal fibers (RF). To understand the pathophysiology of Alexander disease and utilize those results to the understanding of normal function of astrocytes in vivo, we have established transgenic mice that express hunan GFAP R239H mutant under the control of mouse GFAP promoter. Immunohistochemistry using human GFAP specific antibody, SMI21, showed that this immunoreactivity was present only in S100 beta positive astrocytes and ependymal cells. Some astrocytes possessed the aggregations with SMI immunoreactivity that was co-labeled by small heat shock protein, alpha B crystalline and HSP25. Contrary to the case of human, transgenic mice showed no abnormality of myelin formation and structure. To examine whether the presence of human GFAP aggregation altered brain functions, we challenged kainic acid administration to transgenic and wild type mice and studied the susceptibility of convulsion and the vulnerability of hippocampal cell damage. Transgenic mice were more susceptible to systemic administration of kainic acid (20 mg/kg) than wild type mice as revealed by the number of mice showing tonic-clonic convultion: 8 out of 11 for transgenic mice and 2 out of 14 for wild type mice. Transgenic mice were more vulnerable to excitotoxicity of kainic acid as revealed by the appearance of Fluoro Jade positive dying hippocampal neurons after 24 hours administration. We concluded that Alexander disease model mice compromised the protective function against kainic acid excitotoxicity.

SYMPOSIA
Current topics of stem cell biology—focusing on neural stem cells

S124 (3S-31C1)
Control of proliferation and differentiation of neural progenitor cells in the cerebellum

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During CNS development, neural stem cells (NSCs) give rise first to various kinds of specified precursor cells, which proliferate extensively before terminally differentiating into either neurons or glial cells. It is still not clear, however, whether the precursor cells are irreversibly determined to differentiate into their particular cell types. Neither is it clear how proliferation of the precursor cells are terminated, although it is widely accepted that control of the proliferation plays a crucial role in determining the number of neurons or glial cells. We have been addressing these issues using the developing mouse cerebellum as a model system. We found that cerebellar granule cell precursors (GCPs) can differentiate into astroglial cells when exposed to Shh and BMP. This indicates that GCPs are not irreversibly committed to neuronal development, but can be induced to differentiate into astroglial cells by appropriate extracellular signals. We also examined the role of cyclin-dependent kinase inhibitors in the control of proliferation of GCPs. Among the inhibitors we examined, only p27 was expressed at significant levels in cells of the granule cell lineage in the developing cerebellum. We found that there was an inverse correlation between BrdU uptake and p27 expression by GCPs. Even in the presence of saturating amounts of Shh, a potent mitogen, the cells eventually stopped dividing and differentiated, expressing p27 strongly. These results suggest that there is an intracellular mechanism that stops GCP division and causes GCPs to differentiate and that p27 is part of this mechanism.

S125 (3S-31C2)
Generation of Cerebellar Neuron Precursors from Embryonic Stem Cells

Muguruma, Keiko; Su, Hong-Lin; Matsuo-Takasaki, Mami; Watanabe, Kiichi; Sasai, Yoshiki (*Neurogenesis and Organogenesis, CDB, RIKEN*)

We report in vitro generation of Math1+ cerebellar granule cell precursors and Purkinje cells from ES cells by using soluble patterning signals. When neural progenitors induced from ES cells in a serum-free suspension culture are subsequently treated with BMP4 and Wnt3a, a significant proportion of these neural cells become Math1+. The induced Math1+ cells are mitotically active and express markers characteristic of granule cell precursors (Pax6, Zic1 and Zipro1). After purification by FACS and co-culture with postnatal cerebellar neurons, ES cell-derived Math1+ cells exhibit typical features of neurons of the external granule cell layer, including extensive motility and a T-shaped morphology. Interestingly, differentiation of L7+/Calbindin-D28K+ neurons (characteristic of Purkinje cells) is induced under similar culture conditions but exhibits a higher degree of enhancement by Fgf8 rather than by Wnt3a. This is the first report of in vitro recapitulation of early differentiation of cerebellar neurons by using the ES cell system.

S126 (3S-31C3)
Regulation of plasticity of neural cells by methy-CpG binding proteins

Kohyama, Jun; Namihira, Masakazu; Nakashima, Kinichi (*NAIST,Nara, Japan*)

It has become apparent that epigenetic modification plays a critical role in the regulation of lineage-specific gene expression. We have previously reported that the change in DNA methylation at the promoter of astrocytic genes, such as glial fibrillary acidic protein (GFAP), controls the switch from neurogenesis to astrocytogenesis in the developing telencephalon. The methylated promoter at midgestation undergoes demethylation as gestation proceed, corresponding to the onset of astrocytogenesis. However, the exon1 of the gene remains hypermethylated even in the adult neural progenitors and in cells differentiated from the progenitors, i.e. neurons, astrocytes and oligodendrocytes. The methyl-CpG binding proteins (MBDs) bind to methylated DNA and suppress the target gene expression. They are strongly expressed only in neurons in the nervous system and the cells do not respond to astrocyte-inducing signals to express GFAP. In contrast, by using Cre-recombinase fate tracing, we show here that oligodendrocytes, in which MBDs are not expressed, expressed GFAP upon stimulation with the astrocyte-inducing cytokines. Overexpression of MeCP2, one of the MBD family proteins, in oligodendrocytes inhibited the GFAP expression by the cytokines, implicating MBDs as key molecules to restrict the transdifferentiation of neural cells. It is well known that astrocytes increase dramtically in number after insult to the nervous systems. Taking the above results into consideration, oligodendrocytes could be a source of the newly generated astrocytes in damaged nervous systems in vivo.

S127 (3S-31C4)

Molecular mechanism of the reversion of oligodendrocyte precursor cells.

Kondo, Toru[1,2] ([1]*RIKEN CDB, Kobe, Japan;* [2]*Univ. Cambridge, Brain Repair Centre, Cambridge, United Kingdom*)

There is increasing evidence that some kinds of glial cells in central nervous system (CNS) can behave as multipotent neural stem cells (NSCs) and generate neurons, astrocytes, and oligodendrocytes in vivo and in vitro. However it is still unknown how such glial cells acquire multipotentiality. Oligodendrocyte precursor cells, which exist in many area in CNS, can also behave as multipotent NSC when the cells are exposed to specific conditions. Recently we have shown that sox2, which is an essential transcription factor in NSCs, is reactivated in the OPC reversion. We have also shown that in the reversion SWI/SNF chromatin remodeling complex is recruited to an enhancer in the sox2 promoter and lysine 4 and 9 of histone H3 in the enhancer are methylated and acethylated, respectively. We propose that the reversion of OPCs to NSCs depends on extensive chromatin remodeling, which is in part mediated by SWI/SNF.

S128 (3S-31C5)

Fate regulation of mouse telencephalic neural precursor cells

Gotoh, Yukiko (*Inst. Mol. Cell. Biosci. Univ. of Tokyo, Tokyo, Japan*)

Cortical neural precursor cells (NPCs) sequentially undergo expansion, neurogenic and gliogenic phases during development, although the underlying mechanisms are poorly understood. We have recently shown that Wnt signaling instructively induces neuronal differentiation of NPCs. Importantly, Wnt signaling does so only in midgestation stage (neurogenic phase) of NPCs but not in early embryonic stage (expansion phase) or in perinatal stage (gliogenic phase) of NPCs. In early embryonic stage, Wnt signaling rather promotes proliferation of NPCs. Here I will discuss possible mechanisms that might account for these stage-dependent responses. Likewise, STAT3-activating ligands induce astrocytic differentiation in late (gliogenic phase) but not in early (expansion and neurogenic phases) NPCs. These stage-dependent responses of NPCs might play a central role in determining the timing of differentiation and the size of final population of each differentiated cell type.

S129 (3S-31C6)

Roles of the bHLH genes Hes1/Hes3/Hes5 in neural development

Kageyama, Ryoichiro (*Inst. Virus Res., Kyoto Univ., Kyoto, Japan*)

Neuroepithelial cells are first generated from the ectoderm, forming the neural plate. These cells undergo symmetric cell divisions to produce more neuroepithelial cells. After neural tube formation, they become radial glial cells, which undergo asymmetric cell divisions, forming one radial glial cell and one neuron (or a neuronal precursor) from each cell division. After production of neurons, radial glial cells finally give rise to glial cells such as astrocytes. Thus, neural stem cells change their characteristics of morphology and competency over time during development. We found that inactivation of the bHLH genes *Hes1* and *Hes5*, known Notch effectors, and additional inactivation of *Hes3* extensively accelerate cell differentiation and cause a wide range of defects in brain formation. In *Hes*-deficient embryos, initially formed neuroepithelial cells are not properly maintained, and radial glial cells are prematurely differentiated into neurons and depleted without generation of late-born cells. Furthermore, loss of radial glia disrupts the inner and outer barriers of the neural tube, disorganizing the histogenesis. We also found that the boundary structures such as the isthmus and the zona limitans intrathalamica are not maintained and that the boundary cells are differentiated into neurons and lose the organizer activity. Thus, *Hes* genes are essential for generation of brain structures of appropriate size, shape and cell arrangement by controlling the timing of neural stem cell differentiation and by maintaining the boundaries with the organizer activity.

SYMPOSIA
Vascular endothelial cells and blood rheology

S130 (3S-32E1)
Purinoceptor-mediated blood flow sensing mechanism in endothelial cells

Ando, Joji; Yamamoto, Kimiko (*Grad. Sch. Med. Univ. Tokyo, Japan*)

Vascular endothelial cells (ECs) alter their morphology, function, and gene expression in response to shear stress generated by blood flow. However, the molecular mechanism of shear stress sensing by ECs has not been clarified. We investigated the mechanism from the aspect of calcium (Ca) signaling. Human pulmonary artery ECs (HPAECs) loaded with the Ca indicator Indo-1/AM were exposed to laminar flow and changes in intracellular Ca concentrations were monitored. A stepwise increase in flow rate elicited a corresponding stepwise-increase in Ca concentrations. Apyrase or EGTA completely abolished the flow-induced increase in Ca concentrations, indicating that ATP and influx of extracellular Ca are essential for the Ca responses. Flow increased the release of ATP from HPAECs in a shear stress-dependent manner. HPAECs predominantly express a subtype of ATP-operated cation channel P2X4, and antisense oligonucleotides targeted to P2X4 abolished the flow-induced Ca influx. Pulmonary microvascular ECs cultured from P2X4-deficient mice showed no flow-induced Ca influx and nitric oxide production. Human embryonic kidney 293 cells became sensitive to flow and show flow-induced Ca influx when transfected with P2X4 cDNA. Flow-induced dilation of skeletal muscle arterioles was markedly suppressed in P2X4-deficient mice. P2X4-deficient mice had higher systolic blood pressure values than wild-type mice. Thus, ECs transduce the signal of shear stress into Ca influx via P2X4, and that the purinoceptor-mediated blood flow-sensing plays important roles in endothelial NO production and vascular tone control.

S131 (3S-32E2)
Stretch-induced Ca increase in endothelial cells

Naruse, Keiji (*Okayama Univ. Grad. Sch. of Med., Dent. and Pharmaceut. Sci., Okayama, Japan*)

Human umbilical endothelial cells (HUVECs) show various responses including morphological changes and protein expressions in response to mechanical stretch. Our previous studies revealed that intracellular Ca increase in response to mechanical stretch via Ca permeable stretch-activated (SA) channel activation is critical in HUVECs cultured on an elastic PDMS (polydimethylsiloxane) membrane. Since recent reports suggest that the transient receptor potential (TRP) channels may form the SA channel, we investigated the involvement of TRPV2 in the stretch-induced Ca increase in HUVECs. Human TRPV2 was isolate from a HUVEC cDNA library. Heterologous expression of the human TRPV2 in COS7 cells resulted in the stretch-induced Ca increase and injection of a TRPV2-specific siRNA in HUVECs abolished the stretch-induced Ca increase.These observations indicate that TRPV2 plays a critical role in the stretch-induced Ca increase in HUVECs.

S132 (3S-32E3)
Maintenace of the Blood fluidity (anticoagulattion)

KOJIMA, TETSUHITO (*Nagoya University School of Health Sciences, Nagoya, Japan*)

Hemostasis is a physiologic mechanism that maintains blood in a fluid state within the circulation. The blood-coagulation cascade has the ability to transduce a small initiating stimulus into a large fibrin clot, which is mediated by cellular components and soluble plasma proteins. The potentially explosive nature of this cascade is counterbalanced by natural anticoagulant mechanisms. The maintenance of adequate blood flow and the regulation of cell-surface activity control the local accumulation of activated blood-clotting enzymes and complexes. Antithrombin is a plasma protein that inhibits the blood serine proteases of the intrinsic and common coagulation pathways. Heparin-like molecules, heparan sulfate proteoglycans, are closely associated with endothelial cells and enhance the action of circulating antithrombin. In this session, the endothelial heparan sulfate and antithrombin system, which plays an important role in the natural hemostatic balance to maintain the blood fluidity, will be discussed through the data from the congenital deficient mouse-models, i.e. KO mice.

S133 (3S-32E4)

Regulatory mechanism of fibrinolysis in the vasculature by vascular endothelial cells.

Urano, Tetsumei; Suzuki, Yuko; Ihara, Hayato; Mogami, Hideo (*Hamamatsu Univ. Sch. of Med., Hamamatsu*)

Tissue plasminogen activator (t-PA), the primary PA in vasculature, is synthesized and secreted from vascular endothelial cells (VECs). Besides other serine proteases involved in both coagulation and fibrinolysis, t-PA has unique characteristics of possessing physiological activity as a single chain form and being secreted as an active form. In blood there also exist plasminogen activator inhibitor type 1 (PAI-1), a member of serine protease inhibitor superfamily (SERPINS), which inhibits the activity of both single chain- and two chain- forms of t-PA by forming an equimolar high molecular weight complex. We have reported that total fibrinolytic activity in plasma is regulated by the balance between these two molecules, showing that increase in PAI-1 level under either physiological or pathological conditions suppresses fibrinolytic activity, whereas the enhanced t-PA secretion accelerates fibrinolysis. Recently, we have studied the dynamics of t-PA secretion from its containing granules in VECs using total internal reflection fluorescence microscopy (TIRFM). We obtained results suggesting that secreted t-PA by regulatory exocytosis stays on the membrane of VECs for certain period of time, and expresses its specific activity on VECs. PAI-1 appeared to modify the dynamics of t-PA secretion, and thus fibrinolytic activity on VECs.Showing these resuls, we discuss how fibrinolytic activity is regulated by t-PA and PAI-1 both in plasma and on VECs. We also want to discuss the physiological relevance of this regulatory mechanism which is naturally modified by many physiological stimuli

SYMPOSIA
Mechanisms of sensory processing under pathological pain conditions

S134 (3S-32E5)

Mechanism of Platelet Thrombus Formation under Blood Flow Condition.

Goto, Shinya; Tamura, Noriko; Ishida, Hideyuki (*Department of Medicine, Tokai University School of Medicine*)

Introduction. Atherothrombosis, including myocardial infarction and ischemic stroke, is a leading cause of death in the modern world. Platelet, forming thrombi at site of ruptured or disrupted atherosclerotic plaque, play crucial role in the onset of atherothrombosis. Mechanism of platelet thrombus formation under blood flow condition may not be the same as platelet aggregation under static conditions. **Method.** Whole blood, containing platelets rendered fluorescent by addition of mepacrine or calcium sensitive dye of Fluo-3, was perfused on the immobilized collagen fibrils at various shear rate conditions. Two-dimensional and three-dimensional growth of platelet thrombi on the collagen fibrils were detected by epi-fluorescent video-microscpy and ultra-fast laser confocal miscroscope equipped with piezo-motor control unit, respectively, Real time visualization of intra-cytoplasmic calcium ion concentration was also achieved with the use of laser confocal microscopy. **Results.** Unlike platelet aggregation under static condition, platelet thrombus growth on the collagen fibrils under blood flow conditions was markedly inhibited by blocking von Willebrand factor binding with glycoprotein Ibα, ADP binding with $P2Y_{12}$ ADP receptor, and so on. Our results also revealed that cyclic increase in intracytoplsmic calcium ion concentration was abolished when $P2Y_{12}$ was blocked. Collagen and thrombin also play important roles in the growth of platelet thrombi. **Conclusion.** Mechanism of platelet thrombus formation under blood flow conditions are different from that of platelet aggregation under static condition.

S135 (3S-33F1)

BDNF derived from microglia involving neuropathic pain

Inoue, Kazuhide; Tsuda, Makoto (*Grad. Sch.Pharma.Sci, Kyushu Univ, Fukuoka, Japan*)

Microglia play an important role as immune cells in the central nervous system. Recently, accumulating evidences indicate the important role of ATP receptors of activated microglia in the neuropathic pain. Neuropathic pain is often a consequence of nerve injury through surgery, bone compression, cancer, diabetes or infection. The expression of P2X4 receptor is enhanced in spinal microglia after peripheral nerve injury model, and blocking pharmacologically and suppressing molecularly P2X4 receptors produce a reduction of the neuropathic pain (Tsuda et al. Nature 424, 778-783, 2003). Several cytokines such as interleukin-6 and tumor necrosis factor in the dorsal horn are also increased after nerve lesion and have been implicated in contributing to nerve-injury pain. ATP can activate MAPK leading to the release of bioactive substances including cytokines from microglia (Shigemoto-Mogami et al., J Neurochem 78, 1339-1349, 2001; Suzuki et al., J Neurosci 24, 1-7, 2004). Thus, diffusible factors released from activated microglia by the stimulation of purinergic receptors may have an important role in the development of neuropathic pain (Tsuda, M., Inoue, K., & Salter, M.W. Trend Neurosci 28, 101-107, 2005). I will discuss the mechanism of P2X4-evoked allodynia with an effect of a neurotrophic factor from activated microglia based on the latest our findings (Nature, in press).

S136 (3S-33F2)

Role of BDNF in inflammatiory hyperalgesia and sprouting

Yoshimura, Megumu; Matayoshi, Satoru (*Grad. Sch. Med. Sci. Kyushu Univ. Fukuoka, Japan*)

Brain-derived neurotrophic factor (BDNF) is known to involved in the development of spinal plasticity underlying inflammation-induced hyperalgesia. An injection of complete Freund adjuvant (CFA) into rat plantar surface produced hyperalgesia, which was significantly attenuated by intraperitoneal administration of anti-BDNF antiserum performed a day before and just after CFA. In vivo patch-clamp recordings from the spinal substantia gelatinosa (SG) neurons of the inflamed rats demonstrated a marked enhancement of excitatory synaptic responses to noxious and non-noxious stimuli, suggesting an increase in the activity-dependent synthesis and release of BDNF in the SG. In the spinal slice preparations, BDNF, but not nerve growth factor (NGF) or neurotrophin-3 (NT-3), acted presynaptically to increase frequency of miniature EPSCs in SG neurons of the inflamed, but not naive rats, through an activation of lidocaine-sensitive, TTX-resistant sodium channels. This effect was observed in slices of the inflamed rat only 2-4 days after CFA injection. On the other hand, the number of monosynaptic A-beta afferent inputs to the SG significantly increased a week after the onset of the inflammation, and this increase was significantly suppressed by treatment with anti-BDNF antiserum. These findings, taken together, suggest that BDNF, which is considered to be released from the sensitized primary afferents, increases the excitability of SG neurons through its action on the presynaptic terminals, and may thereafter trigger plastic changes in the spinal sensory transmission to develop hyperalgesia/allodynia during inflammation.

S137 (3S-33F3)

Molecular mechanisms for neuropathic pain - lysophosphatidic acid as the initiator

Ueda, Hiroshi (*Nagasaki University Graduate School of Biomedical Sciences,Nagasaki,Japan*)

Here I report the initiation mechanisms underlying neuropathic pain (NP) following nerve damages. In this experiment, we used partial sciatic nerve ligation (PSNL) to induce NP in mice. The abnormal chronic pain including hyperalgesia and tactile allodynia was observed 3 days after the PSNL and lasted for more than 2 weeks. We found many nociceptive fiber-specific changes in protein and/or gene expression of molecules involved in pain transmission. They include the novel expression of Ca channel alpha2-delta1 subunit in myelinated fiber DRG neurons, which may account for hyperalgesia. At the same time, we found the loss of function in unmyelinated fiber-mediated pain transmission. They include the down-regulation of substance P in dorsal horn of spinal cord. In addition to these phenotypic changes in the expression, we observed marked demyelination and remyelination in dorsal root, a phenomenon which is closely related to allodynia. All these changes were abolished in lysophosphatidic acid (LPA) receptor (LPA1) knock-out mice, and mimicked by the intrathecal single injection of LPA. In ex vivo culture system of dorsal root, the addition of LPA caused demyelination and sprouting/remyelination, which were accompanied with down-regulation of myelin basic protein expression. We also found that LPA causes microglia activation in the spinal cord. I will discuss how LPA plays the initiation role in many molecular events observed in PSNL-induced NP.

S138 (3S-33F4)

Changes in brain dynamics by chronic pain: Molecular mechanisms of the suppression of morphine-induced rewarding effects and the aggravated anxiety

Narita, Minoru; Niikura, Keiichi; Kuzumaki, Naoko; Suzuki, Tsutomu (*Dept. Toxicol., Hoshi Univ. Sch. Pharm. Pharmaceut. Sci., Tokyo, Japan*)

It has been widely recognized that chronic pain could cause physiological changes at supraspinal levels. Here, we found that chronic pain caused a dramatic down-regulation of μ-opioid receptor function to activate its coupling with G-proteins of ventral tegmental area (VTA), and produced of the suppression of morphine-induced rewarding effect. Using the fluoro-gold (FG) microinjection into the VTA, numerous FG-labeled cells were detected in the lateral preoptic nucleus (LPO) and dorsolateral hypothalamus (DMH) of nerve-ligated rats. Subpopulations of β-endorphin-positive fibers in the LPO and DMH were co-labeled by FG. Furthermore, we found that chronic pain caused a dramatic down-regulation of cortical δ-opioid receptor function to activate its coupling with G-proteins, which is associated with the increased δ-opioid receptor phospholylation, and produced anxiety-like behaviors in mice, as characterized by both the light-dark and elevated plus-maze tests. These data provide direct evidence that the endogenous opioid-containing neuron projecting from the pain processing regions may be continuously activated by nerve ligation, resulting in the long-lasting down-regulation of μ- or δ-opioid receptors. This phenomenon may lead to the suppression of the morphine-induced rewarding effect and emotional disorders including aggravated anxiety under chronic pain-like state.

SYMPOSIA
Behavioral analysis of genetically modified mice in the postgenomic era [Workshop Organized by Program Organizing Committee]

S139 (3S-34G1)
Behavioral analyses of genetically manipulated mice for synaptic plasticity-related genes

Kojima, Nobuhiko; Shirao, Tomoaki (*Dept. of Neurobiol. and Behav., Gunma Univ. Grad. Sch. of Med., Maebashi, Japan*)

To understand molecular mechanisms of learning and memory is one of major topics in neuroscience research. To identify genetic components underlying synaptic plasticity and memory processes, I have been so far focusing on analyzing behavioral phenotypes, especially learned fear, of several strains of genetically manipulated mice for plasticity-related genes. One example is Fyn-overexpressing mice exhibiting hyper-tyrosine-phosphorylation of the NMDA receptor (NR). These mice showed NR activity-dependent impairment of fear conditioning, suggesting that this tyrosine kinase is a key molecule that controls conditioned fear through NR phosphorylation. Another is knockout (KO) mice for drebrin A, a F-actin binding protein in the dendritic spines. Context-dependent fear conditioning and MK-801-induced hyperlocomotion were changed in these KO mice. These results suggest that drebrin A has a pivotal role in the regulatory mechanism of NR function. The mutant mice for ICER, a CRE-binding transcriptional repressor, also showed the phenotype on fear conditioning. In ICER-overexpressing mice, long-term retention of fear memory was impaired, while the short-term memory remained intact. ICER-KO mice conversely showed a better performance in the retention of conditioned fear. These results suggest that ICER acts as a negative regulator for memory consolidation. Thus, these three examples demonstrate that the learned fear is a good target of behavioral analysis of mutant mice for genes being critical for synaptic plasticity and it should be included in the behavioral test battery.

S140 (3S-34G2)
Comprehensive behavioral test battery for genetically engineered mice: A proposal to use it as a "hub" for large-scale neuroscience

Miyakawa, Tsuyoshi (*Grad. Sch. Med. Univ. Kyoto, Kyoto, Japan*)

Identification of the functions of the genes at system level is one of the greatest challenges in the post genome-sequence era. To reveal functional significance of the genes expressed in the brain, we have been utilizing a comprehensive behavioral test battery on various kinds of genetically-engineered mice. The behavioral test battery covers a broad range of various behavioral domains, such as learning and memory, sensory-motor functions, emotions and motivation. During the past two years, we have assessed behaviors of more than 30 different strains of mutant mice and, surprisingly, we found at least one significant behavioral abnormality in more than 90% of those strains. This fact not only demonstrates that our strategy is useful in elucidating the functions of the genes expressed in the brain but also implies that a large part of the genes expressed in the brain may have some functions. In this workshop, I'd like to introduce some examples of our work using the test battery on mutant mice and to make a proposal to use it as a "hub" for large-scale neuroscience. Establishing a collaborative network among experts in the field would be necessary for greater success of this approach. The potential issues related to implementing such collaborative network or consortium will be also discussed.

S141 (3S-34G3)
Studies of emotional and socio-sexual behaviors in genetically modified mice

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We have been studying neuroendocrinological bases of emotional and socio-sexual behaviors. Our studies using two types of estrogen receptor knockout mice during the last ten years have proven that the knockout mouse model is a powerful tool to delineate the relationship between genes, hormones, and behaviors. Since our main research interest, as behavioral scientists, is to understand brain mechanisms of emotional and socio-sexual behaviors, we have been focusing on developing test paradigms that are able to measure the behaviors of interest most appropriately and reliably. On the other hand, progress in molecular biology has enabled production of enormous numbers of genetically modified animals. As a result, interest and demand for analyses of behaviors as one of the phenotypes of these animals, have rapidly grown for molecular biologists. In this talk, I will first overview our findings in behavioral characteristics of estrogen receptor knockout mice and then discuss the problems in standardization of behavioral testing paradigms. Particularly, I will talk about differences between "how to measure" and "what is measured".

S142 (3S-34G4)

Mouse behavior analysis support service at RIKEN Brain Science Institute (BSI)

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Research Resources Center (RRC) in RIKEN Brain Science Institute (BSI) provides the mouse behavior analysis support service. As a base of experimentation, RRC provides the common laboratories for mouse behavior analysis in the laboratory animal facility in BSI. This laboratory area consists of 12 separated rooms, and one or several kinds of devices for the behavioral experiment are set up in each room. BSI researchers can use these rooms and devices freely following a guidance. The mouse behavior analysis support service section offers user guidance on use, maintains, develops and updates the laboratories and the equipped experimental apparatuses. Another important part of this service is consultation. We help to plan for behavior experiments, paradigms and/or methods, data analysis and so on. Furthermore, a primary behavioral screening of mutant mice based on a "behavioral test battery" is currently provided on a trial basis. In this workshop, the advantage and disadvantage of the mouse behavior analysis support service at BSI will be discussed from the point of view of standardization of the mouse behavior analysis at a large-scale research facility like BSI.

S143 (3S-34G5)

G-substrate, a putative downstream component of NO-cGMP-PKG cascade, plays a important role in cerebellar-dependent long-term memory

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Cerebellum plays an important role in non-declarative memories such as motor memories. Cellular substrate for the cerebellar-dependent memory is long-term depression (LTD). NO-cGMP-PKG (cGMP-dependent protein kinase) pathway is shown to be involved in the induction of cerebellar LTD. NO absorbing reagent prevents cerebellar-dependent learning such as the adaptation of perturbed locomotion and VOR adaptation. Furthermore, each component of NO-soluble guanylate cyclase-cGMP-PKG pathway has been shown to be essential for the induction of LTD. However, the downstream component of PKG was not identified.

Recently we molecularly cloned and characterized G-substrate, localized specifically in cerebellar Purkinje cells, as a downstream component of PKG. Further, we have generated a mice lacking G-substrate gene. Homozygous mice are vital as expected from the restricted localization of G-substrate. The behavioral analyses were conducted on the G-substrate gene knockout mice. Significant difference was not observed between the control and G-substrate knockout mice in general behaviors. However, a specific impairment was observed in long-term horizontal optokinetic response (HOKR), a cerebellar-dependent memory, without any impairment in short-term HOKR. In addition, we observed G-substrate, with shuttles between nuclear and cytosol of Purkinje cells. G-substrate may have a role in transcription and translation in the nuclear that is essential for the long-term memory.

S144 (3S-34G6)

Transponder-based approaches to behavioral phenotyping in mice

Lipp, Hans-Peter (*Institute of Anatomy, University of Zurich, Zurich, Swiss Confederation*)

Extensive behavioral testing of mice including common laboratory strains and genetically modified lines has revealed that many measures of hippocampus-dependent memory and learning are confounded by reactions of mice towards handling and test conditions. Moreover, very little is known about cognitive activity of mice in their natural environment. Therefore, we have developed transponder-based techniques permitting to assess learning and cognition in social settings, first in outdoor pens and later in large cage units called "Intellicage". Data presented will show how hippocampal malfunction is manifested in the water maze, under outdoor conditions and in Intellicages, and how transponder-based technology can be used for fully automated assessment of memory and learning in large number of mice.The main conclusions for high-throughput phenotyping are:- Mutation, strain and hippocampal lesion effects can easily be discovered within a social group - testing single mice in isolation is methodologically not necessary.- Automated testing is 10 to 40 times more economic than manual testing.- Comparability across labs is improved and standardization is much facilitated.- Transponder-based automated systems can be used for both, screening by laypersons and sophisticated analysis by behavioral experts.Since all testing is done without human interference by computers, different groups can easily share data over the web. This will enable researchers to perform comparative analysis of animals having undergone identical test protocols in different locations. Supported by Swiss National Science Foundation and NCCR "Neural Plasticity and Repair".

SYMPOSIA
Basic approach for therapy of failing heart models

S145 (3S-35H1)

Translocation and cleavage of myocardial dystrophin as a common pathway to advanced heart failure: a scheme for the progression of cardiac dysfunction and the novel treatment

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The progression mechanism from cardiac dysfunction to advanced heart failure (HF) including dilated cardiomyopathy (DCM) should be clarified to establish a novel treatment. We have identified a gene mutation in δ-sarcoglycan (SG) that causes hereditary DCM in both animals (Sakamoto *et al., PNAS*, 1997) and humans (Tsubata *et al., JCI*, 2000). TO-2 hamsters with hereditary DCM show age-dependent cleavage and translocation of myocardial dystrophin (Dys) from sarcolemma (SL) to myoplasm, enhanced SL permeability *in situ*, and a close relation between Dys loss and hemodynamics. Dys disruption is not an epiphenomenon but directly causes advanced HF, because *in vivo* transfer of the missing gene to degrading cardiomyocytes ameliorated all of the pathological features and improved the disease prognosis (Kawada *et al., PNAS,* 2002). Furthermore, acute HF after isoproterenol toxicity and chronic HF after coronary ligation in rats both time-dependently cause Dys disruption in the degrading myocardium (Takahashi *et al., CVRes.*, 2005). Dys cleavage was also detected in human hearts from patients with DCM of unidentified etiology, supporting a scheme of vicious cycle consisting of SL instability, Dys cleavage, and translocation of Dys from the SL to the myoplasm (Toyo-oka *et al., PNAS*, 2004), irrespective of an acute or chronic process and a hereditary or acquired origin. Activation of endogenous calpain will be discussed for the Dys disruption.

S146 (3S-35H2)

Molecular cloning of cardiac troponin I isoform

Suzuki, Hideaki; Takeda, Nobuakira (*Jikei Univ. Sch. Med., Tokyo, Japan*)

Background and Objectives: Laminin, which is a major component of the extracellular matrix, is known to increase in the myocytes in the diabetic heart and dilated cardiomyopathy. The laminin gamma 1 chain promoter contains a transcriptional element denoted bcn-1 that is inducible and active (Suzuki H, et al. J Biol Chem 271:1996). To elucidate the molecular pathogenesis of the increase of laminin in the cardiac muscle cells of cardiomyopathy, we have carried out the yeast one-hybrid screen using bcn-1 as a bait, and cloned Smarce 1r protein as a molecular partner which interacts with bcn-1 protein. Next, we repeated two-hybrid screen using Smarce 1r as a bait and cloned MLF11P as a partner (Suzuki H, Exp Clin Cardiol 9:2004).Methods: The yeast two-hybrid screen analysis was carried out again using MLF11P protein (amino-acids 1-318) as a bait. A human heart cDNA library was screened by the yeast mating method for overnight culture.Results: We isolated two final positive clones encoded the same protein, which is an alternative-RNA splicing form of the human cardiac troponin I (TnI) protein and we call this as a spliced form of TNI (STNI). The mRNA expression pattern of STNI is heart-specific. The STNI shares several sequence similarities with the human cardiac TNI but lacks troponin T binding protein.Conclusions: We report the heart-specific segment of the human cardiac troponin I isoform which lacks troponin C binding portion. These results suggest that STNI might be involved in the molecular pathogenesis of the increase of laminin in the cardiomyopathy.

S147 (3S-35H3)

A mouse knock-in model of dilated cardiomyopathy associated with δK210 mutation in cardiac troponin T and its potential pharmacotherapy

Morimoto, Sachio (*Kyushu Univ. Grad. Sch. Med., Fukuoka, Japan*)

Dilated cardiomyopathy (DCM) is characterized by cardiac dilation and systolic dysfunction, which often leads to severe heart failure and sudden death. However, little is known about the pathogenic mechanism for DCM, and no therapeutical method is established at present except for cardiac transplantation. We created a knock-in mouse model of DCM caused by a deletion mutation δK210 in cardiac troponin T and explored its molecular pathogenic process and potential pharmacotherapy. Mutant mice developed enlarged hearts with bi-ventricular dilation and systolic dysfunction and suffered sudden death frequently, recapitulating human DCM with this mutation. Skinned cardiac muscle fibers from mutant mice showed a decreased Ca^{2+} sensitivity of force generation, confirming our previous hypothesis that decreased contractility of cardiac muscle is a primary pathogenic mechanism of this mutation. Surprisingly, however, intact cardiac muscle fibers from mutant mice showed no significant reduction in isometric force per cross-sectional area. Analyses of Fura-2 loaded cardiomyocytes revealed that this was due to an increase in the amplitude of intracellular Ca^{2+} transient. Biochemical analyses, including DNA microarray, strongly suggested that Ca^{2+} transient was increased through down-regulation of a specific isoform of phosphodiesterase (PDE4B) and associated increase in cAMP in cardiomyocytes of mutant mice, which could compensate for the decreased myofilament Ca^{2+} sensitivity but at the same time would increase the risk for arrhythmia leading to sudden death due to a Ca^{2+} overload.

S148 (3S-35H4)

Gene therapy by SERCA2a for failing hearts in spontaneous type II DM model rats

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OLETF rat is a model of spontaneous non-insulin-dependent diabetes mellitus (DM), accompanying diastolic dysfunction associated with abnormal Ca^{2+} handling and decrease in sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) expression. The aim of this study was to examine whether SERCA2a gene transfer can restore left ventricular (LV) function in this DM in terms of LV mechanical work and energetics. DM rats were randomized to receive adenovirus carrying SERCA 2a gene (DM+Serca) or β-galactosidase (DM+Gal), or saline (DM+Sa) by catheter-based (cross-clamping) technique. LV mechanoenergetics was assessed in cross-circulated whole heart preparations 3 days after the infection. In DM, end-systolic pressure ($ESP_{0.1}$) was low and end-diastolic pressure ($EDP_{0.1}$) was high at 0.1 ml intraballoon water. In DM+Serca, however, $ESP_{0.1}$ increased and $EDP_{0.1}$ decreased. LV relaxation rate in DM+Serca was faster than that of DM+Gal/Sa groups. Oxygen cost of LV contractility in DM was higher than that of normal, indicating energy wasting in Ca^{2+} handling during E-C coupling. The O_2 cost of LV contractility decreased to non-DM rat level in DM+Serca, although it remained high in DM+Gal and DM+Sa. These results indicate that SERCA2a overexpression by SERCA2a gene transfer improves not only LV mechanics but also energetics in DM rat hearts.

S149 (3S-36B1)

Neuronal mechanisms of respiratory rhythm generation

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Is respiratory rhythm generated by pace-maker neurons or by network mechanisms? Although this longtime and important question has not been solved yet, recent studies provide evidence supporting pace-maker theory at least under some experimental conditions and clarified characteristics of respiratory pace-maker neurons. In respiratory rhythm generation, a term of pace-maker neurons indicates neurons that possess cellular property intrinsically producing rhythmic burst (but not tonic) activity. In pace-maker theory, it is hypothesized that intrinsic burst generating neurons are embedded in a network composed of neuronal population with same active phase and contribute to produce basic synchronized rhythm, whereas inhibitory synaptic connections are not necessary for the rhythm generation. At least two respiration-related rhythm generators have been suggested to exist in the medulla and to produce intrinsic periodic burst activity. One is an inspiratory (Insp) neuronal network (i.e., Insp rhythm generator), which is localized predominantly in the pre-Boetzinger complex of the ventrolateral medulla. Another is a pre-inspiratory (Pre-I) neuronal network (i.e., Pre-I rhythm generator), which generates activity prior to Insp bursts and is located in the more rostral ventrolateral medulla including the para-facial respiratory group (pFRG). We have shown that pFRG-Pre-I neurons serve as a primary rhythm generator, triggering inspiratory burst periodically in the brainstem-spinal cord preparation from newborn rat. In this presentation, we focus on mainly burst generating properties of Pre-I neurons and the ionic and synaptic mechanisms.

SYMPOSIA
Pacemaker mechanism of the rhythmic activity of cell and tissue

S150 (3S-36B2)

Role of interstitial cells in generating spontaneous activity of smooth muscles

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Many smooth muscles exhibit spontaneous electrical and mechanical activity. This has been considered to "myogenic" activity for many years, however, it is in fact generated by specialized cells, namely interstitial cells of Cajal (ICC). In the gastrointestinal tract, a network of myenteric ICC (ICC-MY) generates pacemaker potentials to initiate spontaneous electrical activity. Intramuscular ICC (ICC-IM) augment the depolarizations by generating ongoing unitary potentials (UPs). However, corporal ICC-IM may be dominant pacemaker as they create a greatest frequency in spontaneous activity. ICC-like cells have been found in other smooth muscle organs and may play a similar role. In the urethra, ICC-like cells generate spontaneous transient depolarizations (STDs), which sum to activate L-type Ca channels. UPs and STDs result from the opening of Ca-activated Cl^- channels. UPs solely depend on inositol 1, 4, 5-trisphosphate ($InsP_3$)-dependent Ca release, whilst the generation of STDs requires both $InsP_3$- and ryanodine-receptors. In corpus cavernosum (CC), where smooth muscle cells are capable of generating spontaneous depolarizations, ICC-like cells express cyclooxygenase 2 and spontaneously produce prostaglandins to reinforce spontaneous activity. Therefore, although ICC or ICC-like cells in different regions or tissues share many similarities, they also have a significant diversity. Interaction between ICC and smooth muscle cells result in a further heterogeneity of spontaneous activity, and thus develops characteristics of individual smooth muscle organs.

S151 (3S-36B3)

In silico analysis of the cardiac pacemaking

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Computers have become the necessities of life science with improvements of information technologies. Thus, Systems Biology, whose aim is to describe the whole biological interactions as a mathematical model and understand the whole mechanisms by simulation, has been emerged. We have started developing a comprehensive cardiac model (Kyoto model), and a cardiac sinoatrial node pacemaker model is developed with the same set of equations as the ventricular cell model. The model successfully reconstructs the experimental action potentials at various concentrations of external Ca^{2+} and K^+, and reaches steady-state and the sensitivity analyses can be applied to a variety set of parameters. Increasing the amplitude of L-type Ca^{2+} current (I_{CaL}) prolongs the duration of the action potential and thereby slightly decreases the spontaneous rate. On the other hand, a negative voltage shift of I_{CaL} gating by a few mV markedly increases the spontaneous rate. When the amplitude of sustained inward current is increased, the spontaneous rate is increased irrespective of the I_{CaL} amplitude. When the spontaneous activity is stopped by decreasing I_{CaL} amplitude, the resting potential is around -35 mV over 1-15 mM $[K^+]_o$ because of the presence of the background nonselective cation current. The unique role of individual voltage- and time-dependent ion channels is clearly demonstrated and distinguished from that of the background current by calculating an instantaneous equilibrium potential during the course of the spontaneous activity.

SYMPOSIA
Regulation of feeding and metabolism: molecular and physiological mechanisms

S152 (3S-36B4)

Circadian rhythms are controlled by Arg-vasopressin in the suprachiasmatic nucleus

Isobe, Yoshiaki; Nishino, Hitoo (*Grad. Sch. Med. Nagoya City Univ. Nagoya, Japan*)

Many physiological functions show circadian rhythms controlled by suprachiasmatic nucleus (SCN) in mammals. From the SCN, ca. 24 hr rhythmicity accompanying the time of day information (phase) is conveyed to the other area of the brain and peripheral organs. Arg-vasopressin (AVP) containing neuron is one of the output neuron from the SCN. VIP and glutamate increased AVP release from the SCN, meanwhile suppressed by GABA (via GABAa receptor) and melatonin (MT2 receptor). The rPer2 mRNA products (CCG protein) promote the rBmal1 mRNA increase, which concerns the AVP mRNA transcription. In the SCN, before and after the 8-hr advance of the LD cycle, AVP mRNA and AVP peptide in the SCN remain coupled with time. After the light pulse (800 lux, 15 min at ZT 22), AVP mRNA increased in the SCN, but not in the paraventricular nucleus (PVN). Following the melatonin injection (1 mg/kg, i.p.), AVP content in the SCN decreased at both light and dark period, while no changes were observed in the PVN. In the SCN, rPer2 mRNA increased at both light and dark period. In the PVN, rPer2 decreased and increased at light and dark period, respectively. While in the pineal gland, rPer2 mRNA increased at 180 min after the melatonin injection. The melatonin-AVP reciprocal system indicates that PVN is temporally coupled with the SCN clock activity. The AVP and melatonin on the locomotor activity and body temperature rhythms are interesting theme. Body temperature decrease caused by the melatonin application (i.p.) during the night is considered to be a decrease of AVP in the SCN. Functional significance of AVP in the SCN would be discussed.

S153 (3S-37C1)

Control of appetite and metabolism by olfactory stimulation: Involvements of histaminergic nerve and biological clock

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Recently, we observed that olfactory stimulation with scent of grapefruit essential oil caused excitations of sympathetic nerves innervating the epididymal (white) adipose tissure, interscapular (brown) adipose tissue and adrenal gland and inhibition of a parasympathetic nerve innervating the stomach. In contrast, olfactory stimulation with scent of lavender essential oil inhibited sympathetic nerves innervating the epididymal adipose tissue, interscapular adipose tissue and adrenal gland and excitated a parasympathetic nerve innervating the stomach. These findings suggest that the scent stimulation with grapefruit oil elevates lipolysis, thermogenesis, adrenaline secretion, thus, the blood glucose and the blood pressure, and suppression of food intake, and that the scent stimulation with lavender oil induced opposite responses. In the experiments examined this, we found evidences suggesting that these are the cases. Moreover, we observed that bilateral electrolytic lesions of the hypothalamic suprachiasmatic nucleus (SCN), a master circadian clock, and histaminergic blockers eliminated these responses. That is, a histamine H3-antagonist, thioperamide, abolished the effects of lavender oil and a histamine H1-receptor-antagonist, diphenhydramine, suppressed the effects of grapefruit oil. Anosmic treatment with zinc sulfate eliminated all of the changes due to olfactory stimulations. These findings suggest that olfactory stimulations with scents of grapefruit and lavender oils affect appetite and metabolism via the functions of the SCN and histaminergic nerve.

S154 (3S-37C2)

Regulation of glucose and energy metabolism by PACAP

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a ubiquitous neuropeptide in the central and peripheral nervous systems. Previously we reported that PACAP potentiates both insulin release from pancreatic β-cells and insulin action in adipocytes, contributing to reduction of blood glucose and energy storage. PACAP38 is localized in pancreatic islets and serves as an endogenous amplifier of glucose-induced insulin secretion via VPAC2 and PAC1 subtypes of PACAP receptors. In an adipocyte cell-line, 3T3-L1 cells, PACAP enhances insulin-stimulated glucose uptake via PAC1 receptors and promotes adipocyte differentiation. In contrast, PACAP stimulates secretion of glucagon and catecholamine and glucose output from the liver, causing elevation of blood glucose. Thus, PACAP regulates the glucose and energy metabolism at multiple processes in several tissues. In this symposium, we present three novel effects of PACAP in the metabolism. (1) The action of PACAP to protect islet cells against lipotoxicity and glucotoxicity. (2) PACAP knock out mice exhibits decreased fat mass and increased insulin sensitivity, suggesting a role of PACAP to facilitate adiposity and decrease insulin sensitivity. (3) PACAP promotes feeding behavior by activating neuropeptide Y neurons in the hypothalamic arcuate nucleus, a feeding center.Based on these well known and newly identified metabolic effects of PACAP, we discuss a possible therapeutic use of PACAP receptor subtype-specific agonists and/or antagonists in the treatment of metabolic syndrome.

S155 (3S-37C3)

The Brain-Adipose axis and Obesity:Identification of nesfatin that causes anorexia

Shimizu, Hiroyuki; Mori, Masatomo (*Department of Medicine and Molecular Science,Gunma Univ. ,Grad.Sch.Med, Maebashi,Japan*)

Many molecules compose the brain-adipose axis that controls appetite. Using a subtraction cloning assay, we searched genes which were activated by a PPAR-gamma activator and identified nesfatin, a secreted protein of unknown function, which was expressed in the appetite-control hypothalamic nuclei and adipose in rats. Intracerebroventricular (icv) injection of nesfatin caused a dose-dependent decrease in food intake, and icv injection of its antibody stimulated feeding. The structure of nesfatin possesses several cleavage sites that may undergo processing by prohormone convertase (PC), and nesfatin was co-localized with PC-2 and PC-3. Western blot analysis demonstrated the presence of nesfatin-1 in the hypothalamic extract. Icv injection of nesfatin-1, but not nesfatin-2 or -3, produced satiety, and injection of an antibody neutralizing nesfatin-1 stimulated feeding. Chronic icv injection of nesfatin-1 reduced body weight, and rats gained body weight after chronic icv administration of an antisense morpholino-oligonucleotide against the nesfatin gene. The present data provide evidence that nesfatin is a novel, secreted anorexigenic molecule in the hypothalamus.

S156 (3S-37C4)

Adipocytokines and metabolic regulation

Ogawa, Yoshihiro (*Dept. Mol. Med. Metab., Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo, Japan*)

Weight gain is associated with infiltration of fat by macrophages, suggesting they are an important source of inflammation in obese adipose tissue. We have recently developed an in vitro co-culture system composed of adipocytes and macrophages and examined the molecular mechanism whereby these cells communicate. Co-culture of differentiated 3T3-L1 adipocytes and macrophage cell line RAW264 results in marked up-regulation of pro-inflammatory cytokines such as MCP-1 and TNF-α and down-regulation of anti-inflammatory cytokine adiponectin. Such inflammatory changes are induced by the co-culture without direct contact, suggesting the role of soluble factors. A neutralizing antibody to TNF-α, which occurs mostly in macrophages, inhibits the inflammatory changes in 3T3-L1, suggesting that TNF-α is a major macrophage-derived mediator of inflammation in adipocytes. Conversely, FFAs may be an important adipocyte-derived mediator of inflammation in macrophages because the production of TNF-α in RAW264 is markedly increased by palmitate, a major FFA released from 3T3-L1. The inflammatory changes in the co-culture are augmented by use of either hypertrophied 3T3-L1 or adipose stromal vascular fraction obtained from obese ob/ob mice.

We postulate that a paracrine loop involving FFAs and TNF-α between adipocytes and macrophages establishes a vicious cycle that aggravates inflammatory changes in the adipose tissue. This study suggests the pathophysiologic implication of the intimate crosstalk between adipocytes and macrophages in the development of inflammatory changes in obese adipose tissue and thus the metabolic syndrome.

SYMPOSIA
Molecular dynamics in cardiac function [YFI (Young Foreign Investigator) Symposium]

S157 (3S-38D1)
An X-ray Diffraction Study on Mouse Cardiac Cross-Bridge Function in vivo : Effects of Adrenergic β-stimulation

Yagi, Naoto[1]; Toh, Ryuji[2]; Shinohara, Masakazu[2]; Takaya, Tomofumi[2]; Yamashita, Tomoya[2]; Masuda, Shigeru[2]; Kawashima, Seinosuke[2]; Yokoyama, Mitsuhiro[2] (*[1]SPring-8/JASRI; [2]Kobe Univ. Grad. Sch. Med.*)

In order to investigate how β-stimulation affects the contractility of cardiac muscle, x-ray diffraction from cardiac muscle in the left ventricular free wall of a mouse heart was recorded in vivo. This is the first x-ray diffraction study on a heart in a living body. After the R-wave in electrocardiograms, the ratio of the intensities of the equatorial (1,0) and (1,1) reflections decreased for about 50 msec from a diastolic value of 2.1 to a minimum of 0.8, and then recovered. The spacing of the (1,0) lattice planes increased for about 90 msec from a diastolic value of 37.2 nm to a maximum of 39.1 nm, and then returned to the diastolic level, corresponding to about 10% stretch of sarcomere. Stimulation of β-adrenergic receptor by dobutamine (20 μg/kg/min) accelerated both the decrease in the intensity ratio, which reached a smaller systolic value, and the increase in the lattice spacing. However, the intensity ratio and spacing at the end-diastole were unchanged. The recovery of the lattice spacing during relaxation was also accelerated. The mass transfer to the thin filaments at systole in a β-stimulated heart was close to the peak value in twitch of frog skeletal muscle at 4 °C, showing that the majority of cross-bridges have been recruited with few in reserve.

S158 (3S-38D2)
Molecular Dynamics in Cardiac Function

Fujita, Hideo[1]; Yamashita, Hiroshi[1]; Sugiura, Seiryo[2] (*[1]Univ. of Tokyo Hosp., Tokyo, Japan; [2]Grad. Sch. Frontier Sci., Univ. of Tokyo, Tokyo, Japan*)

Clinical trials have demonstrated the adverse long-term effect of inotropic drugs for the treatment of heart failure. Because most of these drugs exert their effects via the increase of intracellular calcium concentration, people have sought a new class of agents working independently of the calcium handling mechanism. We have studied the function of cardiac myosins with various molecular structures to elucidate their role in diseased conditions, but, at the same time, such studies suggested the possible new mechanisms for modulating cardiac contractility at the crossbridge level. Studies on mutant myosins implicated in familial hypertrophic cardiomyopathy, replacement of a single amino-acid located in the C-terminus thus being far from the functional domains responsible for ATP hydrolysis or actin-binding introduced severe functional defect. On the other hand, myosin light chains seem to modulate crossbridge kinetics without changing ATPase activity of myosin. These calcium-independent (downstream) mechanisms for the modulation of cardiac contractility will be discussed with additional observations.

S159 (3S-38D3)
Transmural heterogeneity of crossbridge dynamics and lattice spacing in isolated rat hearts

Shimizu, Juichiro[1]; Mohri, Satoshi[2]; Miyasaka, Takehiro[2]; Okuyama, Hiroshi[3]; Toyota, Hiroko[3]; Tsujioka, Katsuhiko[3]; Takaki, Miyako[1]; Kajiya, Fumihiko[4]; Yagi, Naoto[5] (*[1]Dept. of Physiol. II, Nara Med. Univ. Sch. of Medicine, Kashihara, Japan; [2]Okayama Univ. Grad. Sch. of Med, Dent, and Pharm., Okayama, Japan; [3]Dept. of Physiol. Kawasaki Med. Sch., Kurashiki, Japan; [4]Dept. of Med. Eng. Kawasaki Med. Sch, Kurashiki, Japan; [5]Japan Synchrotron Radiation Research Institute, Sayo-gun, Hyogo, Japan*)

To analyze the fundamental mechanism of the Frank-Starling's law, we studied the left ventricular (LV) transmural difference in the time course of crossbridge dynamics (CBD) and the end-diastolic myosin filament lattice spacing (EML) in the isolated crystalloid-perfused isovolumically contracting rat hearts (n=11) paced at 2 Hz using X-ray diffraction at SPring-8. We recorded x-ray diffraction patterns of the epicardial (EPI) and deeper (DEEP) myocardium region of the LV free wall along with LV pressure (LVP) at the end-diastolic pressure of either 0 or 20 mmHg by adjusting LV volume. We analyzed transmural CBD from the X-ray diffraction patterns according to the transmural variations of regional myofilament orientation. The developments of CBD of both EPI and DEEP and normalized LVP were synchronous during contraction, but not during relaxation. The CBD decay was significantly faster in DEEP than EPI. The LVV increase significantly potentiated LVP development and more prolonged the CBD decay in EPI than DEEP associated with more reduction of EML in EPI than DEEP. From studies on CBD and EML, we were able to propose a possible underlying mechanism for the Frank-Starling's law in the whole heart.

S160 (3S-38D4)

Effects of Length-Dependent Changes and Ischaemia on Cardiac Cross-Bridge Cycling

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We demonstrate using synchrotron x-ray radiation that length-dependent mechanisms influence cross-bridge cycling in *in situ* rat hearts, and then describe the effects of ischaemia-reperfusion on contractility. All experiments were performed in real time using synchrotron radiation at Spring-8 and x-ray diffraction techniques. Mass transfer of myosin to actin during contraction was inferred from the change in diffraction intensity ratio (intensity of 1,0 reflection / 1,1 reflection) derived from the myosin-actin filaments in fibres. Sustained volume loading by infusion of sodium lactate (40-60 ml/h for <5 min) evoked a rightward shift in left ventricular volume and significant decreases in epicardial myosin spacing, consistent with sarcomere stretching. Increases in stroke volume were correlated with increases in mass transfer and myosin spacing change. Local comparisons of intensity ratio and myosin spacing cycles during ischaemia-reperfusion indicate that 1) mass transfer in the damaged area was reduced by moderate ischaemia, and sometimes unsynchronised, 2) compensatory increases in contractility were detected in non-ischaemic areas (and during reperfusion), and 3) myosin spacing increases were reduced and delayed or reversed under severe ischaemia. These findings suggest that non-functional fibres are stretched under high cardiac output during acute ischaemia.

S161 (3S-39E1)

Single-molecule analyses of the interactions of neurotrophins and the receptors on the growth cone

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The growth cone is a motile structure located at the distal tip of the nerve fiber. The 2.5S nerve growth factor (NGF) promotes the axonal growth and the survival of sensory neurons by reacting with their growth cones. We observed the behavior of single molecules of NGF on the growth cone of sensory neurons by using a fluorescent NGF, Cy3-NGF. Upon the application of 0.4 nM of Cy3-NGF, the growth cones responded within one minute of adding the stimulus by expanding their lamellipodia. Only 40 molecules of Cy3-NGF, which occupied less than 5% of the estimated total binding sites on a single growth cone, were required to initiate the motile responses. After binding to the receptor, Cy3-NGF displayed lateral diffusion on the membrane of the growth cones. The behavior of Cy3-NGF was shifted to a one-directional rearward movement toward the central region of the growth cone. The one-directional movement of Cy3-NGF displayed the same rate as the rearward flow of actin and the movements could be stopped by the application of the potent inhibitor of actin polymerization, latrunculin B. Molecules of Cy3-NGF were internalized in the vicinity of the central region of the growth cone during this rearward trafficking, as Cy3-NGF remained in the growth cone after the removal of Cy3-NGF from the receptors on the surface of growth cones. These results suggested that actin-driven trafficking of the NGF-receptor complex is an essential step to the accumulation and endocytosis of NGF at the growth cone.

SYMPOSIA
The new evolution of molecular imaging—To spy spatiotemporal mechanism on cells [Workshop Organized by Program Organizing Committee]

S162 (3S-39E2)

Molecular imaging and functional analysis of RNG105: an RNA-binding protein responsible for regulation of local translation in neurons

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Local translation in neuronal dendrites plays a key role in activity-dependent synaptic modifications, and is needed for long-term synaptic plasticity. RNA granules, which consist of clusters of ribosomes and RNAs, are responsible for transport of mRNAs to the dendrites and local translational control.

We identified RNG105 (RNA granule protein 105) as a novel component of the RNA granules in dendrites of hippocampal and neocortex neurons. The RNG105-localizing RNA granules contain mRNAs, the translational products of which play key roles in synaptic plasticity. RNG105 is an RNA-binding protein and has ability to repress translation both *in vitro* and *in vivo*. Time-laps fluorescence imaging revealed that dissociation of RNG105 from the RNA granules is induced by BDNF (brain-derived neurotrophic factor) stimulation. In contrast, even after the BDNF stimulation, ribosomes remain in/near the RNA granules. The RNG105 dissociation is concomitant with the induction of local translation of the mRNAs located in the RNA granules. These findings suggest that RNG105 is a translational repressor in the RNA granules and becomes dissociated from the granules by synaptic stimulation, which cancels the translational repression of the mRNAs in the RNA granules.

We also want to show our recent progress in the study of RNG105 knockout mice and identification of RNG105-associated components of

S163 (3S-39E3)

Single-molecule imaging of biomolecular functions

Funatsu, Takashi (*Grad. Sch. Pharm. Sci. Univ. Tokyo, Tokyo, Japan*)

Single-molecule imaging is a useful technique for analyzing functions and interactions of protein molecules. I will show some instances how this technique is applied to the biological studies. The first example is the study of chaperonin assisted protein folding. GroEL mediates the folding of nascent or denatured proteins in the E.coli collaborating with co-chaperonin GroES. We visualized GroEL-GroES interaction at the single molecule level [1]. Release of GroES from GroEL occurred after a rag period (~3s), that was not recognized in previous bulk-phase studies. Furthermore, we succeeded in observing the refolding of denatured GFP in the GroEL-GroES complex, and found that GFP could not start to refold for 3s after GroES binding. This observation suggests the presence of a new kinetic intermediate "cis ATP*-complex" in the GroEL-GroES reaction pathway. It is important for the efficient encapsulation of non-native protein into the GroEL cavity. The second example is the transport of mRNA within a living cell. Fluorescently labeled mRNA was injected into the nuclei of living cells and was visualized by fluorescence microscopy. The injected mRNA molecule were in equilibrium of two states, Brownian motion with diffusion coefficients of 0.2 $\mu m^2/s$ and corralled in a restricted area for 20 s. These results suggested that mRNA travels from the site of synthesis to nuclear pore by diffusion process.

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SYMPOSIA

New frontiers of research in synaptic plasticity—cellular and molecular mechanisms underlying reorganization of synapses in the cerebellum and visual cortex

S164 (3S-39E4)

Visualization of microtubule dynamics in cells

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Microtubule, a polarized hollow tube having plus and minus ends, is highly dynamic structure repeating growth and shortening, especially at its plus end. In cells, the parameters of microtubule dynamics are spatio-temporally regulated by a number of microtubule-binding proteins that stabilize or destabilize microtubules, and thereby asymmetrical microtubule networks are generated.

The generation of a polarized microtubule organization is critically important for proper cellular functions, such as cell division and migration. To explain how microtubules set up and make contacts with cellular structures, a "search-and-capture" mechanism has been proposed, in which the microtubule plus ends dynamically search for and capture specific sites, such as mitotic kinetochores and cell cortex. To date, several classes of proteins called "microtubule plus-end-tracking proteins" or "+TIPs" have been shown to be associated with "growing" microtubule plus ends in a wide range of organisms from fungi to humans to play critical roles in the "search-and-capture" mechanism. Some of +TIPs highlight every growing microtubule plus ends, while some of them accumulate at the microtubule-capturing structure created at the specialized sites, and the complex formation of these molecules serves a link between the microtubule plus end and variety of cellular structures.

In this talk, I will introduce the tools to visualize microtubule dynamics in living cells and overview our current understanding of the +TIPs.

S165 (3S-40F1)

Activity-dependent maintenance of climbing fiber synaptic function in the cerebellum

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Synapses undergo activity-dependent changes not only during development but also in adulthood. Mechanisms underlying these dynamic changes have been studied intensively. However, it is largely unknown how the strength of once matured synapse is maintained stably in the brain. We show that significant weakening in the strength of excitatory climbing fiber (CF) to Purkinje cell (PC) synapse followed chronic inhibition of neuronal activity by tetrodotoxin or persistent blockade of postsynaptic AMPA receptors by their selective antagonist, NBQX. These treatments reduced glutamate concentration transients at synaptic clefts and decreased the frequency of quantal excitatory postsynaptic current (EPSC). In contrast, neither the amplitude of quantal EPSC nor the release probability was changed. Our morphological examination demonstrates selective reduction of CF innervation at PC shaft dendrites after NBQX-treatment. We thus conclude that in the mature cerebellum, AMPA receptor-mediated neuronal activity in PCs maintains CF's functional release sites and its innervation of PC shaft dendrites.

S166 (3S-40F2)

Analysis of functions of parallel fiber-Purkinje cell synapses by using virus vector-mediated gene transfer in young adult cerebellum

Kohda, Kazuhisa; Kakegawa, Wataru; Yuzaki, Michisuke (*Dept. of Physiol., Keio Univ. Sch. Med.*)

Parallel fibers (PFs), axons of cerebellar granule cells, transmit multimodal sensory information via mossy fiber inputs originating from the pontine nuclei. Each PF forms synapses with multiple Purkinje cells (PCs) whose plasticity is believed to play cardinal roles in motor learning. In addition, PF-PC synapses were reported to be dynamically reorganized in an activity-dependent manner even in adulthood. Several lines of evidence have suggested that the δ2 glutamate receptor (GluRδ2) is one of the key molecules that regulate functions of PF-PC synapses; GluRδ2-null mice show ataxia and loss of long-term depression (LTD), a putative cellular model of cerebellar information storage. In addition, GluRδ2-null mice display half the number of PF-PC synapses. Despite their importance, the mechanisms by which GluRδ2 participates in PF-PC synaptic functions have been elusive, mainly because it is not activated by glutamate analogs. To gain insight into GluRδ2's mechanisms, we developed a Sindbis virus vector that express a wild-type GluRδ2. By introducing this virus vector into GluRδ2-null cerebellum in vivo, we could rescue several abnormal phenotypes, such as impaired LTD and enhanced paired-pulse facilitation of excitatory postsynaptic currents at PF-PCs synapses. This virus-based method has several advantages over non-viral conventional gene expression methods and transgenic techniques. Based on studies expressing a mutant GluRδ2, in which several functional domains were mutated, GluRδ2's mechanisms in controlling PF-PC synapses will be discussed.

S167 (3S-40F3)

Rapid reorganization of synaptic input in visual cortex by serine proteases

Mataga, Nobuko; Hensch, Takao (*Neuronal Circuit Development, Brain Science Institute, RIKEN*)

Loss of responsiveness to an eye deprived of vision is mediated by rapid functional disconnection followed by slow axonal rearrangement within kitten and rodent visual cortex (Antonini and Stryker, 1993; Antonini et al., 1999). We hypothesized that extracellular serine proteases (tissue-type plasminogen activator (tPA)-plasmin system) act to degrade cell-adhesion molecules or extracellular matrix proteins to permit earlier synaptic remodeling dependent on visual experience. Indeed, functional ocular dominance (OD) plasticity was reversibly impaired in tPA knockout mice (KO) (Mataga et al, PNAS, 2002). Moreover, we identified a robust anatomical change in layer 2/3 of mouse visual cortex by brief monocular deprivation (MD) (Mataga et. al., Neuron, 2004). Protrusions on the apical dendrite of pyramidal cells increased steadily in number with postnatal age, but were rapidly and transiently lost after MD only during the physiological critical period (CP). Targeted disruption of tPA or its upstream regulation by glutamic acid decarboxylase (GAD65) reversibly prevented MD-induced spine pruning in vivo. The tPA-plasmin system may, thus, rapidly couple physiological perturbation of sensory input to early structural rearrangement of synaptic input.

S168 (3S-40F4)

Orientation map reorganization induced by persistent exposure to a dynamic or stationary single orientation

Tanaka, Shigeru; Tani, Toshiki; Ribot, Jérôme (*RIKEN BASI, Wako, Saitama, Japan*)

To examine the plastic changes in orientation selectivity of developing visual cortex, we manipulated visual experience of kittens for 1-7 weeks under a freely moving condition, mounting cylindrical-lens-fitted goggles to present uni-axially elongated images of their environments, or spherical-lens-fitted goggles to present a stationary black-and-white oriented grating. We performed intrinsic signal optical imaging from those animals to reconstruct cortical representation of orientation preferences. For exposure to either a dynamic or stationary single orientation through the goggles, the extreme over-representation of exposed orientation was found in the visual cortex immediately after 1-2 weeks of continuous goggle rearing. For kittens exposed to a dynamic single orientation for a longer time, reorganized orientation maps were preserved although the degree of over-representation was somehow reduced. However, for kittens persistently exposed to a stationary oriented grating for long time, the over-representation effect almost disappeared or in some cases the orientation maps paradoxically exhibited the over-representation of the orientation orthogonal to the exposed orientation. It is suggested that the consolidation of reorganized orientation maps requires the experience of moving visual stimuli with behavioral relevance.

SYMPOSIA
Social brain and physiology: Neural mechanism for predictive environmental cognition

S169 (3S-41G1)
Brain activity during Social Interaction between Japanese monkeys

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We are surrounded by rich environment which include varieties of social properties. Our brains are handling such information for choosing and performing behaviors. Social meaning of each behavior is always changing time to time so that the brain has to take much effort to extract social parameters mined in the environment. Although we are highly social animal, we know very little about how the brain is manipulating such social parameters. There is almost no neurophysiological study focused on social brain functions. Therefore we tried to see how social parameters are affecting on brain function at single neuronal level by using Japanese monkeys known for their complex social behaviors.

Two Japanese Macaques were used. We implanted multiple electrodes chronically (24 and 30 for each monkey) in prefrontal and parietal cortices. During the experiment, we altered monkeys' relative spatial positions so as to manipulate their social spaces. In some cases, their peri-personal spaces were made either overlapped or separated. Their behaviors were monitored by using motion capture technique and video. These techniques allowed monkeys relatively free movement during recording.

We found that monkeys altered their behaviors dramatically simply by changing social structure between them. While monkeys were showing social interaction, prefrontal and parietal neurons showed varieties of response patterns depending on social context. Prefrontal neurons tended to respond earlier and parietal responses followed, suggesting two areas are managing different aspects of social functions in the brain.

S170 (3S-41G2)
Self and other representation in the parietal cortex based on sense of body

Murata, Akira (*Dept.Physiol, Sch. Med., Kinki Univ. Osaka-sayama, Japan*)

Recently, it is claimed that automatic simulation of inner state in other's brain by observation of action is very important neural mechanism for social interaction. The idea is on the lines of simulation theory. Mirror neurons, which were found in the ventral premotor cortex and inferior parietal cortex of the monkey, are considered to be neuronal correlates of this simulating mechanism. On the other hand, self-other representation in the brain should be necessary in social interaction. Recent imaging experiments suggest that motor control system may be involved in recognition of agency of action or ownership of one's own body parts. We speculate the mirror neurons in the parietal cortex of the monkey would be also involved in monitoring own body action. Actually, we found that some neurons related to the hand manipulation task in the parietal cortex responded to the movie of own hand action. These neurons were also active during observation of other's hand action. Further, we also found that this visual response was less active in the delayed feedback than in the real time. These results suggest that matching between efference copy and sensory feedback (visual and somatosensory) may occur in the parietal cortex. This may be neural bases of self-other distinction. Further, I will also present visual-somatosensory bimodal neurons have visual receptive field on the corresponding other's body parts. I would like to discuss functional property of inferior parietal cortex for the self-other distinction and matching.

S171 (3S-41G3)
Ethological approach to understand the behavioral transmissions in social contexts.

Kikusui, Takefumi (*Grad. Sch. Agr. Univ. Tokyo, Tokyo, Japan*)

Social stimuli during the neonatal and juvenile periods are known to affect various aspects of physiological and behavioral development in rodents. For example, maternal behavior and partner preference in the adulthood are determined by the social environments in which the animals are reared. These phenomena are so called "non-genomic transmissions". In this study, we investigated the long-lasting influences of earlier weaning on adulthood behavioral traits in rats and mice. Subjects were weaned from their mothers one week earlier than the normal weaning period. To assess anxiety levels at ages 8 weeks and 22 weeks, both early weaned and control mice pups were subjected to several behavioral tests, and it was found that the early-weaned animals had a sustained increase in anxiety levels compared to the control groups. Social behavioral tests performed in other sets of mice revealed that early weaned pups engaged in more fights under several conditions, including co-housing them with other mice, although neither isolation-induced aggression nor territorial aggression differed from normally weaned mice. In addition, early weaned females repressed maternal behavior. Similar results were obtained in rats as well. Concurrently, neurochemicals that are responsible for the behavioral and endocrine responses to stress were affected by the early weaning manipulations. These results suggest that the absence of mother-pup interactions during the last several days of pre-weaning period may lead to a persistent increase in anxiety and aggression during adulthood in rodents.

S172 (3S-41G4)

Neuropsychological study for abnormal social interaction

Kato, Motoichiro (*Department of Neuropsychiatry, Keio University School of Medcine, Tokyo, Japan*)

Interpersonal activity is essential in making humans the uniquely social beings that we are. The underlying neural grounds of the social interaction are the fusiform region, amygdala, superior temporal sulcus, orbitofrontal and dorsolateral prefrontal cortex, which are of great interest to neuroscientists. To anticipate possible futures and coordinate thought and action for achieving desired outcomes, the prefrontal lobes play a pivotal role. The dorsolateral prefrontal cortex is essential to guide the behavior by thought and language, while the orbital and ventromedial regions have been considered to be the neural correlates for affective evaluation of the consequences of our action. The brain region that is implicated in gaze processing, STS, has repeatedly been activated when viewing gaze in the normal brain. We have presented a case, MJ, in a recent report, with a circumscribed lesion in the right superior temporal gyrus, due to a cerebrovascular accident, who manifested a puzzling difficulty in obtaining eye-contact. As the STG comprises a part of the STS, we investigated her ability in processing gaze. Indeed, MJ demonstrated a unique impairment in discriminating gaze direction, which is the first neuropsychological evidence that establishes STS as a gaze processor, so often implicated in animals and human neuroimaging studies.

S173 (3S-42H1)

Comprehensive behavioral analysis of Ca^{2+}/calmodulin-dependent protein kinase 4 knockout mouce

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Ca^{2+}/calmodulin-dependent protein kinase 4(CaMK4)is a protein kinase that activates the transcription factor, cAMP response- element binding protein (CREB). CaMK4 has been hypothesized to play a significant role in synaptic plasticity and in learning and memory. However, functions of CaMK4 in a variety of behaviors, e.g., motor function, nociception, fear, anxiety, depression, learning and so on, have not yet been fully elucidated. To gain more insight into behavioral significance of CaMK4, we subjected CaMK4 -/- mice to a battery of behavioral tests, including neurological screen, light/dark transition, open field, elevated plus maze, social interaction, rotarod, hot plate, prepulse inhibition, Porsolt forced swim, 8-armed radial maze, Barnes maze, fear conditioning, latent inhibition, and passive avoidance tests.

CaMK4 -/- mice exhibited increased social interaction in home cage. They did not display any deficit in spatial reference memory and working memory tests, but had mild performance deficit in fear conditioning tests. These results indicated selective and specific involvement of CaMK4 in regulating emotional behaviors.

SYMPOSIA
What we learn about the physiological roles of Ca^{2+}/ calmodulin-dependent protein kinases (CaMKs) from genetically engineered mice

S174 (3S-42H2)

Assessments of depression and the sensitivity to antidepressants in calcium/ calmodulin-dependent protein kinase IV-knockout mice

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Calcium/calmodulin-dependent protein kinase IV (CaMKIV) is expressed abundantly in the nuclei of neurons and thought to regulate gene expressions mediated by the transcriptional factors such as CREB. We have been characterized CaMKIV comparing with other subtypes CaMKI and CaMKII in the hippocampal neurons. The basal CaMKIV activity is kept low by constant inactivation by associated protein phosphatase 2A, and the activation is transient because calcineurin inactivate CaMKIV after the neuronal stimuli, while the activities of CaMKI and CaMKII are sustained. In other words, CaMKIV is hard to be switched on and is easy to be switched off. Based on this fact, we thought the functions of CaMKIV are reflected in the animals after the chronic stimulation rather than the acute one. Recently, we found that chronic treatments of the rats with antidepressants increased CaMKIV activity and CREB phosphorylation in the prefrontal cortex and the hippocampus, suggesting the importance of CaMKIV in the effects of antidepressants. These results led us to perform the behavioral assessments of anxiety, depression and the sensitivity to antidepressants in CaMKIV-knockout mice by some experimental paradigms including the forced swim test, the tail suspension test and the novelty-suppressed feeding test. From the results of these experiments, it was suggested that CaMKIV is involved in some of the depression-related behaviors and the sensitivities to antidepressants.

S175 (3S-42H3)

Comprehensive brain-behavior phenotyping of Ca^{2+}/calmodulin-dependent protein kinase IIα heterozygous knockout mice

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Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is a ubiquitous serine/threonine protein kinase that is abundant in the brain as a major constituent of the postsynaptic density and critically involved in synaptic plasticity, learning and memory. Several behavioral abnormalities of CaMKIIα mutant mice were reported, but systematic assessments of CaMKIIα mutant mice have not been well conducted. To analyze the behavioral effects of CaMKIIα deficiency, we subjected CaMKIIα heterozygous knockout mice to a comprehensive behavioral test battery. The mutant mice showed increased locomotor activity, markedly decreased anxiety, decreased depression-related behavior, increased offensiveness, selective and severe spatial working memory deficit, and dramatic periodic change of locomotor activity in home cage. To identify the mechanism underlying the behavioral abnormalities of CaMKIIα mutant mice, gene expression analysis and biochemical analysis of the brain of the mutant mice were conducted. The potential involvement of CaMKIIα in pathogenesis/pathophysiology of psychiatric disorders, such as schizophrenia, bipolar disorder, and personality disorders, will be discussed.

S176 (3S-42H4)

Increase in anxiety and aggression in transgenic mice overexpressing αCaMKII in forebrain.

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Previous studies have shown that αCalcium/Calmodulin dependent protein kinase II(αCaMKII) plays important roles in not only learning and memory but also aggressive and fear response in mice. To further understand roles of αCaMKII in brain function, we have generated transgenic mice overexpressing αCaMKII in forebrain. Interestingly, these mutant mice showed increase in anxiety in open field and elevated zero maze tests and increase in offensive aggression in resident-intruder test. Increase in anxiety observed in these transgenic mice suggests that expression level of CaMKII positively correlates with expression of anxiety related behavior. We next examined effects of administration of selective serotonin reuptake inhibitor (SSRI) on anxiety related behavior observed in these mutant mice. Treatment of these transgenic mice with SSRI suppressed anxiety-related behavior in both tests. These results raise the possibility that these mutant mice is a mouse model of anxiety disorder, that allows to develop therapeutic drugs . In addition, we have examined the expression profile of these mutant mice with or without the treatment of SSRI and tried to find out the anxiety-related genes.

S177 (3S-42H5)

The role of Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) in neuronal activity revealed by inactivated CaMKIIα knock-in mouse

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Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) is one of the most abundant protein kinase in the central nervous system and a key mediator of intracellular Ca^{2+} in response to various stimuli. CaMKII is involved in many neuronal functions including the regulation of neuronal activity. Previous studies reported episodes of epileptic seizure in CaMKIIα knock-out mice and manifestation of epileptic discharges in cultured neurons treated with anti-sense oligonucleotide to CaMKIIα. However, which protein function of CaMKII, i.e., protein kinase activity, calmodulin-binding capacity or multimeric structure interacting with other proteins, is responsible for stabilizing neuronal activity remains to be elucidated yet. To clarify specifically the role of protein kinase activity of CaMKII, we engineered knock-in mice with the inactivated α subunit of CaMKII by replacing Lys-42 with Arg-42. CaMKIIα protein level was unchanged, but CaMKII activity was specifically decreased in these mutant mice. Spontaneous death rate was higher, and pentylenetetrazole injection resulted in higher seizure-induced mortality in homozygous mutants. Spontaneous seizure was sporadically observed in homozygous and heterozygous mutants, but rarely in wild type controls. Cytochrome oxidase staining revealed decreased neuronal activity in nucleus accumbens in homozygous mutants. These results indicate that protein kinase activity of CaMKII, i.e., protein phosphorylation by CaMKII, is important for maintaining basic and normal neuronal activity *in vivo*.

Oral Presentations

ORAL
Cellular & molecular physiology

O1 (1O-01C1)
UBIQUITIN SIGNALS IN REGULATION OF DNA REPAIR

Dikic, Ivan; Hoeller, Daniela; Bienko, Magda; Crosetto, Nicola; Zapart, Gregorz; Haglund, Kaisa (*Institute of Biochemistry II, Goethe University, Frankfurt, Federal Republic of Germany*)

The attachment of a ubiquitin (Ub) to a substrate serves as an important regulatory modification implicated in receptor endocytosis, virus budding, gene transcription, DNA repair and replication, etc. The discovery of Ub-binding domains (UBDs) has indicated how Ub can regulate such distinct cellular functions. We have recently cloned two novel Ub-binding domains named UBM (Ub binding motif) and UBZ (Ub binding Zn finger), which are evolutionarily conserved in Y-family translesion polymerases (pols). These domains are required for binding of pols to Ub, their accumulation in replication factories and interaction with monoubiquitinated PCNA. In addition, Ub-binding domains of Y-family polymerases play essential roles for in vivo translesion synthesis, which is the major pathway by which mammalian cells replicate across DNA lesions. Interestingly, novel Ub-binding domains are found in several other proteins implicated in regulation of DNA repair and replication.In addition to binding Ub, several UBDs promote monoubiquitination of host proteins. Biochemical, biophysical and mathematical evidence support the concept whereby monoubiquitination of the Ub binding proteins facilitates intramolecular interactions with the UBDs, thus preventing them from binding in trans to ubiquitinated targets. Monoubiquitination of Ub-binding proteins thus represents a regulatory mechanism that inhibits their capacity to bind to and control functions of ubiquitinated targets in vivo.

O2 (1O-01C2)
Stimulation of the gastric H,K-ATPase activity by CLC-5 Cl^- channel

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In the stomach, protons are actively secreted by the gastric H,K-ATPase, but it is unclear what molecule contributes to the secretion of Cl^-. We have previously shown that CLC-2 may not be responsible for the Cl^- transport in the gastric acid secretion. However, we found that CLC-5 was expressed in the gastric parietal cells. Here, we examined the interaction between CLC-5 and the gastric H,K-ATPase. We constructed a tetracycline-regulated expression system of CLC-5 in the HEK293 cells stably expressing the gastric H,K-ATPase. The H,K-ATPase activity and the $^{86}Rb^+$ transporting activity were examined by using SCH 28080, a K^+-competitive inhibitor of the H,K-ATPase. Expression of CLC-5 in the HEK293 cells significantly increased the H,K-ATPase activity by $24.1 \pm 8.9\%$ ($n = 6$) and $^{86}Rb^+$ transporting activity by $28.2 \pm 5.9\%$ ($n = 5$). The expression level of H,K-ATPase in the plasma membrane was not affected by CLC-5 in the HEK293 cells. Furthermore, we found that expression of CLC-5 significantly up-regulated the phosphorylation level of H,K-ATPase by $48 \pm 18\%$ ($n = 4$). These results suggest that CLC-5 may be a modulatory subunit of the gastric H,K-ATPase.

O3 (1O-01C3)
Change in intracellular chloride concentration caused by regulatory volume decrease is the primary hypotonic signal in renal epithelial A6 cells

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Our recent study indicates that hypotonicity-induced decreases in intracellular Cl^- concentration ($[Cl^-]_i$) could act as a signal to regulate Na^+ reabsorption through changes in αENaC mRNA expression in renal epithelial A6 cells. This result suggests that the change of $[Cl^-]_i$ is one of the important signals to cell function. However, currently reported techniques for the measurement of $[Cl^-]_i$ by using halide-specific fluorescent dyes lack sufficient sensitivity and accuracy. One reason for problems in the use of these dyes for measurement of $[Cl^-]_i$ during hypotonicity-induced regulatory volume decrease (RVD) is a change of intracellular dye concentration during RVD, since a fluorescent intensity of these indicators depends not only on $[Cl^-]_i$ but also on intracellular concentration of dyes. In this study, we have developed a new method for measuring $[Cl^-]_i$ by using a cell analyzer Quanta. This flow cytometer can simultaneously measure the exact cell volume by Coulter principle and the fluorescent intensity. The concentration of Cl^- in A6 cells diminished during RVD by 72% (from 47.3 mM to 13.3 mM). This reduction of $[Cl^-]_i$ was blocked by inhibition of RVD with quinine (K^+ channel blocker) or NPPB (Cl^- channel blocker). These results suggest that a change in external osmolality is converted into the change in $[Cl^-]_i$, and that the change of $[Cl^-]_i$ is the primary hypotonic signal in A6 cells. This work was supported by Grants-in-Aid from JSPS (17390057, 17590191 and 17790154).

O4 (1O-01C4)

P2Y receptor-induced increase in sensitivity of adipogenic hormones to preadipocytes

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The effect of extracellular ATP on adipogenesis was investigated using 3T3-L1 cell line. Incubation of cells with ATP (1-100 μM) for 5 min induced membrane ruffling and migration (chemokinesis). In this cell line, growth arrest is required before initiation of differentiation, and growth-arrested post-confluent cells can be converted to adipocytes by the presence of the adipogenic hormones dexamethasone, 3-isobutyl-1-methylxanthine and insulin. On the other hand, those hormones alone do not trigger differentiation in proliferating cells. ATP did not induce differentiation when applied alone to either proliferating or post-confluent cells. In contrast, proliferating cells (density<50%) preincubated with ATP for 5 min and subsequently given the adipogenic hormones in the continued presence of ATP underwent adipocyte differentiation mediated through phospholipase C-coupled P2Y receptors. These adipocytes were found to show very similar characteristics, including morphology and intracellular triglyceride accumulation, compared with adipocytes differentiated from post-confluent preadipocytes with those adipogenic hormones. When proliferating cells were preincubated with ATP prior to the addition of the adipogenic hormones, gene expression of adipose protein 2 was markedly increased in 6 days, while the expression level stayed very low without ATP pretreatment. These results suggest that extracellular ATP renders preadipocytes responsive to the adipogenic hormones during the growing phase.

O5 (1O-01C6)

The screening of novel inhibitors for Ca^{2+}-independent abnormal contraction of vascular smooth muscle which have similar inhibitory effects to eicosapentaenoic acid

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We previously identified sphingosylphosphorylcholine (SPC) and Fyn as upstream signal molecules of Rho-kinase-mediated Ca^{2+}-independent abnormal contraction of vascular smooth muscle (VSM) and found that eicosapentaenoic acid (EPA) can selectively inhibit such abnormal events without affecting Ca^{2+}-dependent normal VSM contraction by blocking the translocation of Fyn to plasma membrane. Moreover, we reported that EPA was clinically and highly effective in preventing vasospasm after subarachnoid hemorrhage. However, EPA is limited to oral administration and thus unsuitable for clinically serious patients unable to ingest orally. We therefore screened novel compounds which could inhibit Ca^{2+}-independent abnormal VSM contraction and could substitute for EPA. Tension study of VSM showed that several compounds inhibited SPC-induced abnormal VSM contraction, which was comparable to the effects of EPA. These results suggest that these newly found compounds would be the candidates for novel therapeutic drugs for vasospasm which could substitute for EPA.

O6 (1O-01C7)

The important role of Fyn tyrosine kinase on the Ca^{2+}-independent contraction of vascular smooth muscle

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Rho-kinase (ROK) is being regarded as the critical signaling molecule of the Ca^{2+}-independent contraction of vascular smooth muscle (VSM). We recently identified that the sphingosylphosphorylcholine (SPC) induced Ca^{2+}-independent contraction of VSM by activating ROK and that SPC activated ROK via activating Src family tyrosine kinase (Src-TK). Since VSM expresses Fyn and c-Src among Src-TK, we analysed which Src-TK is involved in this SPC/ROK-mediated Ca^{2+} sensitization. The inhibitors of Src-TK (PP1 and PP2) abolished all the reactions of Ca^{2+}-independent contraction, activation of Src-TK and ROK, and tyrosine phosphorylation of p60 protein induced by SPC. SPC induced the translocation of Fyn from cytosol to the plasma membrane of VSM cells, but not that of c-Src. In order to examine directly the ability of Fyn to induce Ca^{2+}-independent contraction, we made recombinant Fyn proteins using a baculovirus system. In beta-escin permeabilized VSM, constitutively active Fyn induced Ca^{2+}-independent contraction which was inhibited by Y27632, while dominant negative Fyn inhibited the contraction induced by U46619+GTP. These findings suggest that Fyn tyrosine kinase plays a pivotal role in the SPC-induced and ROK-mediated Ca^{2+}-independent contraction.

O7 (1O-01C8)

Glucagon-Like Peptide 1 Activates Protein Kinase C in a Ca^{2+}-Dependent Manner in Insulin-Secreting Cells

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Introduction : GLP-1, a cAMP mobilizing agonist, is an insulinotropic peptide released from the intestinal L cell in response to a meal. However, the underlying mechanisms of the stimulatory effect of GLP-1 on insulin secretion remain fully elucidated. Aim: The present study was conducted to examine whether GLP-1 can activate PKCalpah and PK-Cepsilon in INS-1 cells at a substimulatory concentration of glucose. Methods: We employed either GFP or DsRed-tagged proteins related to PKC signaling pathway using epifluorescence microscopy and total internal reflection fluorescence microscopy. Results: We first showed that GLP-1 translocated endogenous PKCalpah and PKCepsilon from the cytosol to the plasma membrane. Then we assessed the phosphorylation state of the PKC substrate, myristoylated alanine-rich C kinase substrate (MARCKS), as a maker of PKC activation. GLP-1 translocated GFP-tagged MARCKS from the plasma membrane to the cytosol and the GLP-1-evoked translocation of MARCKS-GFP was blocked by PKC inhibitors. The above observations were verified in three different ways using live cell imaging technique.In addition to these results, PKC inhibitors reduced forskolin-induced insulin secretion in INS-1 cells and rat islet beta cells. Conclusion: GLP-1 can activate PKCalpah and PKCepsilon, and the GLP-1-activated PKCs may contribute considerably to insulin secretion at a substimulatory concentration of glucose.

O8 (2O-08E4)

Ghrelin attenuates glucose-induced insulin release via inhibition of cyclic AMP productions in rat islet β-cells

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Ghrelin, isolated from the human and rat stomach, is the endogenous ligand for the growth hormone secretagogue receptor (GHS-R). We have reported that GHS-R was expressed in rat pancreatic islets and that ghrelin suppressed glucose-induced insulin release via activation of voltage-dependent delayed rectifier K^+ channels and attenuation of glucose-induced action potentials, leading to suppression of glucose-induced Ca^{2+} signaling in β-cells. In this study, we aimed to determine the involvement of cyclic AMP productions, another major signalling pathway for insulin release, in the ghrelin-induced suppression of insulin release. Both GHS-R blockade and anti-ghrelin antiserum markedly enhanced 8.3 mM glucose-induced insulin release in rat perfused pancreas and isolated islets. GHS-R blockade and anti-ghrelin antiserum also enhanced 8.3 mM glucose-induced cyclic AMP productions in rat islets. Conversely, exogenous ghrelin (10 nM) suppressed insulin release and cyclic AMP productions. In the presence of either dibtyryl cyclic AMP or adenylate cyclase inhibitor MDL12330A, ghrelin failed to attenuate glucose-induced insulin release. This study suggests that ghrelin suppresses glucose-induced cyclic AMP production as well as cytosolic Ca^{2+} response. These abilities of ghrelin to impede cyclic AMP and Ca^{2+} signaling routes at least partly account for the inhibition of glucose-induced insulin release.

O9 (2O-11H6)

Myosin light chain kinase stimulates smooth muscle ATPase activity with its non-kinase activity by binding to the myosin heads: a study with protein-engineering

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Myosin light chain kinase (MLCK) is a multifunctional regulatory protein of smooth muscle contraction. The well-established mode for its regulation is to phosphorylate the 20kDa myosin light chain (MLC20) to activate myosin ATPase activity. MLCK exhibits myosin-binding activity in addition to this kinase activity. The myosin-binding activity also stimulates myosin ATPase activity without phosphorylating MLC20. In order to study this non-kinase activity of MLCK and its active site. We engineered two MLCK fragments, one contained the myosin-binding domain but devoid of a catalytic domain and another further deleted a calmodulin (CaM) domain. The former fragment stimulated myosin ATPase activity by Vmax= 5.53 ± 0.63- fold with Km = 4.22 ± 0.586μM (n = 4). Similar stimulation figures were obtained by measuring the ATPase activity of HMM and S1. We failed to observe the stimulation with the latter fragment. Similar stimulating effect were obtained by measuring the ATPase activity of phosphorylated myosin, HMM and S1. Binding of the fragment to both HMM and S1 was also verified, indicating that the fragment exerts stimulation through the myosin heads. We conclude that the non-kinase stimulation of MLCK are involved in the mode for activation of myosin. The CaM domain is one of the active site for non-kinase activity of MLCK fragment.

O10 (2O-11H7)

Development of cardiac fibrosis in sphingosine kinase 1 transgenic mice

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Sphingosine-1-phosphate (S1P) is a plasma lysophospholipid with diverse activities, and is released in a large amount from activated platelets. Our laboratory as well as others have identified the existence of the G protein-coupled S1P receptor family, which include ubiquitously expressed S1P1, AGR16/Edg5/S1P2 and S1P3. In an attempt to get more insight into roles of S1P in vivo, we have generated transgenic (TG) mice that overexpress a major S1P synthetic enzyme sphingosine kinase 1 (SPHK1) in diverse tissues, with up to several ten fold increases in the SPHK1 activity. Although previous reports suggested the involvement of SPHK1 in cell proliferation and transformation, the TG mice show normal growth and no obvious increase in spontaneous malignancy. Importantly, TG mice with a high but not a low level of SPHK1 expression in the heart show age-dependent, progressive cardiac fibrosis with development of dilated cardiomyopathy in a limited population. Transgenic heart tissues show elevated activities in both Rac1 and RhoA small molecular weight G proteins and enhanced superoxide generation in responses to phorbol ester. Treatment of TG mice with an HMG-CoA reductase inhibitor or an antioxidant N-2-mercaptopropyonylglycine, but not an angiotensin II type 1 receptor blocker, resulted in alleviation of cardiac fibrosis. These results provide evidence for a pathophysiological role of SPHK1 and probably S1P.

O11 (2O-11H8)

Cellular mechanism of experimental autoimmune uveoretinitis

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[BACKGROUND] Experimental autoimmune uveoretinitis (EAU) is an organ-specific autoimmune disease that is induced in animals sensitive to retinal antigens. The recruitment of leukocytes is crucial for ocular inflammation in EAU, whereas target cells and effector cells have not yet been clearly characterized.[PURPOSE] Isolation and characterization of target cells in EAU[METHODS] EAU was induced in B10 mice by immunization with 50 μg human interphotoreceptor retinoid binding protein peptide 161-180 in emulsion with CFA supplemented with 3.5 mg/ml *M. tuberculosis*(1:1,vol/vol). Disease severity was assessed clinically by funduscopic examination. Retinal cells and ocular infiltrating cells were obtained by enzyme digestion from the eyecups of normal and EAU mice,respectively, and were separated into different types of cells by Percoll density gradient centrifugation. To assess which types of cells were target and effector cells in EAU, we determined cytotoxic activity of infiltrating cells against retinal cells by ^{51}Cr release assay.[RESULTS] Two kinds of retinal cells were isolated as ^{51}Cr incorporationg cells. They appeared to be retinal pigment epithelial (RPE) cells and monocytic cells judging from their morphological features under an electron microscope. The identification of effector cells responsible for EAU is under investigation in our laboratory.[CONCLUSIONS] We isolated RPE cells and monocytic cells as monodispersed growing cells or a candidate for target cells in EAU.

O12 (3O-14E1)

Localization of the short-chain fatty acid receptor, GPR43, in the rat intestine

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Short-chain fatty acids (SCFAs), including acetate, propionate and butyrate, are the products of bacterial fermentation from indigestible dietary fibers in the large intestine. SCFAs have been known to play a variety of physiological and pathophysiological roles for intestine. We have recently reported the mechanism of SCFA-induced responses to the motility of the rat distal colon (Ono et al. Jpn J Physiol 65: 69-76, 2004; Mitsui et al. Neurogastroenterol Motil 17: 585-594, 2005). These results suggest that SCFAs are sensed at mucosa and modulate the colonic motility through the enteric nervous system as a neural reflex. However, the mechanism of the sense of SCFA is currently unknown. In 2003, two orphan G protein-coupled receptors, GPR41 and GPR43, have been identified as the SCFA receptors (Brown et al. J Biol Chem 278: 11312-11319, 2003; Le Poul et al. J Biol Chem 278: 25481-25489, 2003). The present study shows the localization of GPR43 in the rat intestine by RT-PCR, Western blotting and immunohistochemistry. The results of the present study indicate that GPR43 is expressed by enteroendocrine cells and mucosal mast cells in the rat intestine.

O13 (3O-14E2)

Catecholamine-induced apoptosis in cultured striatal neurons in 2 weeks old mice.

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[Background] cAMP is produced from ATP by adenylylcyclase. Despite various G-protein coupled receptor (GPCR) signals share cAMP as second messenger, physiologicaleffects of stimulation may not be identical amongreceptors. In addition, the role of cAMP in developing apoptosis is not well understood in neuronal cells, especially during development. [Method] We first examinedthe effects of stimulating two GPCR on neuronal cell apoptosis using neonatal striatal neurons. Then, weinvestigated developmental changes of such effects in striatal neurons obtained from 2 week old mice by a newly developed culture technique in our laboratory. Cultured striatal cells were incubated with isoproterenol or dopamine, then, apoptosis was evaluated. [Result] In neonatal neurons, neither isoproterenol nor dopamine stimulation induced apoptosis. In contrast, in neuronsfrom 2 week old mice, TUNEL staining and DNA fragmentation ELISA revealed that dopaminergic receptor stimulation significantly increased the number of apoptotic cells.Western blot analysis revealed that only isoproterenol stimulation increased phosphorylation of Akt and MAP kinase. In contrast, both isoproterenol and dopamine stimulation increased cAMP. Accordingly, only dopamine stimulation induced cellular apoptosis while isoproterenoldid not, presumably due to cytoprotective effect through Akt and/or MAP kinase activation. [Conclusion] The role of cAMP in developing neuronal apoptosis differs among GPCRas well as in developmental stages.

O14 (3O-14E3)

Chloride ions play important roles on the cell death caused by the plasma membrane permeability increase.

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Too much increase of the plasma membrane permeability causes necrotic death in the many types of cells. Detail mechanisms of this type of cell death are still unclear. We use amphotericin B (amB) as the membrane pore formation agent and investigate the ionic dependency on the cell death caused by amB. First we apply propidium iodede (PI) to the HeLa cells together with 10 μg/ml amB and observe the staining nucleus by PI using the fluoresent microscope. 2 hours after apllication of amB, we could observe the PI signal in the nucleus, indicating that the large pores were formed after the application of amB. These pores should be larger than the pores formed by amB because PI did not enter into the cells just after amB application. Cl^- replacement by gluconate or Cl^- channel blocker, DIDS (0.5 mM) inhibited the staining of nulueus after amB application. Next we stained the lysosomes in the HeLa cells by FL-labelled pepstatin A. In the control condition, fluorescent dots were observed around the nucleus and such dots gradually disappered after amB application. Low Cl^- condition or DIDS application inhibited the disappearance of the fluorescent dots by amB. These results suggest that the Cl^- ions can enter into the cell after amB application and those ions may couse the disruption of lysosome, which enhances the membrane permeability increase by the attacking membrane proteins by the lysosomal enzymes.

O15 (3O-14E4)

Role of plasma membrane Ca^{2+}-ATPase (PMCA) in contraction-relaxation processes of the bladder using PMCA gene manipulation

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Role of PMCA in smooth muscle contractility was investigated using the bladder isolated from PMCA gene manipulated mice: PMCA4 null mutant (*Pmca4*$^{-/-}$) and PMCA1 and PMCA4 double gene targeted (*Pmca1*$^{+/-}$*4*$^{-/-}$) mice. Western blot shows the loss of PMCA4, a major isoform, but not PMCA1, sarco-endoplasmic reticulum ATPase (SERCA) and Na^+/Ca^{2+} exchanger (NCX), in the muscle layer preparation of *Pmca4*$^{-/-}$ and *Pmca1*$^{+/-}$*4*$^{-/-}$. Half-times of contraction and relaxation upon treatment with 80 mM KCl were determined. Surprisingly, half-times of contraction in *Pmca4*$^{-/-}$ and *Pmca1*$^{+/-}$*4*$^{-/-}$ muscles tended to be prolonged, when compared with that in WT muscle. Relaxation half-times were also prolonged in the gene manipulated muscles, as expected. On the other hand, inhibition of SERCA or NCX marginally shortened the contraction half-time and prolonged the relaxation half-time in muscles of all tested genotypes. Using relaxation half times, the contribution of PMCA to relaxation was calculated to be 25%, SERCA 20% and NCX 70%. PMCA and SERCA appeared to function additively, but the function of NCX might overlap with those of other components. FuraPE3 signal shows that the basal level of $[Ca^{2+}]i$ slightly increased in *Pmca1*$^{+/-}$*4*$^{-/-}$ muscle. In summary, the gene manipulation of PMCA indicates that PMCA, in addition to SERCA and NCX plays a role in both excitation-contraction coupling and Ca^{2+}-extrusion-relaxation relationship, i.e., Ca^{2+} homeostasis, of the bladder smooth muscle.

O16 (3O-14E5)

Study of the role of Fyn in the sphingosylphosphorylcholine-induced formation of stress fibers and filopodia-like protrusions in NIH3T3 fibroblasts

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We previously showed that Fyn, a member of Src family tyrosine kinase (Src-TK), was involved in the activation of Rho-kinase (ROK) to develop Ca^{2+} sensitization of vascular smooth muscle contraction induced by sphingosylphosphorylcholine (SPC). Recently we found that SPC can stimulate the formation of stress fibers and filopodia-like protrusions in NIH3T3 fibroblasts through Src-TK/ROK-dependent and independent pathway, respectively. Then, we further investigated the role of Fyn in the SPC-induced formation of stress fibers and filopodia-like protrusions with RNA interference method. Three different Fyn siRNAs were designed and transfected into NIH3T3 cells using Lipofectamine 2000. As control, a non-silencing siRNA and a positive MAPK1 control siRNA were transfected in parallel. The non-silencing AF 488-labeled siRNA was used to monitor transfection efficiency. Our results showed that transfection efficiency was high above 90% and the down-regulation of Fyn expression was confirmed in western blot with concentration-dependency and incubation time-dependency. SPC-induced stress fiber formation was partially inhibited by Fyn siRNAs, but not by other control siRNAs. The formation of filopodia-like protrusions induced by SPC was not affected by Fyn siRNAs. These findings suggest that Fyn plays a role in SPC-induced stress fiber formation, but not in the formation of filopodia-like protrusions in NIH3T3 cells.

O17 (3O-13D1)

Effects of osmolarity change on fluid secretion by the perfused submandibular glands in normal and low-AQP5 rats

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Osmolarity changes created by sucrose during the perfusion of isolated rat submandibular glands (SMG) *in vitro* alter secretion rates much more than predicted by the osmotic theory of fluid production. However, these are in accord with a theory involving AQP5 feedback control of paracellular fluid transfer (Hill & Shachar-Hill 2002). The changes in transport rate can be predicted with parameters determined earlier for this gland (Murakami et al. 2001) and a model of the SMG system is presented. Experiments were performed with SMG from genetically selected rats that have very low levels of AQP5 as determined by Western blotting (Murdiastuti, K. et al. 2002). The fluid secretion rates after osmolarity changes were those expected for the osmotic theory. We suggest that control of paracellular flow has been lost in these low AQP5 rats which have reverted to osmotic fluid production. Retrograde injection of Hg ions into the duct partially inhibited AQP5, leading to a concentration-dependent reduction in flow rates. However, reduction of fluid secretion after osmolarity changes was still close to that of normal rats. The results suggest the involvement of a feedback loop including AQP5 and paracellular fluid transport.

ORAL
Transport across cell membrane

O18 (3O-13D2)

Ethanol alters anion composition of the fluid secreted by guinea-pig pancreatic ducts

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We reported that 1 mM of ethanol (relevant to blood level after social drinking) potentiated secretin-stimulated fluid secretion in interlobular ducts isolated from guinea-pig pancreas. In this study, we examined anion composition of the secreted fluid during the hypersecretion. Isolated ducts were cultured overnight, during which time both ends of the ducts sealed. The lumen was punctured and injected with solutions containing BCECF-dextran or ABQ-detxtran (plus Cl-NERF-dextran as ratiometric reference). Luminal pH (pH_L) and luminal Cl^- ($[Cl^-]_L$) were estimated by microfluorometry. Images of luminal fluorescence were obtained at 1-min intervals and the rate of fluid secretion was calculated from the increment of luminal volume. During stimulation with secretin (10 nM), the rate of fluid secretion was 2.28±0.14 nl min^{-1} mm^{-2} (per unit area of epithelium, n=5, mean±SE), pH_L increased due to HCO_3^- secretion, and $[Cl^-]_L$ steadily decreased. The net Cl^- transport calculated from $[Cl^-]_L$ and luminal volume was nearly zero. Ethanol (1 mM) increased secretin-stimulated fluid secretion to 4.11±0.22. pH_L slightly decreased and $[Cl^-]_L$ was stable in the presence of ethanol. The net Cl^- transport was 0.265±0.042 nEq min^{-1} mm^{-2}. The increase of fluid secretion by ethanol was almost equivalent to fluid secretion accompanying Cl^- efflux. Ethanol induced Cl^- secretion during secretin stimulation without affecting HCO_3^- secretion, which resulted in the increase of Cl^- of secreted fluid from zero to about 70 mM.

O19 (3O-13D3)

Functional characterization of L1156F CFTR: a newly identified mutation in Japanese patients with chronic pancreatitis

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Cystic fibrosis transmembrane conductance regulator (CFTR) is a cAMP-regulated chloride channel that plays an important role in bicarbonate transport in the exocrine pancreas. Accumulating evidence suggests that CFTR mutations are associated with a subset of patients with chronic pancreatitis. We have identified a new point mutation (L1156F) in exon 18 in patients with chronic pancreatitis. In order to elucidate a disease associated with this mutation, we examined the function of L1156F-CFTR. The mutation was introduced in pCMV-wild type (WT) CFTR plasmid by site directed mutagenesis. Chloride channel activity was measured in HEK293 cells expressing either WT or L1156F-CFTR by whole cell current recording. The amount of CFTR proteins expressed was analyzed by immuno-blotting using an anti-CFTR antibody. When stimulated with 10μM forskolin, WT and L1156F generated a chloride current of 1534±72 and 476±45 pA (n=5), respectively. The introduction of the L1156F mutation did not affect the expression of CFTR protein compared with the WT. In conclusion the L1156F mutation reduces the CFTR chloride current by 69%. The lack of lung and intestinal symptoms and the chronic pancreatitis in these patients further highlight that low activity of CFTR is sufficient for normal lung and intestinal function and the particular susceptibility of the pancreas to mutations in CFTR.

O20 (3O-13D4)

Expression of K^+-Cl^- cotransporters in gastric parietal cells

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For gastric acid (HCl) secretion, protons are actively secreted by H^+,K^+-ATPase in the apical membrane of gastric parietal cells, but it has not been established what molecule contributes to Cl^- secretion. K^+-Cl^- cotransporter (KCC) plays a significant role in epithelial transport and cell volume regulation. Four KCC isoforms have been cloned. KCC1, 3, 4 are widely expressed and KCC2 is neuron specific. However, the expression of KCC isoforms in stomach has not been reported. Here we examined whether the KCC isoforms are expressed in gastric parietal cells. Western blot analysis showed that KCC3 and KCC4 were expressed in isolated gastric mucosa of rats and mice. Immunohistochemistry in the isolated gastric mucosa showed that KCC3 was expressed in the basolateral membrane and KCC4 was expressed in the apical membrane of the gastric parietal cells. Interestingly, KCC3 and KCC4 were abundantly expressed in the parietal cells located at luminal segment of the gland. Because luminal segment parietal cells are much more active in HCl secretion than those of the basal segment, KCC3 and KCC4 may be involved in the mechanism of HCl secretion. We constructed the T-REx system for KCC3 in the LLC-PK1 cells. Tetracycline-induced expression of KCC3 protein significantly increased ouabain-sensitive Na^+,K^+-ATPase activity.

O21 (3O-13D5)

Properties of a novel splicing variant of ATP1AL1, a human non-gastric proton pump

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Rat non-gastric H^+,K^+-ATPase is highly expressed in the distal colon and may be associated with K^+ conservation in the colon. But physiological function of human non-gastric H^+,K^+-ATPase (ATP1AL1) has not been clarified yet. Here, we have cloned a normal type (NT) and a novel splicing valiant deleting exon4 (Δexon4) of ATP1AL1. Then, the stable cell lines expressing gastric H^+,K^+-ATPase β-subunit (gHKβ) were transfected with the pcDNA4/His-ATP1AL1 cDNA (NT or δexon4) construct. The activities of the K^+-dependent ATPase and the $^{86}Rb^+$ uptake of ATP1AL1 were estimated by subtracting 1 mM ouabain-sensitive activity from 5 μM ouabain-sensitive activity. These activities of ATP1AL1 were also measured by using 100 μM SCH 28080, an inhibitor of gastric H^+,K^+-ATPase. We found that δexon4 is expressed in the plasma membrane of the cells. Similar to the case for NT, the gHKβ was required for expression of δexon4. We found that δexon4 has no activities of the K^+-dependent ATPase and the $^{86}Rb^+$ uptake. When NT and δexon4 were co-transfected into the stable cell lines expressing gHKβ, the $^{86}Rb^+$ uptake activity was significantly lower than that in the cells transfected with NT alone. Apparently, δexon4 had no effect on the level of expression of NT in the cells. These results suggest that δexon4 exerts a dominant negative effect on NT.

ORAL
Heart & circulation

O22 (3O-12C1)

Inward rectifier K^+ current I_{K1} and cardiac repolarization: a simulation study using the Kyoto model

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I_{K1} is known to be responsible for the late rapid repolarization phase of action potential in cardiac ventricular myocytes, yet its role in the slow repolarization phase remains unclear. The amplitude of I_{K1} is determined by voltage-dependent block of the channel by internal spermine (SPM) and Mg^{2+}. During repolarization, the release from the Mg^{2+} block in the presence of SPM induces a significant transient component of I_{K1} in voltage-clamp experiments. We developed a new model of the I_{K1} channel, which includes the high- and low-affinity modes of blocks by SPM and by Mg^{2+} (Yan & Ishihara, J. Physiol. 563, 2005), and examined the participation of this dynamic gating of I_{K1} channel to action potential repolarization by incorporating it into the Kyoto cardiac ventricular cell model. The model shows that the Mg^{2+}-induced transient component of I_{K1} appears during the slow phase of repolarization. Thus, changes in the I_{K1} density significantly alter the action potential duration (APD), as has been demonstrated in experiments. A decrease in the Mg^{2+} concentration or an increase in the SPM concentration prolongs APD by reducing the transient component of I_{K1}. Under this pathological condition, if the rapid component of delayed rectifier K^+ current is blocked, APD is markedly prolonged. This model study predicts that the internal spermine and Mg^{2+} are important factors affecting the occurrence of early afterdepolarization and arrhythmia.

O23 (3O-12C2)

Axial Stretch Acutely Increases Ca^{2+} Spark Rate in Rat Ventricular Myocytes

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The slow response of myocardial contractility to stretch has been linked to a nitric oxide mediated stretch-induced increase in ryanodine receptor Ca^{2+} release, revealed as an elevated diastolic Ca^{2+} spark rate in rat ventricular myocytes exposed to 10% stretch for 10 min (Vila Petroff et al. 2001). Here, we investigate whether stretch-induced changes in Ca^{2+} spark rate may occur more rapidly. Isolated rat ventricular myocytes were exposed to 10% axial stretch using computer-controlled piezo-manipulated carbon fibres, attached to the centre and one end of a cell. Controlled and reversible stretch was applied selectively to a half-cell only, allowing the non-stretched part to serve as control. Diastolic spark rate was studied using a Zeiss 510 system and software detection of signal deviation from background by > 2 S.D. Within 10 s, axial stretch transiently increased Ca^{2+} spark rate by $31 \pm 6.5\%$ ($n = 8$, $p < 0.05$), followed by return to background levels within 1 min. The response was not blunted by 1 mM L-NAME (nitric oxide synthase inhibitor; $n = 7$). We conclude that: i) axial stretch acutely raises diastolic Ca^{2+} spark rate in rat ventricular myocytes; ii) underlying mechanisms differ from those involved in the slow response to stretch. This study is supported by the British Heart Foundation and Eisai Co., Ltd.

O24 (3O-12C4)

β-adrenergic stimulation does not enhance Na/Ca exchange current in guinea-pig, mouse and rat ventricular myocytes

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The effect of β-adrenergic stimulation on cardiac Na/Ca exchange current (I_{NCX}) has been controversial. Recently, 25 - 400% increase by isoproterenol of I_{NCX} was reported. To reexamine this effect, we measured I_{NCX} in voltage-clamped guinea-pig, mouse and rat ventricular cells. When I_{NCX} was defined as a 5 mM Ni^{2+}-sensitive current in guinea-pig ventricular myocytes, 1 μM isoproterenol apparently augmented I_{NCX} by about 32%. However, this increase was probably due to contamination of the cAMP-dependent Cl^- current (I_{CFTR}), because Ni^{2+} inhibited the activation of I_{CFTR} by 1 μM isoproterenol, with a half-maximum concentration of 0.5 mM under the conditions where I_{NCX} was suppressed. 5 or 10 mM Ni^{2+} did not inhibit I_{CFTR} activated by 10 μM forskolin, an activator of adenylate cyclase, suggesting that Ni^{2+} acted upstream of adenylate cyclase in the β-adrenergic signaling pathway. Furthermore, in a low Cl^- bath solution, 1 μM isoproterenol did not significantly alter the amplitude of Ni^{2+}-sensitive I_{NCX} at +50 mV, which was close to the reversal potential of I_{CFTR}. No change in I_{NCX} amplitude was induced by 10 μM forskolin. When I_{NCX} was activated by external Ca^{2+}, it was not significantly affected by 1 μM isoproterenol in guinea-pig, mouse or rat ventricular cells. We concluded that β-adrenergic stimulation does not have significant effects on I_{NCX} in guinea-pig, mouse or rat ventricular myocytes.

O25 (3O-12C5)

Lysosomal degradation may accelerate down-reguration of cardiac gap junction protein Connexin-43 (Cx43) via protein kinase C (PKC)-mediated hyper-phosphorylation

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It is well accepted that gap junctions (GJs) play a pivotal role in the intercellular spread of electrical flows between cardiac myocytes. Dysfunction of GJs can thus be one of major arrhythmogenic factors. In this study, we have obtained the evidence that in both type I and II diabetic hearts, myocardial intercellular communication through GJs is impaired via PKC-mediated hyper-phosphorylation of the GJ protein Cx43. Western blot and immunohistochemical analyses of the PKC-activated diabetic hearts indicated that the expression level of Cx43 is significantly reduced compared with control hearts, with altered distribution of sparse or sporadic pattern at the intercalated disk. These alterations were ameliorated by treatment with lysosomal inhibitors as well as PKC inhibitors, but could not be prevented by the proteosmal inhibitor ALLN. These results strongly suggest that facilitated lysosomal degradation of Cx43 via PKC-mediated hyper-phosphorylation may underlie the down-regulation of Cx43 protein in rat diabetic hearts. This mechanism may in part account for the reported vulnerability of rat diabetic hearts to ventricular fibrillation.

O26 (3O-12C6)

New function of creatine kinase in myocardium

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Present study was designed to demonstrate intracellular radial gradients of myoglobin oxygen saturation and ATP in single cardiomyocytes with elevated oxygen demand. Intracellular ATP concentration was indirectly assessed from intracellular free Mg^{2+} concentration using a Mg^{2+} sensitive ratiometric fluorescent dye mag-fura-2 (MF2). A bright field fluorescence microscopy was newly manufactured for simultaneous measurements of myoglobin light absorption (spectrophotometry) and MF2 fluorescence (fluorometry). Uncoupler of oxidative phosphorylation (1 μM CCCP) was used to increase oxygen flux. While significant intracellular gradients of myoglobin oxygen saturation was demonstrated in CCCP treated cells at physiological extracellular Po_2, no significant heterogeneity was found in MF2 fluorescence. In contrast, in cardiomyocytes treated with 0.5 mM iodoacetamide (a blocker of creatine kinase) in addition to CCCP, gradients of MF2 fluorescence from the sarcolemma to the cell core (indicating radial gradients of ATP) were demonstrated. Such MF2 fluorescence gradients were not demonstrated when extracellular oxygen concentration was elevated to 20%. From these results, it is concluded that significant gradients of ATP may be produced in the isolated single cardiomyocyte when mitochondrial oxygen consumption is moderately elevated at physiological extracellular Po_2. However, such potential ATP heterogeneities are effectively buffered in the normal cardiomyocyte by ATP supplementations from creatine phosphate. Thus, present results propose a new function of creatine kinase in the myocardium. This study was supported by JSPS KAKEN #15390061.

O27 (3O-12C7)

Essential role of phosphoinositide 3-kinase class IIα in Ca^{2+}-dependent Rho GTPase activation and contraction in vascular smooth muscle cells.

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We previously demonstrated that excitatory agonists such as noradrenaline (NA) and membrane depolarization induce Ca^{2+}-dependent activation of Rho GTPase in vascular smooth muscle (VSM) cells, resulting in inhibition of myosin phosphatase (MP) through the mechanisms involving Rho kinase-mediated phosphorylation of its regulatory subunit MYPT1/MBS. We found that phosphoinositide 3-kinase class IIα (PI3K-C2α) plays an essential role in NA-induced Rho activation and contraction in differentiated VSM primary cultured-cells. In the present study we show that ionomycin, a Ca^{2+} ionophore, induced contraction with stimulated phosphorylation of MYPT1. Ionomycin-induced MYPT1 phosphorylation and contraction was inhibited by the Rho kinase inhibitor Y-27632. Silencing PI3K-C2α, but not PI3K p110α, expression by small interfering RNA (siRNA) in differentiated VSM cells inhibited ionomycin-induced phosphorylation of MYPT1, consequent reinforcement of 20-kDa myosin light chain (MLC) phosphorylation and contraction. Consistent with this, the PI3K inhibitors Wortmannin and LY294002 inhibited both MLC phosphorylation and contraction. These findings indicate an essential role of PI3K-C2α in Ca^{2+}-dependent, Rho/Rho kinase-mediated negative control of MP and VSM contraction.

O28 (3O-12C8)

Identification of dynamic and static characteristics of a baroreflex system using a neural cascade

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Background: Identification of dynamic and static characteristics of a given biological system promotes the understanding of the system behavior under a variety of circumstances. **Purpose:** To estimate the dynamic and static characteristics of a baroreflex neural arc from pressure input to efferent sympathetic nerve activity, we developed a system identification method using a *neural cascade*. **Method:** A "neuron" used in a neural network can represent the dynamic linear element followed by a nonlinear transfer function. By connecting two neurons in series, we can represent a system comprised of dynamic-linear (L1), static-nonlinear (NL), and dynamic-linear (L2) subsystems. Because the contamination of noise to the observed output resulted in biased estimates of the system characteristics, we added an iterative noise cancellation procedure where the noise was estimated by an autoregressive model. **Results:** In a simulation study, the *neural cascade* effectively identified the dynamic and static characteristics of an L1-NL-L2 system. The baroreflex neural arc is known to have derivative characteristics followed by a sigmoidal nonlinearity. When applied to the actual input-output data of the baroreflex neural arc obtained from rabbits, the *neural cascade* could identify the derivative characteristics followed by the sigmoidal nonlinearity. **Conclusion:** The *neural cascade* proposed in the present study may provide a useful method to simultaneously identify the dynamic and static characteristics of a biological system.

O29 (3O-17H1)

Antioxidative effect of estrogen in hypertensive Dahl salt-sensitive rats

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Estrogen deficiency in the menopause is associated with an increased cardiovascular risk. Endogenous estrogen has been suggested to exert vasoprotective effects through decreasing vascular oxidative stress. To investigate the mechanism of the decreasing in oxidative stress by estrogen, we examined superoxide production and antioxidant enzyme expression in aorta in ovariectomized Dahl salt-sensitive (DS) rats. Female DS rats (8 weeks old) were ovariectomized (OVX group) or sham-operated (sham group). Estrogen pellets were subcutaneously implanted in ovariectomized rats for estrogen treatment (OVX + E group). After 4 weeks of salt-loading (8% NaCl diet), blood pressure was increased in OVX group compared with sham and OVX + E groups. Superoxide production in aortic ring was higher in OVX group than in sham and OVX + E groups. Increase in superoxide production was abolished by pretreatment with diphenyleneiodonium, a NADPH oxidase inhibitor. Expression of mRNA of p22phox, a NADPH oxidase subunit, increased in aorta from OVX group compared with sham group. In contrast to p22phox, mRNA expressions of antioxidant enzymes extracellular superoxide dismutase (ecSOD) and glutathione peroxidase (GPX) were decreased in OVX rats. Expression levels of p22phox, ecSOD and GPX in OVX + E rats were not different from that in sham group. These data suggest that estrogen deficiency in ovariectomized DS rats enhances oxidative stress through increased NADPH oxidase expression and decreased antioxidant enzymes, and promotes vascular injury by salt-loading.

O30 (1O-06H2)

Age-dependent roles of an ATP-sensitive potassium channel Kir6.2 in the hypoxic ventilatory response in the mouse

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Acute hypoxia elicits a biphasic ventilatory response, initial augmentation and subsequent depression. Although oxygen-sensitive channels of type I cells in the carotid body are considered to be involved in the initial augmentation, the underlying cellular mechanism for the subsequent depression, hypoxic ventilatory decline (HVD), has not been fully elucidated. The purpose of the present study is to examine the role of an ATP-sensitive potassium channel, Kir6.2, in the hypoxic ventilatory response including HVD in the mouse. We serially measured minute ventilation volume (Ve) of the Kir6.2-knockout mouse (Kir6.2-/-: n = 5) exposed to hypoxia (12% O_2 in N_2: 10min) in an unanesthetized unrestrained state by whole body plethysmography in the 2nd and 4th postnatal weeks. Percent changes from the baseline Ve in the room air were calculated and compared with that in the C57BL/6 mouse (n = 10). In the 2nd postnatal week, there was no difference in the hypoxic ventilatory response between the C57BL/6 and Kir6.2-/- mice. Meanwhile, in the 4th week, the initial augmentation lasted longer, and HVD was much weaker in the Kir6.2-/- than in the C57BL/6. It is concluded that Kir6.2 is involved in the hypoxic ventilatory response including HVD in an age-dependent manner in the mouse.

ORAL
Respiration

O31 (1O-06H3)

Changes in electroencephalogram and cerebral blood flow during frequent yawns in human

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Our previous studies showed that frequent yawns can be evoked by microinjection of L-glutamate into the paraventricular nucleus (PVN) in an anesthetized, spontaneously breathing rat. The yawning response was characterized by an arousal shift in the ECoG to lower voltage and faster rhythms. We focused the present study upon frequent yawns in human. Yawns occurred once a few minutes for more than 5 minutes in this study. We monitored electroencephalogram (EEG) and cerebral blood flow (CBF) using near-infrared spectroscopy (NIRS). We found a shift in the EEG to lower voltage and faster rhythms and an increase of CBF in ventromedial prefrontal cortex (PFC) during and immediately after yawning responses. These results suggest that the activation of ventromedial PFC may be associated with an arousal/yawning response in human.

O32 (1O-06H4)

Metabolic changes in cerebral cortex and spectral analysis of EEG during long-term breath holding in professional divers

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We investigated metabolic changes in prefrontal and parietal cortices and EEG changes during a long-term breath holding (BH) in professional divers. He/she performed BH for 2 to 7 minutes following a preparatory period of approximately 5 minutes. Such BH procedures were repeated 3 times in this study. We measured metabolic changes in cerebral cortex using near-infrared spectroscopy (NIRS) and made spectral analysis of EEG before, during and after BH. Concentration of deoxygenated hemoglobin (deoxyHb) showed a gradual increase during BH in both prefrontal and parietal cortices. There is a linear relationship between the maximal level of deoxyHb and the duration of BH. In contrast, concentration of oxygenated hemoglobin (oxyHb) of BH decreased along with prolonged duration of BH. Voluntary abdominal breathing (VAB) was performed during the preparatory period before BH. Spectral analysis of EEG was focused on the high-frequency alpha (HF-alpha) band (10-13Hz) in this study. The higher power of HF-alpha band was observed before and immediately after the onset of BH, and thereafter the HF-alpha power exhibited a gradual decrease until the end of BH. Since previous study showed that VAB produced increase in HF-alpha power and urinary 5-HT level (fumoto et al, 2004), we suggest that the higher HF-alpha power evoked before BH may contribute to the tolerance to cerebral hypoxia during BH.

O33 (1O-06H5)

Ventilatory long-term facilitation following intermittent hypoxia is state-dependent in rats

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Ventilatory long-term facilitation (vLTF) following acute intermittent hypoxia (AIH) has been reported to variable extent in unanesthetized rats. However, none of these studies reported sleep-state, a critical variable in many physiological functions. We hypothesized that vLTF would be preferentially expressed in sleeping vs. awake Lewis rats following AIH. The sleep-wake state of unrestrained rats was determined from implanted EEG and nuchal EMG electrodes. Tidal volume (VT), frequency (f), minute ventilation (VE) and CO2 production (VCO2) were determined in unanaesthetized male Lewis rats via plethysmography before, during and after AIH (five, 5-min exposures, 10.5% O2; 5-min normoxic intervals) or acute sustained hypoxia (25-min exposures, 10.5% O2, ASH). VE, VT and f in quiet wakefulness (QW) or NREM sleep were normalized to its own baseline value during the corresponding state during baseline, pre-hypoxia conditions. LTF was observed in VE after AIH, but not ASH. Following AIH in NREM, VE gradually increased and reached maximum level at 20 min post-hypoxia, remaining at that level for at least 60 min (26.6±5.2% baseline). The main contributor to vLTF was VT (13.5±2.3%), with a lesser increase in f (7.4±1.7%). The corresponding increase in VE/VCO2 was 35.5±2.4% baseline. In QW, significant vLTF was not observed. The duration, magnitude, and pattern in vLTF in NREM were similar to phrenic LTF in anesthetized rats. In conclusion, vLTF is highly state and pattern sensitive in unanesthetized rats. (Supported by NIH HL65383, HL07654, HL68255).

O34 (1O-06H6)

Factors affecting expired minute volumes of low molecular weight compounds

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Chemical compounds in breath originate mainly from volatile metabolic products in health and diseases. Among them, hydrogen and methane are believed to represent the conditions of gastro-intestinal systems in the presence of bacterial flora. On the other hands, carbon monoxide and nitric oxide are well-known bioactive chemicals to play important roles in physiological and pathophysiological conditions. Because matrix analysis based on simultaneous measurements of exhaled chemicals with such low mass numbers may give us important information of physical conditions, we conducted the following experiment.Nine hundreds and forty two subjects aged from 20 to 88 years old volunteered for the present study. After tooth brushing and gargling, fasted subjects breathed purified artificial air via a mouthpiece in a sitting position for 12 min, during which exhaled air was collected via one-way valve into the Douglas bag. Minute ventilation volume and concentrations of carbon dioxide and oxygen in exhaled air were monitored by respiratory metabolic analyzer with the chemical sensors. Concentrations of hydrogen, methane, carbon monoxide and nitric oxide in the bag were analyzed by gas chromatography and chemiluninescence methods. We will present the summarized results of the relationships between minute expired volumes of these compounds and such physical conditions as age, gender, laboratory data, scores of health-related questionnaires, diseases and disorders by using the multivariate analysis.

O35 (1O-06H7)

Acute effects of thixotropy conditioning of inspiratory muscles on end-expiratory chest wall volume

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Thixotropy is a passive property of the skeletal muscle that depends on the muscle's immediate history of contraction and length change. We showed that inspiratory muscle thixotropy affects the end-expiratory position of the rib cage. The present study aimed to test whether changes in end-expiratory chest wall volume (Vcw) occurs after thixotropic inspiratory muscle conditioning in normal subjects (n = 32). We first examined effects of the conditioning on end-expiratory Vcw of succeeding five breath cycles with respiratory induction plethysmography. Subjects participated in the conditioning at three different Vcw (60% inspiratory capacity [IC] + end-expiratory Vcw of baseline breathing [EEB], EEB, and residual volume [RV]) giving one of two levels of inspiratory effort (no effort or maximal inspiratory effort) with airway closure in the sitting position. End-expiratory Vcw increased after conditioning at 60% IC + EEB and decreased after conditioning at RV. We then measured the time course of changes in spirometrically determined IC, which confirmed thixotropic changes in end-expiratory Vcw. A decrease in the IC was found at 60 s after conditioning performed at the higher volume. However, the decrease disappeared until 180 s. Conditioning at the lower volume was followed by an increase in the IC, which was maintained even after 180 s. Furthermore, the thixotropic inflation/deflation of the chest wall was proved by the helium-dilution FRC and esophageal pressure measurements. In conclusion, thixotropy conditioning of inspiratory muscles changes end-expiratory Vcw.

O36 (1O-06H8)

Genetic or pharmacological ablation of orexin attenuated and supplementation ameliorated hypercapnic chemoreflex

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We examined whether the chemoreceptor reflex in prepro-orexin knockout mice was blunted or not, and if so, whether supplementation of exogenous orexin restore the abnormality. In addition, we studied whether pharmacological blockade of orexin in the wild-type mice resulted in a similar abnormality. A cannula for intracerebroventricular injection to the lateral ventricle was implanted to the isoflurane-anesthetized mice together with electrodes for recording electroencephalogram and electromyogram. Ventilation was recorded by whole body plethysmography after recovery period of at least 7 days. After recording of baseline breathing for 1 hr, orexin-A, -B, an orexin receptor antagonist, or vehicle was intracerebroventriculary injected and hypercapnic or hypoxic gas mixture was introduced into the recording chamber for 10 min. Data were examined for only awake period because sleeping distorts chemoreflex sensitivity. Hypercapnic ventilatory responses but not hypoxic responses were attenuated in orexin knockout mice as compared to those in the wild-type littermates. Similar abnormality was reproduced in wild-type mice treated with orexin antagonist. Intracerebroventricular injection of orexin partially restored the hypercapnic chemoreflex in the mutant mice. Our findings suggest that orexin plays a crucial role for CO_2-sensitivity at least during awake periods.

O37 (3O-14E6)

Kinetics of tissue plasminogen activator (tPA) exocytosis from endothelial cells and its modulation by PA inhibitor-1 (PAI-1).

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Background: Vascular endothelial cells (VECs) contribute to keep the patency of vasculature through the regulated expression and secretion of various molecules having either anti-coagulatory or high fibrinolytic activity. tPA, the primary PA in the vasculature, is secreted from VECs as an active form and express fibrinolytic activity in blood. In blood there also exist its specific inhibitor of PAI-1. Though the impaired tPA secretion as well as the elevated plasma concentration of PAI-1 are considered to be risk factors for thrombosis, precise mechanism underlying in tPA secretion is not clarified. Here, we analyzed the dynamics of tPA secretion from its containing granules and its modulation by PAI-1 using total internal reflection fluorescence microscopy(TIRFM). Method: An established cell-line of VECs was cultured and transfected with tPA-GFP. The dynamics of tPA-GFP secretory granules near the plasma membrane was analyzed by TIRFM. Results: 1) The dynamics of tPA-GFP granules including its opening and tPA-GFP secretion were successfully monitored by TIRFM. 2) Once tPA-GFP granules open, they kept open and tPA-GFP was released slowly. The secreted tPA-GFP was detected as tPA-GFP-PAI-1 complex in cultured medium. 3) The velocity of tPA release was facilitated by supplementary added PAI-1, which resulted in the increase in tPA-PAI-1 complex in supernatant. Conclusion: tPA-GFP is beneficial tool to investigate its exocytotic dynamics. PAI-1 seems to facilitate tPA release as an inactive complex-form, which suppresses fibrinolytic activity on VECs.

ORAL
Blood

O38 (3O-14E7)

The colony-forming cell assay for human hematopoietic progenitor cells harvested by a novel continuous-flow cell separation

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A novel cell separation method which can continuously separate the cells according to their densities has been developed for application to the transfusion medicine. In the past the performance of this method was examined on separation of human buffy coat, peripheral blood and co-cultured cell suspensions. Flow cytometry analysis on separation of human buffy coat revealed that CD34-positive cells, which were assumed to be hematopoietic progenitor cells, were distributed around density = 1.065. Recently, the colony-forming cell assay was performed on human hematopoietic progenitor cells separated from peripheral blood by the present method. Five polymer media with densities of 1.060, 1.065, 1.070, 1.075 and 1.080, prepared with sterile isotonic Percoll media and PBS, were used for the separation, and the fractionated cells were cultured in a methylcellulose-based medium containing hSCF, hGM-CSF, hIL-3 and hEPO. Colony-forming unit-erythroid (CFU-E) were appeared on day 7. Burst-forming unit-erythroid (BFU-E), CFU-GM (granulocyte, macrophage) and CFU-GEMM (granulocyte, erythroid, macrophage, megakaryocyte) were observed on day 14. BFU-E, CFU-GM and CFU-GEMM were colonies that were found in the fourth fraction (density = 1.075), among those no CD-34 positive cells being detected by flow cytometry analysis. These results suggest that the method might enable to harvest many types of human hematopoietic progenitor cells according to minute differences in their densities.

O39 (3O-14E8)

The interaction of synthetic peptide derived from staphylokinase with plasminogen

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Staphylokinase (SAK) expresses plasminogen activator (PA) activity by forming a complex with plasmin. In this report, the interaction of nonadecapeptide derived from staphylokinase with plasminogen, was investigated. The effects of synthetic peptides on plasminogen activation were estimated by using a chromogenic substrate assay and ^{125}I-labeled plasma clot lysis assay. The binding of peptides to Glu-plasminogen was estimated by using IAsys resonant mirror biosensor. The synthetic nonadecapeptide (SAK22-40) corresponding to Glu22-Leu40 of SAK amino acid sequence did not show any PA activity in the presence of plasmin. However, SAK22-40 enhanced Glu-plasminogen activation by t-PA. SAK22-40 bound to plasminogen in a concentration-dependent manner. Although this binding ability was not inhibited in the presence of anti-K1-K3 (plasminogen fragment containing kringle 1 to 3 domains) IgG or anti-K4 (plasminogen fragment containing kringle 4 domain) IgG, it was partially inhibited by anti- mini plasminogen IgG. The substitution of Lys35 to Ala in SAK22-40 did not show the enhancement of PA activity by t-PA. The t-PA activity was enhanced in the presence of cultured endothelial cells, and it was further enhanced by SAK22-40. These findings indicate that the synthesized nonadecapeptide, SAK22-40, binds to B-chain of Glu-plasminogen and enhances PA activity by t-PA. The mechanism by which SAK22-40 enhances t-PA activity seems to be different from that by which COOH-terminal Lys of fibrin does.

O40 (2O-08E5)

Low ghrelin contents in the blood and the stomach of CCK-A and B receptor gene knockout mice

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In mammals including humans, a brain-gut hormone, cholecystokinin (CCK) mediates the satiety effect via CCK-A receptor (R). However, we generated CCK-AR gene deficient (-/-) mice and found that the daily food intake, energy expenditure, and gastric emptying did not change compared with those of wild-type mice. We also generated CCK-BR (-/-), CCK-AR (-/-) BR (-/-) mice. Daily food intake and ghrelin contents in the blood and the stomach were investigated. Male mice at 6-8 months of age were used. Mice, deprived of food for 18 hr with free access to water, were injected i.p. (0.1ml/mouse) with either vehicle or CCK-8 (0.3, 1.0, and 3.0 n mol/mouse). Thereafter, daily food intake was measured. Additional animals were sacrificed by guillotine, the blood was obtained, and the stomach was removed to measure ghrelin contents. Administration of CCK-8S significantly decreased food intake in wild-type and in CCK-BR (-/-) mice. However, no significant inhibition was observed in CCK-AR (-/-) and in CCK-AR (-/-) BR (-/-) mice. When mice injected with vehicle were compared, food intake in CCK-AR (-/-) BR (-/-) mice was significantly lower than that in wild-type and CCK-BR (-/-) mice. Moreover, the ghrelin contents in the blood as well as in the stomach were significantly lower in CCK-AR (-/-) BR (-/-) mice than wild-type mice. CCK-Rs may be involved in the regulation of ghrelin biosynthesis and secretion.

ORAL
Gastrointestinal functions

O41 (2O-08E6)

Monocarboxylate transporter 1 plays a role in short chain fatty acid absorption in caprine rumen

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Despite the importance of short chain fatty acids (SCFA) in maintaining the ruminant physiology, the mechanism of SCFA absorption is still not fully studied. Therefore, this study was carried out to elucidate the mechanism of SCFA transport in the caprine rumen epithelia by investigating the possible involvement of monocarboxylate transporter 1 (MCT1) as well as to delineate the precise localization and the level of MCT1 protein along the caprine gastrointestinal tract. To achieve these objectives, molecular studies including: RT-PCR, Western blotting, and immunohistochemistry as well as functional studies (in vivo and in vitro) were used. RT-PCR studies revealed the presence of mRNA encoding for MCT1 in all regions of the caprine gastrointestinal tract. By immunoblotting analysis on membrane protein extract, a 45-kDa-band corresponding to MCT1 was detected in all parts of the forestomach and abomasum as well as along the entire length of the intestine. The MCT1 protein level was found most abundantly in forestomach, at intermediate levels in large intestine and abomasum, and lower levels in small intestine. MCT1 is immunolocalized mostly to the stratum basale and stratum spinosum of the caprine rumen epithelia. The MCT1 inhibitor, pCMB exerted a significant influence on acetate flux in vitro as well as short-chain fatty acids absorption in vivo. The results obtained provide evidence, for the first time, for transepithelial transport of SCFA via MCT1 across the caprine ruminal epithelia.

O42 (2O-08E7)

The role of bowel fermentation in appetite control

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Disordered eating has been investigated extensively. The large bowel has been studied less. The objective is to find its influence on appetite. Particularly, the relationship between large bowel fermentation and appetite is not known. Hundred patients with no or some colons and the age sex BMI matched controls were given a breakfast followed by lactulose. The hydrogen breath and appetite levels were determined. Energy intake of all patient groups was less than their matched controls. At 180 min, in proctocolectomised group less hunger and higher satiety levels than that of controls group were observed. The satiety levels of subtotal/hemicolectomised significantly less at 240 and 330 min. The breath hydrogen level at -30 min and 60 min is positively correlated with the satiety levels at 120 min proctocolectomy group. The breath hydrogen level -30 min and 60 min is negatively correlated with the satiety levels at 240 min subtotal removal of the rectum group. The breath hydrogen level -30 min is positively correlated with the satiety levels at 180 min, 300 min and 330 min in total rectal removal group. The subjects who have had large bowel surgery had lower hunger and higher satiety levels than controls implicated that large bowel plays a role in appetite regulation. The breath hydrogen influences the satiety in proctocolectomy and total rectal removal positively and negatively in sub/hemi colectomy, and the hunger only in sub/hemi colectomy in one time point.

ORAL
Muscle physiology

O43 (2O-08E8)

Stimulatory effect of a congener of beer (N-methyltyramine) on pancreatic secretion in conscious rats

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Alcoholic beverages contain several congeners. N-methyltyramine (NMT) was isolated from beer as a factor to stimulate gastric acid secretion. We examined whether NMT stimulated pancreatic secretion in conscious rats. Methods: Male Wistar rats (330g) were prepared with cannulae for draining bile and pancreatic juice separately, with two duodenal cannulae, with a gastric cannula, and with an external jugular vein cannula. The rats were placed in modified Bollman-type restraint cages. After the 4-day recovery period, the experiments were conducted in unanesthetized rats. Different concentrations of NMT solutions (5, 25, and 50 micro-g/kg/3ml/3min) were infused into the stomach. NMT content in beer was 2mg/L, so that if 50kg body weight man consumes a bottle of beer, 25 micro-g/kg NMT will be ingested. To examine the mechanism, the effects of proton pump inhibitor, CCK-BR antagonist, CCK-AR antagonist and atropine were administered prior to the NMT infusion. The effect of intravenous infusion of NMT (2.5 micro-g/rat) was also determined. Results: Intragastric administration of NMT significantly increased pancreatic exocrine secretion in a dose dependent manner. Atropine abolished the stimulatory effect of NMT, but others did not. Intravenous infusion of NMT did not affect pancreatic secretion. Conclusions: NMT stimulates pancreatic secretion via cholinergic gastro-pancreatic reflex. Therefore, the stimulatory effect of beer on pancreatic secretion was produced by not only ethanol but also a congener, NMT.

O44 (2O-11H1)

Crowding problem in skinned muscle: muscle compression with organic solutes of small molecular weight.

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We found that organic solutes potently compress the myofilament lattice of skinned skeletal muscle. This compressing effect cannot be ascribed to the ordinary osmotic compression due to the filtration of the solutes by the lattice, because some solutes smaller than ATP in molecular weight could effectively compress the muscle. Another candidate for the compressing force comes from the entropic aggregating force of the macromolecules constituting the myofilament lattice. As Asakura-Oosawa theory and its derivatives describe, the aggregation of macromolecules in the presence of smaller particles depends on the exclusion of the particles from the very vicinity of the macromolecule surface. To estimate the effective exclusion volume for the organic solutes in the myofilament lattice, we examined the compressing effects of a series of organic molecules from mono- and poly-hydric alcohols. The results clearly indicated that the compressing efficiency of the alcohols depends primarily on the number of CH_2 group that is not directly attached by the hydroxyl group. That is, the unitary component for the compressing effect is a single CH_2 group. Since the molecular size of the CH_2 group is very close to that of the water molecule (OH_2; 0.31 nm^3), any macromolecular aggregation force due to the exclusion of solutes would not work, unless each CH_2 group forms a larger complex with surrounding water molecules or water molecules form a stable cluster larger than the bare CH_2 group. The possibility of the CH_2-OH_2 complex formation and the stable water cluster formation will be discussed.

O45 (2O-11H2)

Diameter measurements of single skeletal myofibrils: AFM studies

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In the present studies, the diameter of single skeletal myofibrils was measured under various physiological conditions by AFM. Our AFM instrument is not suitable for precise diameter measurements of specimen having the diameter range of micrometer like single myofibrils. Therefore, we modified our AFM system by incorporating precise piezo systems so as to be useful for the present purpose.

Single myofibrils were prepared by homogenizing glycerinated muscle fibers of the rabbit psoas muscle. Myofibrils were fixed on the surface of cover slip coated with aminosilanes. A small glass sphere was adhered to the tip of AFM cantilever to smoothly touch to the surface of myofibrils. The piezo stage could be moved in the horizontal and the vertical directions in the range of 10 μm with the accuracy of ± 2 nm. The diameter of myofibrils was determined by comparing the distance moved for the glass sphere fixed to AFM cantilever to touch the top surface of myofibrils and that to touch the surface of the cover slip. The error of the diameter measurements was < ± 5 nm.

The diameter of single myofibrils thus obtained was 1.119 ± 0.042 μm (n=5) in the relaxed state and 1.026 ± 0.039 μm (n=5) in the rigor state. Thus the diameter of myofibrils in the rigor state was smaller by 8% than that in the relaxed state. We also further examined the diameter of single myofibrils under other physiological states. The results thus obtained will be compared with the data for the lattice spacing of actomyosin filaments of muscle fibers reported by X-ray diffraction studies.

O46 (2O-11H3)

Effects of BDM on the transverse stiffness of myofibrils and muscle fibers studied by atomic force microscopy

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It is generally accepted that the contraction of muscle fiber is reversibly inhibited by 2,3-butanedione monoxime (BDM). Since the muscle contraction takes place by the interaction between thin actin filaments and myosin heads of thick filaments, it was suggested that BDM directly inhibits the actin-myosin interaction. But detail mechanisms of the suppression of contraction by BDM are still unknown. In this study, we examined how BDM affects the transverse stiffness of myofibrils and muscle fibers by AFM.

Myofibrils and muscle fibers prepared from psoas muscle of rabbit were used. The transverse stiffness was measured as in our previous studies. Modified AFM cantilevers having a glass rod attached to the tip of AFM cantilever were prepared and used for measurements to minimally alter the actin-myosin lattice structure. The tip of AFM cantilever was approached to the surface of preparations attached to coverslip, and force-curves were measured to obtain the transverse stiffness.

The transverse stiffness of myofibrils decreased in the order of rigor state > contracting state > contracting state (+BDM) > relaxed state. Remarkably the transverse stiffness of myofibrils was much greater in contracting state (+BDM) than in relaxed state. For muscle fibers, the transverse stiffness decreased in the same order as above for myofibrils. These results indicate that cross-bridges are formed in contracting state (+BDM), and suggest that the suppression of muscle contraction by BDM is not simply due to the inhibition of cross-bridge formation.

O47 (2O-11H4)

The effects of temperature on the generation of pacemaker potentials recorded from the mouse small intestine.

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Interstitial cells of Cajal distributed in myenteric region (ICC-MY) generate spontaneous electrical activity called pacemaker potential in gastrointestinal tract. Pacemaker potentials recorded from mouse small intestine have two components: rapidly rising primary component and following plateau component. In the present study the effects of temperature on the generation of pacemaker potentials were studied with conventional microelectrode techniques. Elevation of temperature increased the frequency and maximum rate of rise (dV/dt_{max}) of pacemaker potentials and decreased their duration, without affecting the resting membrane potential and amplitude. CPA (3μM), an inhibitor of internal Ca pump, abolished pacemaker potentials at around 28°C, while CPA only shortened the duration of pacemaker potentials with the depolarization of the membrane at around 40°C. KCN (30μM), an inhibitor of metabolic process in mitochondria, decreased the frequency of pacemaker potentials without changing the amplitude and dV/dt_{max}. These results suggest that the sensitivity to temperature is different between two components (primary and plateau) of pacemaker potentials. The primary component may be generated by the activation of temperature sensitive Ca permeable channels. On the other hand, the plateau component seems to be generated by temperature insensitive mechanisms. The results also suggest that mitochondrial function seems to be uninvolved in the formation of two components of pacemaker potentials.

O48 (2O-11H5)

Tyrosine-kinase-related Mechanism Confers Smooth Muscle L-type Ca^{2+} Channel to a Second Open State

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In contrast to cardiac myocytes, sympathetic stimulation does not largely enhance L-type Ca^{2+} channel current in smooth muscle cells. In the present study, we assessed possible mechanisms underlying this discrepancy, using a whole-cell clamp technique. In guinea-pig detrusor cells, only L-type Ca^{2+} channels occur. During depolarizations of large positivity, the conformation of the majority of Ca^{2+} channels is converted from the normal (O_1) to a second open state (O_2), in which Ca^{2+} channels do not, or only slowly inactivate during depolarization. This feature of the O_2 state produces U-shaped inactivation. In order to estimate the population of Ca^{2+} channels that can be converted to the O_2 state, we applied a paired pulse protocol: Test steps with and without preconditioning step (+80 mV, 4s) were alternately applied. Extracellular application of genistein decreased the amplitudes of both conditioned and unconditioned test inward currents (A_{cond} and A_{uncond}), accompanied by significant reduction of A_{cond}/A_{uncond}, while genistin, an inactive analogue, did not. Intracellular application of genistein caused similar or more pronounced effects, when ATP was removed from the patch pipette. This result is consistent with the fact that ATP antagonizes the inhibitory effect of genistein on tyrosine kinase activity. It is concluded that even under normal conditions smooth muscle L-type Ca^{2+} channels are already in a "stimulated mode° due to a tyrosine-kinase-related mechanism(s).

ORAL
Ionic channels & receptors

O50 (2O-09F2)

Voltage sensors influence ciguatoxin effects on $Na_V1.4$ Na^+ channels

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The synthetic ciguatoxin, CTX3C has four distinct effects on Na channels:1) to speed up time-to-peak Na current (I_{Na}), 2) to suppress peak I_{Na} amplitude, 3) to shift the activation curve in the hyperpolarizing direction, and 4) to delay recovery from "slow inactivation." In this study, we explored possible sites modulating CTX effects. Mutant channels N434A, L437A or A438K lacked effect 3 of CTX3C. These sites in D1S6 selectively modulate activation mechanisms *via* CTX binding. In separate experiments, we systematically replaced positively charged amino acids (Arg or Lys) with a neutral residue (Gln) in each S4 segment of the four domains. In all domains, neutralization of positive charges did not suppress the hyperpolarizing shift of the activation curve induced by the toxin (effect 3). However, CTX3C(3μM) did not suppress I_{Na} in the four mutants with neutralized charges in D2 (lack of effect 2). Interestingly, some of the D2 mutants even exhibited an increase in I_{Na} in the presence of CTX3C: I_{Na} grew progressively larger as the location of the mutations approached the extracellular face of the membrane. CTX3C is a membrane-spanning molecule having a length of 30 Å and possessing a relatively constrained structure. Thus, CTX3C may affect the voltage sensor by accessing the sodium channel from its exterior surface through the membrane lipid phase.

O49 (2O-09F1)

Molecular regions underlying the voltage-dependence of L-type Ca^{2+} channel $Ca_V1.3$ which activates and inactivates at the lower-voltage relative to $Ca_V1.2$

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$Ca_V1.2$ (α1C) and $Ca_V1.3$ (α1D) are pore-forming subunits of cardiac L-type Ca^{2+} channels. We have previously shown that $Ca_V1.3$ activates and inactivates at more negative voltages than those of $Ca_V1.2$, thus contributing to the threshold and the duration of the pacemaker action potential in SA nodal cells. To elucidate molecular regions underlying the unique voltage-dependence of $Ca_V1.3$ in comparison with $Ca_V1.2$, we examined chimeras of $Ca_V1.2$ and $Ca_V1.3$, and found that repeat II of $Ca_V1.3$ contains a critical domain for the negative shift of its voltage-dependence. In the present study, we further localized the critical regions of the unique voltage-dependence in repeat II of $Ca_V1.3$. We introduced a series of point mutations in $Ca_V1.2$ and $Ca_V1.2/Ca_V1.3$ chimera. The mutant Ca^{2+} channels were transiently expressed in BHK6 cells and analyzed in patch-clamp experiments. As a result, F618L at the outside of three Rs in IIS4 reversed the negative shift of the steady-state inactivation of $Ca_V1.2/Ca_V1.3$ chimera channel, indicating that F618 is one of regions underlying the voltage-dependence of $Ca_V1.3$. These results suggest that the difference in a single amino acid in IIS4 between $Ca_V1.2$ and $Ca_V1.3$ confers the unique voltage-dependence in cardiac L-type Ca^{2+} channels.

O51 (2O-09F3)

Structural and functional correlation of the pore region of KCNQ3 channel in the brain neurons

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Heterotetramers of two kinds of KCNQ subunits form voltage-dependent K channels called M channels in the brain neurons. The outward current from M channels repolarizes the action potential. It is reported that point mutations in KCNQ3 cause a neuronal channelopathy. In this study, we primarily examined the functional consequences by mutations. Whole-cell currents were measured from the mutant KCNQ3 channels expressed in HEK293 cells. **1**-1) Homomeric channels composed of mutant KCNQ3 lacked currents. **1**-2) Heteromeric channels composed of wild-type KCNQ2 and mutant KCNQ3, which were mimicked to the native M channels, were lower in conductance than those composed of wild-type KCNQ2 and wild-type KCNQ3. **1**-3) The activation curve of heteromeric channels composed of wild-type KCNQ2 and mutant KCNQ3 was shifted to the right than that composed of wild-type KCNQ2 and wild-type KCNQ3. We also reconstituted the three-dimensional protein structure of the mutant K channels. **2**-1) The selectivity filter of wild-type KCNQ3 channel was structurally supported by chemical bonds. **2**-2) The mutation, however, lead to a loss of chemical bonds. Such a structural alteration may result in the lower conductance in mutant KCNQ3 channels.

O52 (2O-09F4)

Cytokines affect activity of an inwardly rectifying K channel in cultured human proximal tubule cells

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An inwardly rectifying K channel with inward conductance of 40 pS is the most frequently observed K channel in cultured human proximal tubule cells. We have previously reported that the activity of this K channel was modulated, at least in part, by nitric oxide (NO) which was mainly derived from inducible NO synthase (iNOS) in these cells. Since cytokines are known to enhance the expression of iNOS mRNA, we explored the effects of interleukin-1β (IL), tumor necrosis factor-α (TNF) and interferon-γ (IFN) on the K channel activity, using the patch-clamp technique and RT-PCR. After 24-h incubation of cells with each cytokine, iNOS mRNA was significantly increased by IFN (100 U/ml) but not by IL (10 U/ml) and TNF (200 U/ml). In cell-attached patches using the IFN-treated cells, a NOS substrate, L-arginine (500 μM) suppressed channel activity, whereas a NOS inhibitor, L-NAME (100 μM) stimulated it. These observations were in sharp contrast to the case with control cells where L-arginine was stimulatory and L-NAME was suppressive. Acute effects of cytokines on channel activity were tested in cell-attached patches using control cells. Addition of IL to the bath suppressed channel activity, which was not restored by the subsequent addition of L-arginine. On the other hand, addition of IFN stimulated channel activity and this stimulatory effect was not abolished by L-NAME. TNF had little effect on channel activity. These results suggested that IFN affected K channel activity through the NO-dependent and -independent pathways whereas the effect of IL was NO-dependent.

O53 (2O-09F5)

Implication of plasma membrane raft in modulation of CFTR channel function

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Cystic fibrosis transmembrane conductance regulator (CFTR) plays a central role in transepithelial fluid and electrolyte transport as a Cl^- channel and also as a regulator of other transporters. CFTR channel function is widely deviated among several different CFTR epithelia whereas any CFTR subtype has never been identified. Recent evidence suggests that ion channels can target to membrane lipid raft and associate with other regulatory proteins. In this study, we have investigated the role of cholesterol, an important component of lipid raft, in the regulation of CFTR channel function using patch-clamp technique.

In cell-attached patches, cholesterol-depletion by cyclodextrin treatment inhibited CFTR channel activity Po from 0.35±0.03 to 0.15±0.02 with decreasing burst duration (T_b) from 2.3±0.3 to 0.31±0.03 msec and interburst duration (T_{ib}) from 3.4±0.6 to 1.8±0.03 msec (n = 5). On the contrary, cholesterol-enrichment by treatment with cyclodextrin-solubilized cholesterol slowed the CFTR channel gating with prolonging T_b to 5.6±1.1 msec and T_{ib} to 10.8±3.5 msec (n = 5). These results indicate that cholesterol plays a critical role in regulation of CFTR channel kinetics. We also found that CFTR channels expressed in Hi-5 insect cell line which is known not to synthesize cholesterol exhibited a similar gating kinetics to those in cholesterol-depleted NIH3T3 cells. We speculate that CFTR channels localize to lipid raft and are tissue-specifically modulated.

O54 (2O-09F6)

Localization and function of isoforms of the Cl- channel-related molecule, CLCA in ductal cells of rat submandibular gland

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A molecular entity for Ca^{2+}-dependent Cl^--transport has not been well characterized in rat salivary glands. We previously identified a new member of the CLCA family, rCLCA, which we isolated from rat ileum by a PCR-based strategy. Here, we report that rCLCA isoforms are expressed in the submandibular gland (SMG). The full length of rCLCA mRNA is 3.3 kb, and the predicted ORF encodes a 903-a.a. protein. The amino acid sequence of rCLCA protein has 83% homology to murine CLCA1 and 2. Transient transfection of HEK293 cells with rCLCA cDNA resulted in a marked Ca^{2+}-dependent Cl^--conductance with an outward rectification in the I-V relationship. Intense immunostaining was detected in the striated ducts of SMG, but not in the acinar cells. Immunoblot of the membrane fraction of the SMG yielded N-glycosylated 137- and 90-kDa bands. Interestingly, we also found the expression of a truncated isoform (60 kDa, presumably 514 a.a.) including the N-terminal 455 a.a. of the full-length form. Comparison of this mRNA with that of the full-length form revealed that exon 10 is truncated in this isoform. The full-length of rCLCA is likely to be responsible for modulation of Ca^{2+}-dependent Cl^- transport in the ductal cells, although the function of the trancated isoform was not defined.

O55 (2O-09F7)

Expression and localization of aquaporin-6 in the rat parotid gland

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Objectives: Aquaporins (AQPs) are 6 trans membrane proteins facilitating water transport via a plasma membrane and are involved in water, urea, glycerol or lipid transport. It has currently demonstrated that AQPs are located in intracellular organelle and their localization is changed by cell stimulation. In this study, we investigated AQP6 in the rat parotid gland.Methods: Plasma membrane and secretory granule fractions were isolated from the rat parotid glands using parcoll gradient. mRNA expression was determined by RT-PCR. Protein expression was determined by western blotting analysis. Morphological localization was observed by confocal microscopy and electron microscopy with immunolabelled ultrathin-cryosection. Results and Discussion: In RT-PCR, 262 bp band of AQP6 was detected in parotid acinar cells. In western blotting analysis using anti-AQP6 antibody, only 29 kDa protein was detected in plasma membrane and secretory granule membrane fractions of the parotid gland. In confocal microscopy, immunofluorescence with anti-AQP6 antibody was positive at the apical sites, especially cell-cell junctional region, in parotid acinar cells. When the glands were stimulated by β-agonist, AQP6 was observed to accumulate at the apical sites. These results suggest that AQP6 expresses in the apical sites and secretory granule membrane in the rat parotid gland as a monomeric form. The change of localization of AQP6 appears to be involved in exocytosis.

O56 (2O-09F8)

Spontaneous Ca Oscillations activate NFAT in undifferentiated Human Mesenchymal Stem Cells

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Previously, we have shown that Ca oscillations modulate the activities of ion channels and the fluctuation of membrane potentials in human mesenchymal stem cells (hMSCs). We have also found an ATP autocrine/paracrine signaling pathway as the mechanism for Ca oscillations. In this study we further investigated whether Ca dependent transcription factors are regulated by Ca oscillations by examining the localization of NFAT and NF-κB through immunocytochemical experiments. NF-κB was found to be more abundant in the cytosol than in the nucleus in most undifferentiated hMSCs (140/151 cells). On the other hand, nuclear staining of NFAT was detected in more than 80% of hMSCs (40/48 cells). The nuclear translocation of NFAT was also confirmed by Western blotting of the cytoplasmic and nuclear fractions. These findings suggest that NFAT is constitutively activated in hMSCs. When an ATP autocrine and Ca oscillations signaling pathway was blocked, the NFAT signals in the nucleus were reduced, indicating the involvement of Ca oscillations in the regulation of NFAT activation. Using adipogenic techniques we differentiated hMSCs into adipocytes. In these adipocytes, no spontaneous Ca oscillations were observed (32/32 cells). In the immunocytochemical experiments, the nuclear staining of NFAT was not prominent. Thus, we concluded that Ca oscillations might play an important role in NFAT activation in undifferentiated hMSCs but not in derived adipocytes.

O57 (3O-15F2)

High water intake compensates for the capacity to excrete a normal potassium load in dominant negative Kir7.1 transgenic mice.

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Recent data have shown that inward rectifier K^+ channel Kir7.1, localized in the basolateral membrane of rat distal nephron (JASN 2000; 11; 1987-1994), may be involved in the development of renal K^+ excretion (Kidney Int 2003; 63; 969-975). To further assess the role of Kir7.1 in kidney K^+ excretion, we have generated transgenic (Tg) mice expressing dominant-negative mutant of Kir7.1 (dnKir7.1 Tg/+). Wild-type (WT) and dn Kir7.1 Tg mice were placed in metabolic cages and their water balance and urine osmolality and concentrations of urine electrolytes (Na^+, K^+, Cl^-) were examined. Both WT and Tg mice fed a K^+-free diet developed hypokalemia (2.4 and 1.7 mEq/l, respectively) at 3 days and after. Water intake was increased in WT, but not in Tg mice. On the other hand, Tg mice fed a normal diet demonstrated a significant polydipsia and polyuria, with a relatively lower urine osmolality as compared to WT mice. Further, urinary K^+ excretion of WT and Tg mice increased in proportion to that their water increase. There was no significant difference in renal K^+ excretion between WT and Tg mice. In conclusion, Tg mice being insufficient with the basolateral Kir7.1 of the kidney distal nephron probably drink more water to compensate for the ability to excrete a K^+ load.

O58 (3O-15F3)

Block of the astroglial inward-rectifier Kir4.1 by the antiarrhythmic agent quinidine

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Neurological research has traditionally emphasized neuronal components of the nervous system. Recent findings suggest, however, that astrocytes harbor greater (patho-)physiological significance than previously thought. Known to provide mechanical support and chemical control of the neuronal environment (neurotransmitter clearance, extracellular K+ siphoning, etc.), newer data now additionally tie astrocytes to pharmaceutical pathways (Nature 433:73) and neurological disorders such as epilepsy (Nat. Med. 11:973), calling for a clearer molecular description of astrocyte function. The inwardly-rectifying K+ channel Kir4.1 (KCNJ10) is exclusively expressed in astrocytes in the brain. Due to its central role in astroglial K+ buffering, Kir4.1 is likely a critical element of astrocyte performance. We examined the effects of various neuroactive and neurotoxic compounds on human Kir4.1 using heterologous expression and the whole-cell configuration of the patch-clamp technique and found that the antiarrhythmic/antimalarial agent quinidine reversibly blocked Kir4.1 current in a time-dependent manner at concentrations slightly above the therapeutic range. Voltages positive to the Nernstian potential strongly enhanced Kir4.1 block, but frequency and number of the supplied depolarizing pulses seemed to be of little importance. It is conceivable that electrochemical alterations caused by astroglial ion channel block are in fact responsible for numerous so-far unexplained neurological effects of commonly administered therapeutic agents.

O59 (3O-15F4)

Single molecule imaging of stretch-activated BKca channels on the plasma membrane of cultured cells: immobilization on focal contacts

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Mechano-sensitive channels (MSCs) have been implicated to mediate the mechano-sensation of various types of cells. It has been well established that, in prokaryotes, MSCs can be activated directly by tension in the membrane. In contrast, the mechanism for eukaryotic MSCs activation has remained unclear. Since isolated eukaryotic MSCs in liposome could not be activated, it is proposed that eukaryotic MSCs may form molecular complex with other accessory proteins such as cytoskeletons for their function. To address this issue, we have investigated lateral mobility of hSAKcaCs (human stretch activated and Ca^{2+}-activated big K channels), which we have recently identified, in the plasma membrane of living cells. We have expressed GFP-tagged hSAKcaCs in HeLa cells and observed individual channels by single fluorophore video imaging using total internal reflection fluorescence microscopy. Most of hSAKcaCs exhibited simple diffusion (diffusion coefficient, 0.4 $\mu m^2/s$ on average), but approximately 25% of the channels was almost stationary. The observation of single channels of hSAKcaC simultaneously with RFP-tagged paxillin showed that these immobilized channels were specifically localized on/near focal contacts. These results suggest that hSAKcaCs may associate with the force-transmitting modules including adhesion molecules and cytoskeletons to form a mechano-sensing device.

ORAL
Neurons & synaptic functions

O60 (1O-04F1)

Long lasting spontaneous Ca^{2+} transients in the striatal cells.

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The striatum plays an important role in linking cortical activity to basal ganglia outputs. We conducted the Ca^{2+} imaging study to investigate the spontaneous activities of the striatum.

Corticostriatal slices of 10-25 days old Sprague Dawley rats were stained with Fura-PE3-AM to measure intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) using a cooled-CCD imaging system.

Long lasting spontaneous $[Ca^{2+}]_i$ transients, which lasted up to about 250 s, were observed. Most cells exhibited irregular frequencies, but some exhibited oscillatory feature. A pairwise correlation analysis revealed that some cells appear to belong to a correlated network. Administration of TTX or of CNQX + AP5 did not block the $[Ca^{2+}]_i$ transients. Therefore, the action potentials and the excitatory synaptic inputs in the striatal network were not involved in induction of the $[Ca^{2+}]_i$ transients. In contrast, the number of active cells, which exhibited the $[Ca^{2+}]_i$ transients, was greatly reduced by the intracellular Ca^{2+} store depletor, thapsigargin, and was reduced little by the administration of the Ca^{2+}-free saline. Therefore, the intracellular Ca^{2+} store is likely to contribute to the $[Ca^{2+}]_i$ transients.

In the mouse, which expressed green fluorescent protein (GFP) in astrocytes (GFAP-GFP mouse), both the GFP positive cells and the GFP negative cells exhibited the $[Ca^{2+}]_i$ transients. These results suggested that both astrocytes and neurons may exhibit the long lasting spontaneous $[Ca^{2+}]_i$ transients in the striatum.

O61 (1O-04F2)

Drebrin A is involved in spine morphology *in vivo*

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Dendritic spines are the major sites of excitatory synaptic transmission, and their morphogenesis plays a pivotal role in neuronal development and plasticity. Recent studies suggest the correlation between spine morphology and spine functions. Diversity in synapse functions is related to the diversity in spine morphology. As one of the candidate which control spine morphology, we focused on a major actin-binding protein in the brain, drebrin A (DA). We hypothesized that spine morphology depends on DA, and we analyzed spine ultrastructure in terms of DA localization using immunoelectron microscopy. We have recently reported that only 75% of spines are DA immuno-positive in adult rat cortex. We first analyzed if there was any morphological differences between DA immuno-positive spine (DPS) and DA immuno-negative spines (DNS). We found that DPS were larger in the spine head area and in the post synaptic density (PSD) length. Next, we analyzed if overexpression of DA changed spine morphology, using DA transgenic mice (Tg) that overexpressed DA in the forebrain. Similar to wild type mice (WT), DPS were larger than DNS in Tg; however, spine head area of DPS was significantly smaller than that of WT. The amount of DA within each spine did not change between two genotypes. These data suggest that overexpression of DA leads to smaller DPS with higher concentration of DA. There were no differences between two genotypes in the ratio of DPS to DNS, the spine density, and the DA localization within spines. These results indicate that DA is involved in spine morphology *in vivo* although its molecular mechanism is not yet clarified.

O62 (1O-04F3)

Effects of maintenance of body temperature on hippocampal neural activity: control of membrane potential through TRPV4 activation

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TRPV4 is one of the member of themo-TRP channels. We screened brain-*TRPV4* mRNA expressions by *in situ* hybridization, and found hippocampus strongly expressed *TRPV4* in addition to choloid plexus, where *TRPV4* expression has been reported. We speculated that TRPV4 might be an important ion channel to regulate hippocampal functions. Therefore, we established a new dissociated neural culture system from neonatal mice hippocampi. TRPV4 protein was localized in soma and dendrites in the culture. We also examined whether functional-TRPV4 was expressed in hippocampal neurons by a Ca^{2+} imaging method with Fura2. The hippocampal neurons responded to the all reported stimulus, such as heat (>32 °C), hypotonic stimulus and 4αPDD, but no response was observed in the *TRPV4*-mutant neurons. We considered that body temperature activates brain-TRPV4, and the activation might contribute to slight depolarization of the resting membrane potential (RMP). Next, we compared the RMP between wild type and *TRPV4*-mutant neurons at 37 °C, and found the wild type RMP was approximately 5 mV higher than the *TRPV4*-mutant RMP. We also performed current-injection experiments in both neurons, and found that *TRPV4*-mutant neurons required much bigger currents to get their firing. We conclude that TRPV4 is activated by body temperature in hippocampus, and produces proper environments for their firing.

O63 (1O-04F4)

Cannabinoid CB1 receptor at excitatory presynaptic site in the hippocampus and cerebellum

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Endocannabinoids mediate short- and long-term suppression of synaptic transmission. Nature of presynaptic cannabinoid receptor has been investigated for both excitatory and inhibitory synapses in various regions of the brain. For inhibitory synapses, importance of type 1 cannabinoid receptor (CB1) is generally accepted. For excitatory synapses, however, results are controversial. In the present study, we used electrophysiological and immunohistochemical techniques, and examined the type of cannabinoid receptors functioning at hippocampal and cerebellar excitatory synapses. Using CB1-knockout mice, we demonstrate predominant contribution of CB1 to excitatory synaptic transmission on CA1 pyramidal neurons in the hippocampus and that on cerebellar Purkinje cells from climbing fibers and parallel fibers. The presence of CB1 at presynaptic terminal was confirmed by immunohistochemical experiments with specific antibodies against CB1. In immunoelectron microscopy of the hippocampus and the cerebellar cortex, densities of CB1-positive signal in excitatory terminals were much lower than in inhibitory terminals, but clearly higher than the background level. These results clearly indicate that CB1 is responsible for cannabinoid-dependent suppression of excitatory transmission in the hippocampus and cerebellum.

O64 (1O-04F5)

Morphological and behavioral analyses of mice lacking adult form of drebrin

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Drebrin A is a neuron-specific actin-binding protein, which is localized in mature dendritic spines. During synapse formation, embryonic isoform, drebrin E is converted into neuron-specific isoform, drebrin A. We have demonstrated that suppression of the upregulation of drebrin A attenuated spine formation in vitro. To investigate physiological differences of drebrins E and A, we generated mice, in which isoform conversion of drebrin did not occur, by targeted disruption of drebrin A specific exon (DAKO). In these mice total amount of drebrin was not changed since drebrin E was overexpressed instead of drebrin A. We first analyzed their dendritic spine morphology on apical dendrites of layer V pyramidal cells in somatosensory cortex using rapid Golgi staining. The number and length of dendritic spine in adult DAKO mice (16-18 week old) were comparable to that in wild-type mice. We next analyzed behavioral phenotypes in DAKO mice. These mice showed impaired context-dependent fear conditioning, a hippocampal NMDA receptor-dependent learning task. Our findings indicate that isoform conversion of drebrin is required for regulation of synaptic function. In contrast, total amount of drebrin is important for regulation of spine morphology.

O65 (1O-04F6)

Dopamine-induced potentiation of the hippocampal mossy fiber synaptic transmission

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Dopamine is thought to play important roles in various brain functions including learning and memory. In the hippocampus, dopamine has been shown to modulate synaptic transmission and plasticity in the CA1 region. However, it remains largely unknown how dopamine affects synaptic transmission in the CA3 region where forms of synaptic plasticity distinct from those in the CA1 region have been demonstrated. In the present study, we show that application of dopamine reversibly potentiates synaptic transmission between the mossy fibers (MFs) and CA3 pyramidal cells. Pharmacological experiments showed that D1-like receptors mediate the potentiation induced by dopamine. This potentiation was accompanied by a decrease in the magnitude of synaptic facilitation and occluded by application of forskolin, an adenylate cyclase activator, suggesting the involvement of presynaptic cAMP-dependent mechanisms. The MF synaptic transmission is kept inhibited by tonic activation of presynaptic adenosine A1 receptors and $GABA_B$ receptors. It is known that dopamine and adenosine counteract each other in some brain regions and that adenosine receptor antagonists can enhance effects of dopamine receptor agonists. At the MF synapse, application of an adenosine receptor antagonist alone, or together with a $GABA_B$ receptor antagonist, enhanced the synaptic transmission, but suppressed the dopamine-induced potentiation. These results suggest that dopamine-induced presynaptic potentiation of the MF synaptic transmission is unmasked by the tonic presynaptic inhibition mainly mediated by adenosine.

O66 (1O-04F7)

Imaging analysis of associative LTP in rat hippocampal CA1 region

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Associative LTP by two independent inputs is known to be an important feature of synaptic plasticity observed in the CA1 of rat hippocampus. However, it is still unclear what kind of physiological role, especially in relation with memory and learning mechanisms, it plays and how it is inducted by two pathways. In order to investigate these questions, the specio-temporal pattern of neuronal activities during LTP induction followed by long lasting later period were studied by using both voltage-sensitive dye optical and extracellular electrical recording. Moreover, the possible contribution of back-propagating action potentials invaded into dendritic trees to the induction mechanisms of the present LTP was investigated under the assumption of STDP (spike timing dependent plasticity) mechanisms. In order to induce associative LTP rat acute slice preparations stained with Di-4-anepps were stimulated by two independent Schaffer collateral pathways with the stimulation protocol reported previously; one pathway is stimulated weakly under threshold (single pulse) while another pathways is strong over the threshold (multiple pulses). Optical signals were recorded and analyzed by using the photodiode array system (Neuro-plexVI; Redshirt Imaging, USA). The obtained results from the present study are discussed from the view point of non-linear summation of two independent inputs and its distribution along the dendrite, together with the possible induction mechanisms obtained from low TTX experiment.

O67 (1O-04F8)

NEURON AND SYNAPTIC FUNCTIONS-A gene expression fingerprint of C. elegans embryonic motor neurons

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NEURON AND SYNAPTIC FUNCTIONS Differential gene expression specifies the highly diverse cell types that constitute the nervous system. With its sequenced genome and simple, well-defined neuroanatomy, the nematode C. elegans is a useful model system in which to correlate gene expression with neuron identity. The UNC-4 transcription factor is expressed in thirteen embryonic motor neurons where it specifies axonal morphology and synaptic function. These cells can be marked with an unc-4::GFP reporter transgene. Here we describe a powerful strategy, Micro-Array Profiling of C. elegans cells (MAPCeL), and confirm that this approach provides a comprehensive gene expression profile of unc-4::GFP motor neurons in vivo.ResultsFluorescence Activated Cell Sorting (FACS) was used to isolate unc-4::GFP neurons from primary cultures of C. elegans embryonic cells. Microarray experiments detected 6,217 unique transcripts of which ~1,000 are enriched in unc-4::GFP neurons relative to the average nematode embryonic cell. The reliability of these data was validated by the detection of known cell-specific transcripts and by expression in UNC-4 motor neurons of GFP reporters derived from the enriched data set. In addition to genes involved in neurotransmitter packaging and release, the microarray data include transcripts for receptors to a remarkably wide variety of signaling molecules.

O68 (2O-10G2)

Re-evaluation of GABA releasing hypothesis from hippocampal mossy fiber terminals

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It has been hypothesized that hippocampal mossy fiber terminals of young rodents release GABA in addition to glutamate. Using whole cell-clamp recordings in mouse slice preparations, I re-examined this possibility with paying attentions about the conditions to evoke monosynaptic GABAergic responses by stimulation of mossy fibers. Strong stimulus to the stratum granulosum of dentate gyrus or stimulus to the stratum lucidum of CA3 region elicited IPSCs in CA3 neurons in the presence of glutamate receptor antagonists 10 μM CNQX and 25 μM D-AP5, and these putative "monosynaptic IPSCs" were abolished by addition of $GABA_A$ receptor antagonist 100 μM picrotoxin. In contrast, weak stimulus to the stratum granulosum never elicited IPSCs in the presence of CNQX and D-AP5. The responses to weak and strong stimuli also displayed differential sensitivity to group II mGluR agonist DCG-IV; application of 1 μM DCG-IV almost abolished the responses to weak stimulus and left substantial responses to strong stimulus which were inhibited by further application of picrotoxin. These results suggested that strong stimulus to stratum granulosum causes monosynaptic IPSCs by stimulating inhibitory interneurons in addition to mossy fibers, and mossy fiber terminals themselves may not release GABA.

O69 (2O-10G3)

Regultation of neurotransmitter release by tomosyn phosphorylation

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Regulation of neurotransmitter release by tomosyn phosphorylation. Sumiko Mochida[1], Takeshi Baba[2], Toshiaki Sakisaka[2], and Yoshimi Takai[2]. [1]Depatment of Physiology, Tokyo Medical University, Shinjuku 160-8402, Japan, and [2]Department of Molecular Biology and Biochemistry, Osaka University Graduate School of Medicine/Faculty of Medicine, Suita 565-0871, Japan.PKA phosphorylation of tomosyn, a SNARE regulatory protein, significantly decreased its binding to syntaxin-1 in vitro, and cAMP stimulation increased the phosphorylation of tomosyn and decreased tomosyn binding to syntaxin-1, resulting in enhanced SNARE complex formation. Overexpression of tomosyn in cultured superior cervical ganglion neurons inhibited neurotransmitter release which could be rescued by the introduction of cAMP into the presynaptic neuron. Expression of tomosyn S724A, a PKA unphosphorylated mutant, or knock-down of tomosyn by siRNA introduction also decreased neurotransmitter release, but was not rescued by cAMP. Under high frequency stimulation, expression of tomosyn S724A and S724D, a PKA phosphorylated mimic mutant, increased the EPSP failure rate and asynchronous EPSPs. These results indicate that tomosyn is a physiologically significant PKA target that controls neurotransmitter release through the regulation of SNARE complex formation.

O70 (2O-10G4)

GABAergic modulation for TEA-induced synaptic plasticity in rat hippocampal CA1, CA3 and dentate gyrus

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Tetraethylammonium (TEA), a K^+ channel blocker, reportedly induces long-term potentiation (LTP) of hippocampal CA1 synaptic responses in an NMDA receptor-independent manner. However, the characteristics of TEA-induced plasticity and modulation by inhibitory interneurons at CA3 and the dentate gyrus (DG) remain unclear. This study recorded field EPSPs from CA1, CA3 and DG to examine the involvement of GABAergic modulation in TEA-induced synaptic plasticity at each region. In Schaffer collateral-CA1 synapses, bath application of 25 mM TEA for 12 min induced LTP in the presence and absence of 100 μM picrotoxin (PTX), a $GABA_A$ receptor blocker, suggesting little modulation by interneurons in the CA1 region. In CA3, associational fiber (AF)-CA3 synapses showed TEA-induced LTP regardless of PTX, but mossy fiber (MF)-CA3 synaptic plasticity were influenced by PTX application; TEA-induced LTP was detected only in the absence of PTX. In DG, synaptic plasticity was modulated by GABAergic inputs, but characteristics differed between lateral perforant path (LPP) and medial perforant path (MPP). LPP-DG synapses showed TEA-induced LTP in the presence of PTX, but no changes in the absence of PTX. At MPP-DG synapses, TEA-induced long-term depression (LTD) was observed in the absence of PTX, but no changes were seen in the presence of PTX. This series of results demonstrated that TEA-induced plasticity at perforant path-DG synapses and MF-CA3 synapses are modulated by GABAergic inputs, and that the effects of GABAergic modulation on plasticity are inhibitory (LPP- and MPP-DG) or excitatory (MF-CA3).

O71 (2O-10G5)

Changes in neuromodulatory effect of adenosine A1 receptors of piriform cortex in amygdala kindled rats

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Considering the anticonvulsant effects of adenosine and the anatomical connections between piriform cortex (PC) and amygdala, in this study, the effect of kindling implementation on adenosine A1 receptor mediated neuromodulation in PC pyramidal neurons was tested at 24 h and 1 month after amygdala kindling. Field potentials were recorded from layer II of PC following stimulation of the lateral olfactory tract. Obtained results showed that N6-cyclohexyleadenosine (CHA; i.c.v.; 1, 10 and 100 μM, as a selective A1 agonist) reduced A1 slope (as an index of EPSP slope) and B1 amplitude (as an index of spike amplitude) of field potentials in both kindled and non-kindled rats. However, its effects were more potent at 24 h, but not 1 month after kindling. Pretreatment of 1,3-dimethyl-8-cyclohexylexantine (CPT; i.c.v.; 50 μM, as a selective A1 antagonist), eliminated effects of CHA (i.c.v.; 10 μM). These results indicate that A1 receptors of PC have anticonvulsant effects on amygdala kindled seizures and the efficiency of the A1 receptor neuromodulation is increased at short- (24 h), but not long-term (1 month) after kindling implementation.

O72 (2O-10G6)

Analysis of gene expressions using RT-multiplex PCR on rat hippocampal neurons in acute slices

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The genome project elucidates that the whole genome possesses approximately 25,000 genes. Although it is thought that single cells express approximately 10,000 genes in various copy numbers in a cell-type specific manner, differences of gene expressions inside and among defined cell-types are not examined at a single cell level. Here, we examined mRNA expressions in acute rat hippocampal slices where the classification of cell types was most extensively examined. CA1 and CA3 pyramidal cells, dentate granule (DG) cells and interneurons were identified from their morphologies and the locations and cellular contents were harvested into patch pipettes. We performed RT-multiplex PCR of 31 genes, including biochemical markers of calcium binding proteins, neuropeptides and neurotransmitter receptors. Detection rates of each mRNA were examined. Except for correlations linked to glutamatergic and GABAergic neuronal markers, strong correlations were scarcely found. When cells were grouped according cell types, however, large differences of detection rates in many genes were observed. χ-square analysis revealed that 37% of tested genes were differently expressed in interneurons and principal neurons and that 30% were differently expressed in pyramidal cells and DG cells. Genes differentially expressed between CA1 and CA3 pyramidal neurons were not found. Dendrogram showing the distances of cell types was obtained by cumulative differences of detection rates.

O73 (2O-10G7)

Regulatory role of small molecule G-protein Arf1 and subsequent phospholipase D in the K^+-current response to dopamine in the ganglion cells of *Aplysia*

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Small molecule G-protein Arf1 in combination with phospholipase D (PLD) is essential for intracellular trafficking of the proteins from endoplasmic reticulum to Golgi apparatus. However, it is recently reported that it also regulate ionic channel activity presumably though recycling of the receptors and ionic channels at the cytoplasmic membrane. To examine possible involvement of Arf and subsequent PLD in regulation of receptor-induced responses in neuron, we recorded K^+-current response induced by dopamine (DA) in the ganglion cells of *Aplysia* under conventional two-electrode voltage clamp. Intracellular application of brefeldin A, a specific blocker of Arf GEF, significantly depressed the K^+-current response to DA. The DA-induced response was also inhibited by injection of 2-(4-fluorobenzoilamino)- benzoic methyl ester (Exo1), an activator of GAP for Arf1. Intracellular injection of N-terminal peptide of Arf1 markedly suppressed the DA-induced response. In contrast, application of those of Arf6 did not affect the response to DA. Furthermore, intracellular application of α-synuclein, a specific blocker of PLD, significantly depressed the K^+-current response to DA. In contrast, all these reagents had no significant effect on the Na^+-current response induced by acetylcholine in the same type of cells. These results suggest that Arf1 and subsequent PLD may regulate the K^+-current response induced by DA.

O74 (2O-10G8)

5-HT_{1B} receptor-mediated presynaptic inhibition at the calyx of Held

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5-hydroxytryptamine (5-HT; serotonin) inhibits transmitter release via activating GTP binding (G) proteins, but the target of this effect in the nerve terminal is not determined. We addressed this question at the calyx of Held synapse in brainstem slices of developing rats. In 5-day-old rats bath-application of 5-HT (10 μM) attenuated the amplitude of evoked EPSCs and facilitated paired-pulse ratio, whereas 5-HT had no effect on the amplitude of spontaneous miniature EPSCs. The 5-HT_{1B} receptor agonist CP93129 mimicked the inhibitory effect of 5-HT, but the 5-HT_{1A} agonist 8-OHDPAT had no effect. 5-HT_{1B} receptor antagonist NAS-181 blocked the inhibitory effect of 5-HT. These results suggest that 5-HT activate 5-HT_{1B} receptors in the nerve terminal, thereby inhibiting transmitter release. In whole-cell recordings from calyceal nereve terminals, 5-HT attenuated voltage-gated Ca^{2+} currents, but had no effect on voltage-gated K^+ currents. Upon repetitive application 5-HT showed tachyphylaxis with its effect on both EPSCs and presynaptic Ca^{2+} currents becoming weaker in the second application. Surprisingly 10 mM BAPTA loaded into the nerve terminal abolished the tachyphylaxis. The presynaptic inhibitory effect of 5-HT was robust at postnatal day 5, but became weaker as animals matured. We conclude that 5-HT_{1B} receptors can mediate presynaptic inhibition of transmitter release in immature calyceal terminals via inhibiting voltage-gated Ca^{2+} channels. Upon repetitive activation 5-HT_{1B} receptors may be internalized or desensitized by a Ca^{2+}-dependent mechanism.

O75 (3O-15F1)

The rate of refilling of synaptic vesicles with glutamate

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At the excitatory synapse neurotransmitter glutamate is released from synaptic vesicles. After exocytosis vesicles are retrieved by endocytosis and recycled for reuse, thereby maintaining synaptic transmission. During recycling vesicles are refilled with glutamate by vesicular glutamate transporters (VGLUTs), using electrochemical gradient produced by vacuolar-type H^+-ATPase (V-ATPase). Multiple recycling mechanisms of different speeds are thought to operate in the nerve terminal. However, the speed of vesicle refilling with glutamate is not known. Here we manipulated vesicular glutamate directly in the nerve terminal, depleting it by whole-cell dialysis and refilling it by the photolysis of caged glutamate. Vesicle refilling, monitored by postsynaptic currents, was abolished by blocking VGLUT or V-ATPase, and attenuated by increasing cytosolic Cl^- concentrations. The refilling time constant ranged 2-13 s depending upon the magnitude of refilling, with 100% refilling time constant being 18 s. This rate is faster than the "full-fusion"-type slow recycling time (1 min), but slower than "kiss-and-run"-type fast recycling time (<1s). We conclude that transmitter glutamate can fully refill vesicles recycled via slow, but not fast, pathways.

O76 (3O-15F5)

Activation of mGluRs during preconditioning low-frequency stimulation determines direction of synaptic plasticity in hippocampal CA1 neurons

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In hippocampal CA1 neurons, delivery of low-frequency afferent stimuli <LFS;80 pulses at 1 Hz> induced LTP in both the field EPSP and population spike <PS>. In the same cells, reversal of LTP in the EPSP and PS was achieved by the same LFS given 20 min after the first LFS <second LFS>. We investigated the effects of metabotropic glutamate receptor <mGluR> antagonists on LTP or LTD induced by the second LFS. When the first LFS was given in the standard solution, both the field EPSP and PS were attenuated by the second LFS. In contrast, when the first LFS was delivered in the presence of MCPG, a broad spectrum mGluR antagonist or 4CPG, a type 1 mGluR antagonist, the field EPSP and PS were enhanced by the second LFS. Thus, activation of mGluRs during preconditioning LFS stimulation determines the direction of synaptic plasticity at CA1 neurons. Then, we investigated the effects of an NMDA receptor antagonist, AP5 on synaptic plasticity induced by the second LFS. When the first LFS was given in the standard solution or in 4CPG but the second LFS was given in the presence of AP5, both the EPSP and PS were enhanced after the second LFS. These results indicate that the synaptic plasticity induced by the second LFS depends on NMDA receptor activation at CA1 synapses. Thus, it is possible that activation of mGluRs during prior synaptic activation resulted in enhancement of NMDA receptor activation, leading to reversal of LTP.

O77 (3O-15F6)

Glutamate NMDA receptors and MAPKs within the amygdala participated in the modulation effect of glucocorticoids on extinction

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The present study was aimed to elucidate the possible mechanism of glucocorticoids on the conditioned fear extinction by using fear-potentiated startle paradigm. We found that (1) Systemic administration of dexamethasone (DEX, 1.0 mg/kg, i.p.) accelerated extinction of conditioned fear. (2) Administration of glutamate NMDA receptor antagonists (±)-HA966 (6.0 mg/kg, i.p.) and intra-amygdala infusion of MK801 (0.5 ng/side, bilaterally) or D,L-2-amino-5- phosphonovaleric acid (AP5, 2.0 ng/side, bilaterally) blocked the DEX facilitation effect. (3) Blockade of corticosteroid synthesis inhibitor metyrapone (25 mg/kg. s.c.) on extinction was removed by co-administration of NMDA receptor agonist D-cycloserine (DCS, 5.0 mg/kg, i.p.). (4) Co-administration of DEX and DCS in a sub-threshold dose provided a synergistic facilitation effect on extinction (0.2 mg/kg and 5 mg/kg, respectively). However, DEX and DCS co-administration did not alter the expression of conditioned fear. (5) The facilitation effect of DEX was blocked by intra-amygdala infusion of mitogen activated protein kinase (MAPKs) inhibitors PD98059 (500 ng/side, bilaterally) or U0-126 (20 μM/side. bilaterally). DEX significantly enhanced the phosphorylation of MAPKs which induced by the extinction training. These results suggested that glutamate NMDA receptors and MAPKs within the amygdala participated in the modulation effect of glucocorticoids on extinction.

O78 (3O-15F7)

Effects of drebrin A knock down on glutamate receptor activities in developing hippocampal neurons

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Drebrin A (DA), an F-actin binding protein, is involved in spine morphogenesis. We have recently demonstrated that knock down (KD) of DA expression attenuates synaptic clustering of PSD-95, and decrease the number of dendritic protrusions in developing hippocampal neurons. Furthermore, *in-vivo* experiments indicate that DA-KD in the rat hippocampus impairs pre-pulse inhibition. These data suggest that DA plays a role in the regulatory mechanism of glutamate receptor activity in addition to that of spine shapes. To investigate the effect of DA-KD on glutamate receptor activities in vitro, 12-DIV hippocampal neurons were treated with antisense oligonucleotide specific to DA (AOD) and reverse AOD (ROD). Then at 14 DIV, NMDA and AMPA currents in mock-treated, AOD-treated, and ROD-treated neurons were measured using whole cell patch-clamp technique. We applied glutamate (1, 10, 30, 100 and 300 μM) in the presence of 50 μM AP5, and NMDA (1, 10, 30, 100 and 1000 μM) in the presence of 20 μM CNQX. The AMPA currents seemed larger in AOD-treated neurons than mock-treated and ROD-treated neurons. There were no clear differences in the NMDA currents between AOD-treated and ROD-treated neurons. The data suggest that drebrin A is involved in the regulatory mechanism of glutamate receptor activities.

O79 (3O-15F8)

Myosin II plays a pivotal role in glutamate-induced translocation of drebrin-binding actin filaments from dendritic spine to parent dendrite

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We have previously shown that glutamate induces the translocation of drebrin and F-actin from dendritic spines to the parent dendrites, which induces the morphological changes of dendritic spines into filopodia-like protrusion. Timelapse imaging of GFP-drebrin expressing neuron revealed that translocation occurred within 60 sec. Since myosin II B is involved in actin-drebrin complex in the brain, contribution of actin-myosin system to the translocation was investigated in this study. In the present study, we analyzed the effect of blebbistatin, a specific inhibitor of myosin II ATPase, on the glutamate-induced translocation of drebrin and F-actin in cultured hippocampal neurons. We prepared the low density cultures of rat hippocampal neurons from 18-day embryo. After pretreatment of 21 DIV neurons with 100 μM (±) blebbistatin for 60 min, we applied 100 μM glutamate for 10 min and analyzed the location of drebrin and F-actin immunocytochemically. Blebbistatin completely inhibited the glutamate-induced translocation of drebrin and F-actin. These data demonstrate that myosin II activity is required for translocation of drebrin and F-actin from dendritic spines to parent dendrites in hippocampal neurons.

O80 (1O-01C5)

Regulatory roles of gonadal hormones on the stimulatory effects of cocaine- and amphetamine-regulated transcript (CART) peptide in midbrain dopaminergic systems

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Dense expression of cocaine- and amphetamine-regulated transcript (CART) mRNA and peptide in the nucleus accumbens (NA) and striatum (ST) are found. NA and ST are the main projection sites of mesolimbic (ML) and nigrostriatal (NS) dopaminergic (DA) systems which are involved in the extrapyramidal motor system and ewarding and emotional behaviors, respectively. Whether gonadal hormones, estradiol (E) and testosterone (T), play the regulatory roles on the stimulation of CART peptide in the MLDA and NSDA systems were examined in Sprague-Dawley rats in this study. DA neuronal activities were determined by measuring the concentration of DOPAC (3,4-dihydroxyphenylacetic acid), the major metabolite of DA, in the NA and ST by HPLC-ECD. Intracerebroventricular administration of CART peptide increased the DOPAC content of NA and ST in ovariectomized (OVX) priming E, but not in OVX only female rats. The stimulation by CART peptide on the DOPAC contents of NA and ST was found only in intact, castrated (CAS) with E or T priming, but not in CAS only male rats. Finally, E and T antagonists blocked T effects, but only E antagonist could block E effects. All these findings indicate that gonadal hormones play the regulatory roles on the stimulation of CART peptide in MLDA and NSDA systems, and suggest that E is through intracellular genomic rather than extracellular non-genomic action.

ORAL
Sensory functions

O81 (1O-05G1)

Nature of Neural Clustering in Inferotemporal Cortex of Macaque Monkey

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Extracellular recordings in inferotemporal (IT) cortex suggest that there are columnar organizations in terms of the optimal stimuli for individual neurons (Fujita, et al., 1992). This finding is consistent with optical imaging experiments showing that a visual stimulus elicits a localized activation of the spots in IT cortex (Wang, et al., 1996, 1998). However, it is also known that object selectivity of nearby cells is not necessarily the same. Thus, our understanding of columnar organizations in IT cortex is still partial.

Here, we identified active spots revealed by optical imaging, and then recorded single unit activities and multiple unit activities (MUAs) from these spots. To quantify the similarity of object selectivity, we calculated correlation coefficients of responses to 100 object stimuli.

We found that only 30% of pairs of nearby single units showed significant correlation in object selectivity. However, many pairs of MUAs (63%) showed significant correlation in object selectivity. The difference in number of significant pairs suggests (1) that there is a common property among single cells, and (2) that averaging of responses of individual cells, such as MUA, decreases cell-to-cell variability in object selectivity and disclose common property among cells within a spot. In fact, when all MUAs in a spot were averaged, single unit activity and the averaged MUA showed significant correlation in object selectivity in 63% of single cells within the spot. However, we could not find such significant correlation between the averaged MUA of a spot and single unit activity recorded from outside of the spot.

O82 (1O-05G2)

Changes in expression of G proteins induced by dexamethasone and chronic pain explain for their inhibitory effects on development of analgesic tolerance to morphine administration

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The inhibitory effect of pain on tolerance development to analgesic effect of opioids is reported to be mediated by stress aspect of pain and activation of HPA axis. We tried to investigate whether the chronic pain and co-administration of dexamethasone (Dex) is able to reverse the tolerance and to evaluate expression of $G_{\alpha}i/o$ and G_{β} subunits of G proteins following chronic pain, chronic Dex, tolerance and their combination.Tolerance was induced by chronic intraperitoneal (i.p.) administration of morphine to male Wistar rats and analgesia was assessed using tail flick test. Lumbar spinal tissues were assayed for expression of G proteins using "semi-quantitative PCR" normalized to beta-actin.Both chronic pain and chronic Dex could reduce and reverse the tolerance. Chronic morphine did not change $G_{\alpha}i/o$ gene expression, while chronic pain and Dex both increased its expression. Expression of Gβ was increased following chronic morphine, but not following chronic pain and Dex. None of these increases were observed when morphine was co-administered with pain or Dex. It seems that the development of tolerance to analgesic effect of morphine is partially mediated by increased G_{β} gene expression. The increase in $G_{\alpha}i/o$ genes expression produced by chronic pain and chronic Dex can facilitate opioid signaling pathway and compensate for morphine-induced

O83 (1O-05G3)

Brain regions that distinguish muscle pain from skin pain

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Recent brain imaging studies have been revealing central processing of muscle pain. However, there is hardly a consensus on a brain region specifically responsible for muscle pain, not for skin pain. To seek out this specific region, we used event-related functional magnetic resonance imaging (MRI) and the 3-T MRI scanner. Twelve healthy male subjects participated in this study. Electric stimulation with 1-ms duration was applied to two sites: the left anterior tibial muscle and the skin just above it. The stimuli consist of three levels in strength both for skin and muscle stimulation, i.e. nonpainful, painful (5/10 in visual analog scale, VAS) and more painful (7/10 in VAS). Group analysis revealed that brain regions activated by muscle stimulation included ipsilateral superior frontal gyrus, postcentral gyrus (primary somatosensory cortex, SI), posterior cingulate; contralateral precentral gyrus, medium dorsal nucleus (thalamus), superior temporal gyrus; bilateral middle frontal gyrus, cingulate gyrus and lenticular nuclei. These regions are similar to those reported by other researchers and some of them are known to be activated during pain. Brain regions specifically activated by muscle stimulation, not by skin stimulation, were ipsilateral culmen, cingulate gyrus; contralateral lenticular nucleus, pons, paracentral lobule (SI), substantia negra, medial frontal cortex and bilateral middle temporal gyrus. These regions may play an essential role to distinguish muscle pain from skin pain in the brain.

O84 (1O-05G4)

P2Y receptor-mediated enhancement of inhibitory synaptic transmission in substantia gelatinosa neurons of the rat spinal cord

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To date, eight subtypes of metabotropic P2Y receptors, $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{12}$, $P2Y_{13}$ and $P2Y_{14}$ receptors, have been identified in mammals. Although numerous studies have demonstrated that ionotropic P2X receptors play a crucial role in facilitating pain transmission in the spinal cord, possible roles for P2Y receptors in nociceptive signaling have received limited attention. In this study, we examined whether the activation of P2Y receptors can modulate synaptic transmission in substantia gelatinosa (SG) neurons of adult rat spinal cord slices using whole-cell patch-clamp recordings. Bath application of UTP (100 μM), an agonist for $P2Y_2$ and $P2Y_4$ receptors, and UDP (100 μM), an agonist for $P2Y_6$ receptors, affected neither excitatory (glutamatergic) nor inhibitory (glycinergic and/or GABAergic) synaptic transmission in all 11 SG neurons recorded. 2-Methylthio ADP (30 μM), an agonist for $P2Y_1$, $P2Y_{12}$ and $P2Y_{13}$ receptors, also did not change excitatory transmission in all 11 SG neurons examined, but significantly increased the frequency and amplitude of spontaneous inhibitory (glycinergic and/or GABAergic) postsynaptic currents in 12 of 52 SG neurons recorded. These results indicate that the activation of 2-methylthio ADP-sensitive P2Y receptors enhances inhibitory but not excitatory synaptic transmission, probably through a direct action on spinal inhibitory interneurons. Thus, in contrast to P2X receptors, P2Y receptors can be involved in an inhibitory effect on pain transmission in the spinal dorsal horn.

O85 (1O-05G5)

Evidence from median nerve somatosensory evoked potentials for the activation of inhibitory cortico-coritcal pathway from area 4 to area 1 during finger movement in man

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Previous studies show that the spinal cord and subcortical components of the somatosensory evoked potentials are not attenuated during movement in man. The objective of the present study was to investigate the effect of simultaneous fractionated finger movement on cortically generated somatosensory evoked potentials. Left median nerve somatosensory evoked potentials were recorded from a scalp array of 21 electrodes over the right scalp in 9 normal subjects aged 18-31 years. They reclined, eyes closed as recordings were made at rest and during fractionated finger movements of the left hand. Latency and peak-to-peak amplitude of the parietal N20 and frontal P20 waves did not show statistically significant difference between rest and movement conditions. This represents activity in the tangentially arranged pyramidal neurons in area 3b. Amplitude of the parietal P25 wave, representing activity in radially arranged area 1 pyramidal neurons was attenuated during finger movement by 70% (P<,0.01; Wilcoxon's test). Fractionated finger movements utilise the corticospinal pathway from the motor cortex. In animal studies it has been shown that there is evidence for an inhibitory cortico-cortical pathway from area 4 (motor) to area 1 (sensory) cortex but not to area 3b. We propose that activity in this pathway is responsible for our findings. This can be considered as a physiological evidence for the function of a cortico-cortical pathway in man.

O86 (1O-05G6)

The dynamics of neural network and microcirculation: interhemispheric interactions and neurovascular coupling

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Interhemispheric neural interactions between bilateral cortical regions are critically dependent on interhemispheric time lag of cortical activation. In somatosensory systems the time lag information is processed for integrating bilateral stimulus inputs. Here we investigated interhemispheric interactions between bilateral somatosensory cortices by simultaneous recording of neural and hemodynamic signals and by analyzing their dependence on stimulus time lag between conditioning stimuli (CS) and test stimuli (TS). We measured electrophysiological signals (local field potentials, 1<LFPs<100 Hz; multiunit spiking activity, 300<MUA<5k Hz) and optical intrinsic signals (586 nm, cerebral blood volume, CBV; 605 nm, oxygenation) in rat somatosensory cortex evoked by electrical pulses to the contralateral hindpaw (TS) while delivering electrical pulses to the ipsilateral hindpaw (CS) under α-chloralose anesthesia. Both responses to CS-TS were normalized by the responses to TS without CS. The results showed that both electrophysiological (LFPs and MUA) and optical (586 nm monophasic and 605 nm biphasic activity) responses were significantly suppressed around 40-60 ms time lag and slightly augmented at 0 ms. Average and trial-by-trial correlation analyses revealed that CBV-related optical signals have high fidelity to integrated MUA and LFP negative components. Activity-related microcirculatory responses may more faithfully reflect neural interactions through brain network than we imagined.

O87 (1O-05G7)

Activation of TRPV4 by hyperosmolality

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TRPV4 is first reported to be a "hypoosmolality-sensing" cation channel. On the following studies with knockout mice (Trpv4-/-), we have reported that response of vasopressin to hypertonicity was exaggerated but another group has reported that it was abolished in Trpv4-/-. Although controversial in response, both reports suggest that TRPV4 can be responsible to hypertonic stimuli. To elucidate "hyperosmolality-sensing" in TRPV4, we designed to re-examine the response in vivo and investigate whether TRPV4 was sensitive to hyperosmolality in cultured neuronal cells. Trpv4-/- and Trpv4+/+ mice were subjected to dehydration from 24 to 96 hrs. Then serum osmolality and water intake were measured. There was not remarkable difference in serum osmolality at any period of dehydration but a significant decrease in serum osmolality of Trpv4-/- at 72 hrs dehydration. Water-crave behavior and amount of water intakes after the dehydrations were not changed. Thus TRPV4 channel may respond to hyperosmolality. Neuronal cell lines with and without TRPV4 were established from a cell line. Hyperosmoliality (500 mOsm) induced robust Ca influx in TRPV4 (+) cells by the method of fluorescence quenching, while not in TRPV4 (-) cells. The influx was partially blocked with genistine, a blocker of tyrosine kinase, and blunted with pBPB, a blocker of PLA2. Therefore, TRPV4 is hyperosmolality-sensng channel through several biochemical cascades.

O88 (1O-05G8)

Signal amplification in the olfactory sensory cilia

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Molecular mechanisms underlying olfactory signal amplification were investigated by monitoring cAMP dynamics in the intact sensory cilia. [cAMP]i increased superlinearly with time during odorant stimuli for over a second. This time course was remarkably different from that obtained with the rapid quench method previously applied to the in vitro preparation, in which [cAMP]i change has been reported to be transient. The superlinear increase of [cAMP]i was due to a gradual increase of cAMP production rate that was consistent with the thermo-dynamical interaction model between elemental molecules, as has been revealed on the rod photoreceptor cell. It thus seems likely that the fundamental mechanism for molecular interactions between olfactory transduction elements is similar to that of the rod. In olfaction, however, cAMP production was extremely small (~200,000 molecules/s/cell at the maximum), in contrast to the cGMP hydrolysis in the rod (250,000 molecules/photon). The observed numbers indicate that the olfactory receptor cell has lower amplification at the enzymatic cascade. Seemingly, such low amplification is a disadvantage for the signal transduction, but this unique mechanism would be essential to reduce the loss of ATP. Transduction by a smaller number of second messenger formations would be achieved by the fine ciliary structure that has a high surface-volume ratio. In addition, it is speculated that this low amplification at their enzymatic processes may be the reason why the olfactory receptor cell has acquired high amplification at the final stage of transduction channels, utilizing Ca^{2+} as a third messenger.

O89 (2O-08E2)

Analysis of bitter taste inhibition by fatty acids

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The effect of unsaturated fatty acids on taste sensitivity was explored by human psychophysical study, molecular biological study and mouse neuroethological study. Inhibitory effects of fatty acids on bitter taste including QHCl and denatonium were demonstrated in perceived taste intensity test in human, the mouse behavioral experiment using a short - term lick test (10 sec) and responses in the chorda tympani (CT) and the glossopharyngeal (GL) nerve in wild type mice. In contrast, fatty acids have no effect on bitter tasting L-amino acids, NaCl, HCl, sucrose and MSG in all study performed. To investigate involvement of Ggustducin in this inhibitory effect of fatty acids, nerve recording from gustducin KO mice and in vitro G-protein activation assay using bovine taste membrane were employed. Results from gustducin KO mice showed no suppression in bitter taste responses both in the CT and the GL. In vitro G-protein activation assay using bovine taste membrane showed that the activation of both gustducin and transducin by denatonium was significantly inhibited by DHA and oleic acid, and that the activation of transducin by rhodopsin was not inhibited. These results suggest that fatty acids specifically inhibit responses to bitter stimuli by suppression of activation of T2R receptors which coupled with Ggustducin and Gtransducin.

O90 (3O-16G1)

Depolarization of isolated horizontal cells acidifies their immediate surrounding by activating V-ATPase.

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It has been suggested that pH of the invaginating synaptic cleft of the cone terminal is related to the membrane voltage of horizontal cells (HCs), low in the dark and high during surround illumination (1). High pH enhances the release of L-glutamate from the cone terminal, resulting in the formation of the centre-surround receptive field and the colour opponency in the visual system. We measured the pH of the immediate external surface (pH_o) of an HC isolated from carp or goldfish retina to study the mechanisms producing the pH change by a ratio imaging technique, using a pH-sensitive lipophilic dye, 5-hexadecanoylaminofluorescein. When HCs were depolarized by application of 20 μM kainate or by high extracellular K^+, pH_o acidified. The amount of pH acidification was monotonically dependent on the depolarization, as much as 0.21±0.05 pH unit by 100 mM K^+ (approx. 94 mV depolarization). Acidification of the HC surface was suppressed by 0.4 μM bafilomycin A1, a specific inhibitor of vacuolar type H^+-ATPase (V-ATPase), suggesting the existence of an outward electrogenic H^+ pump enhanced by the HC depolarization. These are consistent with the hypothesis that proton released from the depolarized HCs can act as the inhibitory feedback transmitters onto cone synaptic terminals. (1):Hirasawa, H. and Kaneko, A. (2003) *J.Gen.Physiol.*,**122**:657-71.

O91 (3O-16G2)

Structural and Functional Properties of Homologous Electrical Synapses between Retinal Amacrine Cells

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Retinal amacrine cells (ACs) regulate activities of retinal ganglion cells, the output neurons to higher visual centers, through cellular mechanism of lateral inhibition in the inner plexiform layer (IPL). Electrical properties of gap junction networks between ACs in the IPL were investigated using combined techniques of intracellular recordings, Lucifer yellow and Neurobiotin injection, dual patch-clamp recordings and high voltage electron microscopy in isolated retinas of cyprinid fish. Six types of gap-junctionally connected ACs were classified after recordings of their light-evoked responses to light flashes. Among them gap junction networks of three types of ACs were studied with structure-function correlation analysis. Cellular morphology of intercellular connections between three homologous cell classes was characterized. High voltage electron microscopy (Hitachi 1250M, NIPS, Okazaki, co-operative program 2005-HVEM02) revealed localization of gap junctions between the dendritic tips of Neurobiotin-coupled cells. Receptive field size, space length constant, response latency and conduction velocity were measured. Simultaneous dual patch-clamp recordings revealed that the lateral gap junction connections between homologous ACs expressed bi-directional electrical synapses passing Na^+ spikes. Lateral inhibition regulated by ACs in the IPL appears to be associated with directional extension of the dendrites and orientation of dendrodendritic gap junctions (J. Integr. Neurosci. 4(3): pp 313-340, 2005).

O92 (3O-16G3)

Voltage-gated ionic channels of cholinergic amacrine cells in the mouse retina

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Recent studies showed that the cholinergic amacrine cells have a unique membrane properties but the membrane properties of the cholinergic amacrine cells have not been characterized systematically. Here, we studied the voltage-gated ionic channels of transgenic mouse line whose cholinergic amacrine cells were selectively labeled with GFP electrophysiologically and immunohistochemically. Voltage-gated K currents were inhibited by 4-aminopyridine (A current) and tetraethylammonium (delayed rectifier). Voltage-gated Ca currents had ω-conotoxin GVIA-sensitive component (N-type) and ω-AgaIVA-sensitive component (P/Q-type). Tetrodotoxin-sensitive Na current and dihydropyridine-sensitive Ca current (L-type) were not observed. The immunoreactivity for K channels subunits (Kv. 3.1 (delayed rectifier) and Kv. 3.3 (A-current)) and Ca channel subunits (α1A (P/Q-type) and α1B (N-type)) was colocalized with GFP signals. Immunoreactivity for Ca channel subunits (α1C (L-type)) did not colocalize with GFP signals. Immunoreactivity for Na channel subunit existed in the nuclear region but not in the cell surface of the GFP positive cells. Our findings indicate that the signal propagation of the cholinergic amacrine cells are mediated by the combination of the voltage-gated K channels (A-type K current and delayed rectifier K current) and the voltage-gated Ca channels (P/Q-type and N-type) in the mouse retina.

O93 (3O-16G4)

Axonal regeneration of cat retinal ganglion cells into the crushed optic nerve with a Rho/ROCK inhibitor

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We examined whether Y39983, a novel Rho/ROCK inhibitor, can make injured RGC axons regenerate into the crushed optic nerve (OpN) of adult cats. **Methods**: In vitro: retinal pieces were cultured with Y39983 for 14 days to count number of TUJ-1-positive neurites. In vivo: the left OpN of anesthetized cats was crushed with 0.2 N tension for 60 s. On day 12, 0.5 mg of WGA-HRP was injected into the vitreous to label regenerated axons. On day 14, the cats were perfused with fixative, the OpN was dissected, embedded in gelatin. Frozen sections were reacted for HRP with TMB. **Results**: Retinal culture: To obtain the optimum concentration for axonal regeneration, we examined effect of Y39983 on neurite outgrowth of cultured retinal pieces. The number of TUJ-1 positive processes was greatest at the concentrations of 3 and 10 μM in central to peripheral retinal areas. Similarly, length of neurites in retinal pieces was longest at 3 and 10 μM. Axonal regeneration in crushed OpN: We injected Y39983 at 10 and 100 μM. An injection of 10 μM Y39983 increased regenerated axons longer than 0.5 to 2 mm from the crush site. The second injection of 10 μM Y39983 at day 7 increased the number 2 to 3.5 fold than the number in one injection. Single or double injections of 100 μM 39983 increased the number of regenerated axons. **Conclusion**: A Rho/ROCK inhibitor, Y39983, enabled injured axons of RGCs of adult cats to regenerate into the crushed OpN.

O94 (3O-16G5)

Spectral cue responses of sustaned response neurons in the primary auditory cortex

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Previous studies have shown that sustained-response neurons in the primary auditory cortex (A1) of awake animals have sensitivities to the spectral-edge and fundamental frequency (F0) and that the sensitivity originates from the specific inhibition pattern of frequency receptive field (FRF). This study investigated cell-type correspondence between the different experiments by estimating the excitatory and inhibitory summation patterns for harmonic components with a given F0. The findings show that there are, at least, four types of sustained-response neurons sensitive to specific spectral features of the complex tone: energy-integrator cells integrate sound energy on the excitatory FRF; high-edge-sensitive cells are sensitive to the spectral high edge on the best frequency (BF); low-edge sensitive cells detect the spectral low edge on BF; F0-sensitive cells are sensitive to two F0s of harmonics corresponding to BF and an octave below, but not to noise with a similar spectral location. The spectral-cue sensitivity originates from specific inhibitory FRF: less dominant inhibition for energy-integrator cells, asymmetric inhibition for high- and low-edge-sensitive cells, and selective inhibition of non-preferred harmonics for F0-sensitive cells. A1 operates as filters with pass bands and reject bands, which correspond to the peak and trough in FRF. The filter specification is well organized for decoding three acoustic features: sound energy in a given spectral region, spectral edges, and F0 of harmonics.

ORAL
Motor functions

O95 (3O-16G8)

Vergence eye movements elicited by non-disparity factors in 2D movies

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We have analyzed traces of vergence eye movements while subjects watch three-dimensional (3D) and two dimensional (2D) movies, and found that 2D images without binocular disparity sometimes evoked convergence similar to those found in the 3D condition. In this study, we investigated factors that drove convergence other than binocular disparity. As previously presented, 3D/2D movies were projected on an 80 inch-screen in a dark room (10 lux) by two liquid crystalline projectors with/without polarized filters to provide binocular disparity, field-sequentially. To monitor the gaze of the subject, we measured binocular eye movements and head movements with a new binocular video oculography (Newopto) and a magnetic motion sensor (Polhemus). A 2D movie representing driver's view of a go-cart (160 sec), which included forward scenes and expanding optic flows, was shown to fifteen subjects. The motion vectors localized to their gaze points were calculated, and the optic flow was estimated based on flame-by-flame analyses of images. The traces of vergence eye movements consisted with the changes in the optic flow. It is suggested that most of factors that drove convergence in 2D movies are closely related to optic flow.

O96 (2O-07D4)

Longitudinal study of the involvement of several motor cortical regions in functional recovery after the Lesion of the Corticospinal Tract at Cervical Spinal Cord in Monkeys

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It was reported that after the lesion of the corticospinal tract (CST) at C5, recovery of the finger dexterity was completed in 1-3 months (Sasaki et al. 2004, Nishimura et al. 2005). In this study, to clarify the neuronal mechanism of the functional recovery, we performed following two experiments; 1) In 3 monkeys, we performed functional imaging by positron emission tomography (PET) during precision grip. At postoperative 1 month (during early recovery), increased activity was observed in bilateral primary motor cortex (M1). At postoperative 3 months (after complete recovery), in addition to bilateral M1, activities in bilateral ventral premotor cortex (PMv) were increased. 2) In 2 monkeys we investigated the effect of reversible inactivation of areas where we found increased activity in the PET study. Inactivation of M1 contralateral (c) to the lesion caused deficit in control of independent finger movements both at postoperative 1 and 3 months. Inactivation of M1 ipsilateral (i) to the lesion caused deficit in precision grip at postoperative 1 month. Inactivation of iPMv also caused deficit in precision grip at postoperative 3 months. These results indicate that bilateral M1 and iPMv play an important role in the functional recovery.

O97 (2O-10G1)

Synaptic mechanisms acting on motoneurons with reference to the basal ganglia control of locomotion

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The midbrain locomotor region (MLR) is located in the lateral part of the mesopontine tegmentum. Either electrical or chemical stimulation of the MLR first increases the level of postural muscle tone and then initiates locomotion. The MLR receives GABAergic basal ganglia output from the substantia nigra pars reticulata (SNr). The present study was designed to understand synaptic mechanisms acting on motoneurons involved in the basal ganglia control of locomotion. Intracellular recording was made from hindlimb motoneurons (n=47) in two types of decerebrate cats (n=11). One was mesencephalic cats decerebrate at precollicular-postmammilarly level, and the other was hypothalamic cats decerebrated at precollicular-premammilarly level. In mesencephalic cats, short trains of stimuli applied to the MLR (3 pulses, 5 ms intervals, 30-50 μA) induced a sequence of EPSPs and IPSPs. Although stimulation of the SNr (20-60 μA, 50-100 Hz) alone neither changed membrane potentials nor input resistance of motoneurons, it greatly reduced the amplitude of the MLR-induced IPSPs, resulting in an enhancement of the EPSPs. In hypothalamic cats, fictive locomotion, sequences of membrane oscillations with depolarizing and hyperpolarizing phases, was induced in motoneurons. SNr stimuli reduced the hyperpolarizing phases and finally stopped the oscillations of both extensor and flexor motoneurons. These results suggest that enhancement of the basal ganglia output to the brainstem may stop locomotion by suppression of the postsynaptic inhibitory drive acting on motoneurons and initiate locomotion by removal of the inhibition.

O98 (3O-16G6)

Simple-spike activity of Purkinje cells in cerebellar dorsal vermis during vergence eye movements

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For pursuit of small objects moving slowly and smoothly in space close to the observer, two independent eye movement systems are used: frontal smooth pursuit and vergence-tracking. Signals for both systems must be synthesized for pursuit of a target moving in three dimensions. Recent studies in our laboratory have demonstrated that among the cerebral cortical pursuit areas, three dimensional (3D) pursuit signals are generated primarily in the frontal eye fields (e.g. Akao et al. 2005). To drive ocular motoneurons, 3D pursuit signals must be sorted into signals for each eye movement system and finally into oculomotor signals. Studies in our laboratory indicate that such conversion was not detected in the cerebeller floccular region (Tsubuku et al. Soc Neurosci Abstr 2004). The cerebellar dorsal vermis is well known as another pathway for frontal pursuit. To examine whether vergence signals are present in this area, we examined simple-spike discharge of vermal pursuit Purkinje (P-) cells in 2 monkeys. Of a total of 64 P-cells that were examined during both frontal pursuit and vergence-tracking, 50% discharged for both, 37.5% only for vergence-tracking, and 12.5% only for frontal pursuit. These results indicate that about 90% of vermal pursuit P-cells discharged for vergence-tracking and that half of them still had 3D pursuit signals. Majority (71%) of these P-cells discharged before onset of vergence eye movements with the typical lead time of 50 ms, suggesting their involvement in the initiation of vergence eye movements.

O99 (3O-16G7)

The effect of the extinction of the initial fixation target on ocular following responses

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Initial tracking responses were larger when smooth pursuit eye movements were executed after a steady fixation and the initial fixation target disappeared before the onset of the pursuit target motion (Miura et al. JJP, 55 (Suppl), 2005). To study whether this phenomenon is common in the genesis of visually-guided reflexive ocular behaviors, we observed human ocular following response, a reflexive eye movement elicited by the motion of a wide-field visual stimulus. At the beginning of each trial, a stationary fixation point (placed at the center of the screen) and a stationary random-dot pattern (covering the wide visual field) were presented. The subjects fixated the fixation point and immediately (no-gap condition) or 200 ms after the extinction of the fixation point (gap condition), the random-dot pattern moved briefly at 20 deg/s rightward or leftward for 0.2s, and then turned off. The latency of the ocular following responses elicited by the random-dot pattern motion was not affected by the presence of the gap. In all 3 subjects, the change in eye position during the open-loop period of the ocular following responses was significantly larger under the gap condition than under the no-gap condition. The effect of the gap on the ocular following responses started about 15 ms after the onset of the ocular following responses. This result suggests that the efficacy of visuomotor transmission for ocular following responses was facilitated by the release from the fixation before the onset of the random-dot pattern motion, as was seen in the smooth pursuit initiation.

ORAL
Higher CNS functions

ORAL
Autonomic nervous functions

O100 (2O-08E3)

Facilitation of plastic function in the brain during food intake

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During food intake 2-3 mM glucose concentration in CSF become twice. When 6 mM glucose is injected into the hippocampus spatial learning and memory are facilitated. We analized this mechanism. In vitro experiments, glucose concentration in Krebs Ringer solution was changed from 3 to 6 mM for15 min and returned to the original 3mM glucose. By the change the amplitudes of CA1 synaptic potentials were augmented more than 3 times and continued for more than 40 min. Furthermore presynaptic transmitter release and postsynaptic responses to NMDA applied at the apical dendrites were also significantly facilitated. Neurochemically phosphorylations of presynaptic synapsin 1-3, postsynaptic PKCα, ERK, CaMK II of CA1 neurons were all significantly facilitated by the glucose change.LTP of CA1 neurons produced by a tetanic stimulation of the Schaffer collateral / commissure whichwas applied just before returning from 6 to 3 mM glucose was significantly facilitated, while only STPwas produced in 3 mM glucose. During LTP meintenance only phosphorylations of MARCKS (related to plasticity) and PKCα were significantly facilitated. We are now studying the effect of blockers of ATP sensitive K channels on CA1 neurons.These evidences indicate the importance of food intake for reinforcement of the higher brain function..

O101 (3O-17H2)

Attenuated defense response induced by stimulation of amygdala in orexin neuron ablated mice

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We have previously shown that the defense response against stressor was attenuated in prepro-orexin gene knockout mice and orexin neuron-ablated mice (Jpn J Physiol 55: S87, 2005). We have proposed that orexin plays as a master switch to elicit multiple efferent pathways of the defense response. It is still open question, however, how information of stressor activates the orexinergic neurons. In this experiment, we examined possible contribution of the amygdala as one of the afferent nuclei to activate orexinergic neurons. In urethane-anesthetized mice, a GABA-A receptor antagonist, bicuculline, was microinjected into the amygdala, of which electrical stimulation induced simultaneous increases in blood pressure, heart rate, and respiratory frequency. Bicuculline dose-dependently induced cardiorespiratory excitation in both orexin neuron-ablated mice and wild-type controls. However, dose-response curve was rightward shifted in the orexin neuron-ablated mice. We conclude that the amygdala constitutes one of the afferent pathways to the orexinergic neurons that involved in the defense response against stressor.

O102 (3O-17H3)

Penile erection during RFEM sleep regulated by the laterodorsal tegmental nucleus

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The cholinergic neurons in the brainstem (laterodorsal tegmental nucleus: LDT) have a role in the regulation of REM sleep. M. Schmidt have suggested that the cholinergic input to the preoptic area is crucial for induction of penile erection during REM sleep. In the present study, we examined the possible involvement of the cholinergic LDT neurons in the regulation of penile erection during REM sleep. Unanesthetized, head-restrained rats were used. Single neuronal activity was recorded through a glass pipette electrode. Penile erection is composed of two components; slow increase of the corpus spongiosum penis (CSP) pressure and sharp peaks riding on the slow increase. Several types of the LDT neurons showed firing patterns in close relation with penile erection including 1) the cholinergic neurons which showed tonic firing increase 20-30 sec before the erection. The firing increase of this type of neurons was well correlated with the slow increase of CSP pressure. 2) the cholinergic neurons which showed phasic firing in synchronous with the sharp CSP peaks. 3) the non-cholinergic neurons which decreased or stopped firing during erection. These results suggest that the cholinergic neurons in the LDT are involved in induction and excursion of penile erection during REM sleep, while the non-cholinergic neurons have inhibitory influences on penile erection.

O103 (3O-17H4)

Effects of basic tastes stimuli on autonomic nerve activity in anesthetized rat.

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This report deals with effects of five basic taste stimuli on the activity of sympathetic and vagal nerves innervating visceral organs in anesthetized rats. Under urethane anesthesia, basic taste stimuli such as sweet (10% sucrose or 5% glucose), or salty (5% NaCl), sour (0.6% acetic acid), bitter (0.2% quinine sulfate)and Umami (2.8% MSG) were applied into the oral cavity for 10 minutes. Efferent nerve activity was recorded from central cut end of sympthetic branches (adrenal and WAT) and vagal branches (gastric and celiac). Results: Sensory stimulations with four basic taste stimuli (salty, sour, bitter and umami) evoked an activation of sympathetic nerve activity to adrenal gland and WAT, however,taste stilulation with sweet taste caused a suppresseion in sympathetic efferents as well as vagalgastric efferents. On the contrary, vagal celiac efferents were activated by sweet taste and suppressed by other stimuli (salty, sour, bitter. and UMami). These observtions suggest that the visceral functions are regulated by taste stimulithrough autonomic outflows.

O104 (3O-17H5)

A- and C- reflexes elicited in ovarian sympathetic nerves by single shock to a somatic afferent nerve include spinal and supraspinal components in anesthetized rats.

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The spinal and supraspinal components of both A- and C- reflexes were studied in the somato-ovarian sympathetic reflex discharges elicited by a single shock either to a spinal (T9-11) afferent nerve or to a limb (tibial) afferent nerve in urethane anesthetized rats. In central nervous system (CNS) intact rats, a single shock to a T9-11 spinal afferent nerve produced early and late A-reflex discharges with latencies of about 51 ms and 117 ms, respectively, and a C-reflex with a latency of about 200 ms in a ovarian sympathetic efferent nerve. After spinalization at the third thoracic level, stimulation of the same spinal afferent nerve produced an A-reflex with the same latency as the early A-reflex in CNS-intact rats and a C-reflex discharge with a latency of about 112 ms. On the other hand, a single shock to a tibial afferent nerve evoked an A-reflex discharge with latency of about 91 ms, and a C-reflex discharge with a latency of about 228 ms in CNS-intact rats. In most cases, the A-reflex could be divided into two subcomponents of different latencies. These A- and C- reflex discharges elicited by stimulation of a tibial afferent nerve were not observed after spinalization. It was concluded that ovarian sympathetic A- and C- reflex discharges evoked by stimulation of a segmental spinal afferent nerve in CNS-intact rats are of spinal and supraspinal origin, and those evoked by tibial nerve stimulation are of supraspinal origin.

O105 (3O-17H6)

Central nNOS activity in Dahl hypertensive rats is normalized in the brainstem and suppressed in the diencephalon

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We have demonstrated the distribution of nNOS neurons in the brainstem and diencephalon, which was upregulated in hypertensive Dahl-salt sensitive (DSS) rats compared with normotensive DSS rats. In this study, we directly compared nNOS activity in the brainstem and diencephalon between hypertensive DSS and normotensive Sprague-Dawley (SD) rats. The DSS and Dahl-salt resistant (DSR) rats were fed on 8% NaCl food (DSS8% and DSR8%) or 0.4% NaCl (DSS0.4% and DSR0.4%). SD rats were fed only on 0.4% NaCl food (SD0.4%). The level of nNOS activity in the brainstem of SD0.4% was almost the same as that of DSS8%, which had been significantly higher than those of DSS0.4%, DSR0.4% and DSR8% (normotensive Dahl rat strain). Although the level of nNOS activity in the diencephalon of SD0.4% was significantly higher than that of DSS8%, which had been almost the same as those of the normotensive Dahl rat strain. All these results indicated that at normal blood pressure, the nNOS neuronal system in both the brainstem and diencephalon of Dahl rat strain might be downregulated compared with SD rat strain. In hypertensive DSS rats, the nNOS neuronal system specifically in the brainstem seems to be reversed to the level in SD rats, although the nNOS neuronal system in the diencephalon stays to be downregulated compared with SD rats.

O106 (3O-17H7)

Feedforward control of human thermoregulation by skin sympathetic nerve activity

Iwase, Satoshi[1]; Sawasaki, Naoki[2]; Michikami, Daisaku[3]; Mano, Tadaaki[4]; Sugenoya, Junichi[1]; Cui, Jian[5] (*[1]Dept. Physiol. Aichi Med. Univ. Aichi, Japan; [2]Tokai Hospital; [3]Ohtsuka Pharmaceutical Co. Ltd.; [4]Tokai Central Hospital; [5]Pennsylvania State University, PA, USA*)

Feedforward control of human thermoregulation by skin sympathetic nerve activitySatoshi IWASE1, Naoki SAWASAKI2, Daisaku MICHIKAMI3, Tadaaki MANO4, Junichi SUGENOYA1, Jian CUI51. Department of Physiology, Aichi Medical University, 2. Department of Surgery, Tokai Hospital3. Ohtsuka Pharmaceutical Co. Ltd.4. Director, Tokai Central Hospital5. Pennsylvania State UniversityTwo ways of thermoregulation has been recognized, feedforward and feedback mechanisms. Feedforward mechanism employs neural afferent pathway, whereas feedback uses convection of the blood stream from the peripheral to the core. We investigated the relation between the microneurographically recorded skin sympathetic nerve activity (SSNA) and the tympanic temperature (Tty) measured as the core. Four exposure conditions, 1) local cold, 2) generalized cold, 3) local heat, 4) generalized warming, were loaded to the subjects and the response of SSNA and Tty were analyzed in time series analysis. The abilities to activate and to suppress SSNA were correlated to the changes in Tty, indicating that the individuals who are excellent in activating/suppressing SSNA are excellent in thermoregulation. The time lag of neural activation in Tty was within 1 min, whereas that of convectional Tty change was approx. 10 min. We concluded that skin sympathetic regulation is critical in rapid thermoregulation in humans.

ORAL
Behavior & biological rhythm

O107 (3O-17H8)

Effect of CO_2 water immersion on cardiac autonomic nerve function in humans

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CO_2 water immersion at 1000 ppm affects thermoregulation through the increased cutaneous blood flow on the immersed skin area. However, the effect of CO_2 water immersion on cardiovascular function remains to be clarified in humans. To examine whether CO_2 bathing affects cardiac autonomic function, we analyzed heart rate variability and measured the cardiac output by Echo (Apio XV, Toshiba, Japan) during CO_2 and fresh water immersion. Tympanic temperature (thermistor thermometry), cutaneous blood flow (laser-Doppler flowmetry) and electrocardiogram (ECG) were monitored continuously. The subjective thermal and comfort sensations were asked every 10-min during experiments. After a rest for 10 min, the subject immersed up to the breast level to CO_2-rich water at 1000ppm or fresh water at thermoneutral water. The results were shown as follows: 1) HF was significantly higher in CO_2 water immersion than in fresh water immersion. 2) LF/HF ratio was significantly lower in CO_2 water immersion than in fresh water immersion. 3) Heart rate was not significantly different between CO_2 water and fresh water immersion. 4) Tympanic temperature was significantly lower during CO_2 water immersion than during fresh water immersion. 5) Cutaneous blood flow in immersed forearm was significantly higher during CO_2 water immersion than during fresh water immersion.

O108 (1O-02D2)

Early lighting condition (ELC) alters circadian rhythm and maternal care of dam with the subsequent disturbance of the offspring's anxiety and memory.

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Although dramatic changes of lighting environments are occurring in modern society, the influence of these changes has not been fully elucidated. It is demonstrated that, early life experiences (i.e. maternal care) induce various changes in the function of circadian rhythm, emotionality and memory. Here, we examined whether ELC affected circadian rhythm and maternal care of dam, and the offspring's circadian rhythm, anxiety and memory. Prolonged dark phase (PDP) was used to study the effects of ELC. Dams and their litters were kept in a PDP cycle (L/D=6:18h; postnatal days 2-14) or under a normal condition (L/D=12:12h). Throughout this period, locomotor activity of dams was measured and all cages were video recorded for behavioral scoring. At age of 10-week, behavioral observations of the offsprings were undertaken. Circadian rhythm of locomotor, avoidant behavior, social interaction and object recognition memory were examined. Under PDP, the morning offset of dams' motor activity was delayed and amounts of maternal care decreased for the first 2-7 days of lactation. In the adult offspring, circadian rhythm of locomotor was not affected. Whereas PDP increased avoidant behavior, PDP decreased social interaction and memory. In conclusion, the PDP mothers showed impaired circadian rhythm and maternal care, and the offsprings exhibited depressive-like behaviors. Therefore, it is conceivable that ELC may alter mother-infant interaction and subsequently change the offsprings' emotionality.

O109 (1O-02D3)

Discrimination between the tastes of sucrose and saccharin by conditioned preference learning in mice.

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We examined the neural mechanism of discriminative taste preference learning, using the procedure with some modifications of flavor-postingestive consequence learning paradigm in C57BL/6 male mice. Wild mice were allowed to drink water for 10 min daily with the two-bottle method after 16 h water- and food-deprivation. When mice were alternately exposed to sucrose (Suc, 0.5 M) or saccharin (Sacch, 5 mM) instead of water during 2 weeks, the amount of 0.5 M Suc consumption markedly increased but that of 5 mM Sacch did not. Because naive mice prefer to 0.5 M Suc much more than 5 mM Sacch, we employed 0.15 M Suc, to which mice showed the same preference as 5 mM Sacch, instead of 0.5 M Suc. The amount of 0.15 M Suc tended to increase with decreasing of the Sacch-consumption only when the intragastric injection of 0.5 M Suc was done. These results suggest that mice can discriminate between subtle difference cues of Suc from Sacch, mediating the association with intragastric sensory feedback in the brain.

O110 (1O-02D4)

Effect of high fat diet to ICR background clock mutant mouse adipocyte tissue

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Major components of energy homeostasis are subjected to circadian regulation that synchronizes energy intake and expenditure. Recently, relationship of circadian clock and lipid metabolism is highlighted. The CLOCK transcription factor is a key component of the molecular circadian clock. Adipocytes play essential metabolic roles not only serving as energy reserves but also secreting hormones and cytokines that regulate metabolic activities. Clock mutant mice were fed with high fat diet for 13 weeks, and lipid metabolism was investigated. Both wild type and Clock mutant mice gained body weight. But, in Clock mutant mice, increases of body weight and of adipocyte tissue were significantly attenuated. In Clock mutant mice, total cholesterol of plasma and liver, and triglyceride of liver were significantly lowered. Again, we examined clock controlled gene mRNA in the adipocyte by real-time RT-PCR. Plasminogen activator inhibitor type 1 (Pai-1) which is related to cardiac infarction was significantly down-regulated in Clock mutant mice. As a summary, we showed that Clock mutant mice may have abnormal lipid metabolism.

O111 (1O-02D5)

The role of GABAergic system in the ventral pallidum on the retrieval of conditioned taste aversion in rats

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It is suggested that the GABAergic system in the ventral pallidum (VP) plays a role in taste palatability. Taste palatability shift occurs as a function of conditioned taste aversion (CTA). To elucidate the role of VP GABAergic system on CTA, we examined the effects of microinjections of a $GABA_A$ receptor antagonist, bicuculline, on the retrieval of CTA memory. We measured consumption of conditioned stimulus (CS) using one-bottle test (Experiment 1) and observed the ingestive or aversive behavior to CS using taste reactivity test (Experiment 2). Rats received 5 mM saccharin or 0.3 mM quinine hydrochloride as CS, immediately followed by an i.p. injection of 0.15 M lithium chloride (20 ml/kg). After this conditioning, vehicle or bicuculline (12.5 - 200 ng) was bilaterally infused into the VP immediately before re-exposure to the CS. In Experiment 1, the bicuculline microinjections significantly increased the intake of the saccharin CS, but not the QHCl CS. In Experiment 2, while the control rats infused with vehicle showed a variety of aversive responses (e.g. gaping, chin rubbing, head shaking, forelimb flails), the rats infused bicuculline failed to show these aversive responses. These results indicate that the blockade of $GABA_A$ receptors in the VP attenuates aversion to saccharin CS, and this may be due to elimination of aversive responses. Thus, it is suggested that the GABAergic system in the VP plays a critical role in the expression of CTA.

O112 (1O-02D6)

Different influence of moderate exercise stress on acute phase proteins in dependency of bright/dim light exposure during the daytime

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The experiment aimed at knowing whether exercise stress on acute phase proteins may be influenced differently, depending on bright (5,000 lx) or dim (50 lx) light exposure during a day.Eight healthy women volunteered as the subjects. The subject entered a bioclimatic chamber at 08:00 h on first day.The light intensity measured at eye level from 08:00 to 18:00 hours was either 50 lx in the dim light condition (first day) or 5,000 lx in the bright light condition (second day), 10 lx from 18:00 to 23:00 hours.Subjects exercised moderately for 20 min by a bicycle ergometer with 60 W intensity.Blood samples were drawn 30 minutes later after the end of exercise on first, second and third day. Concentrations ofα1-antichymotrypsin (ACT), transferin (Tf), α2-macroglobulin (α2-M) and haptoglobulin (Hp) were analyzed by the usage of immunoelectrophoresis. Interleukin-6 and TNFα concentrations were analyzed by ELISA kits. There did not exist any significant differences for acute phase proteins on first day between exercise and no exercise. ACT was significantly higher ($p<0.05$) on 3rd day morning than on 2nd day morning, suggesting that an increase of ACT due to the exercise stress was amplified by bright light exposure during one diurnal day before the exercise stress was applied to the subjects.

O113 (1O-02D7)

Local administrations of muscimol into Nif (Nucleus interfacialis nidopallialis) alter song grammar of the Bengalese finches (*Lonchura striata* var. *domestica*).

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Songs of passerine birds (like zebra finch) are learned motor behavior which used by males to attract females and to protect territories. Generally, bird songs are consist of several different song notes (elements), and these notes are produced in a fixed temporal order. Among the passerine birds, male Bengalese finches (BF) sing complex song which follows finite state syntax. The song control system of BF consists of a set of discrete nuclei including the HVC, RA and Nif. Previous lesion study (Hosino and Okanoya 2000) showed that Nif lesioned BFs sung simpler songs, with less phrases to phrases branching than that of prelesion birds. This finding suggests that Nif-HVC connection plays important role in generating song grammar in BFs. In this study, we perfused Nif with muscimol (GABA agonist) for 30 minutes via microdialysis probes as a perturbation on Nif-HVC system. Following a local administration of muscimol into Nif, song grammar of BF was modified. Some of chunks in their finite state grammar disappeared and pronounced elongation of introductorily notes' duration was observed in first 3 hours. In addition, we also recorded stuttering like repetitions of song notes. Nif is also known as one of auditory relay nucleus to HVC. Some of drug effects, therefore, are possibly caused by disruption of auditory feedback. Further detailed studies are needed to know function of Nif-HVC connection in relating to generate song grammar.

ORAL
Neurochemistry

O114 (1O-02D8)

Effects of postnatal stress by cinchophen injection on motor behavior in adult rat

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To study the ontogenic change in stress susceptibility during postnatal brain development, we have previously examined the ontogenic changes of Fos expression in the paraventricular nucleus of the hypothalamus (PVN) after intraperitoneal (i.p.) injection of cinchophen. We have obtained evidence that Fos was induced by cinchophen in PVN after postnatal day (P)10. Such differential responses to stress have led us hypothesize that such stressful stimuli at various time point during postnatal development may differentially affect motor behavior in adult rat. To test this hypothesis, we applied an open field test and a rotarod test using rats that received i.p. injection of cinchophen on P5, P7, P10 and P15. There were no significant differences of body weight and muscle strength between them. In open field test, female rats that received cinchophen injection on P10 tended to stay longer in the center of the field at P30, suggesting that they showed anxiety to a novel environment. In rotarod test, there was no significant difference among all groups. These results indicate that postnatal stress by cinchophen may affect in part their motor behavior related to anxiety in adult rat.

O115 (2O-07D3)

Na^+/HCO_3^- cotransporter 4 (NBC4) predominantly expressed in choroid plexus is involved in cerebrospinal fluid production

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Secretion of HCO_3^- at the apical side of the epithelial cells of the choroid plexus is an essential step in the formation of cerebrospinal fluid (CSF). Anion conductance with a high degree of HCO_3^- permeability has been observed and suggested to be the major pathway for HCO_3^- transport across the apical membrane, but the molecular entity remains unknown. We identified the first molecular entity of apical choroid plexus HCO_3^- transport, a novel variant of NBC4 (NBC4g). Electrophysiological studies and pH-recovery assay showed that NBC4g has the electrogenic, DIDS-sensitive, and cAMP-dependent HCO_3^- transport activity. Furthermore, the contribution of NBC4g to choroid plexus HCO_3^- transport was demonstrated by RNAi-mediated knockdown of NBC4g using primary cultured cells; treatment with siRNA of NBC4g led to a similar degree of reduction in the transport activity as that observed by treatment with DIDS. Thus, our data strongly indicate that NBC4g is the long-sought transporter responsible for the HCO_3^- secretion from the choroid plexus into the ventricle thereby controlling the H^+ buffering and pH of the CSF.

ORAL
Endocrine glands & hormones

O116 (1O-01C5)

A possible novel toxic index for dioxins/ PCBs -Is dioxin more toxic than OH-PCB?

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Polychrolinated biphenyls (PCBs) and dioxins (PCDD, PCDF, coplaner-PCB) are the environmental chemicals that may affect the growth and homeostasis of many organs. WHO determined toxic index of dioxins as toxicity equivalent factor (TEF). However, we reported previously that the most toxic dioxin (TCDD) did not affect on thyroid hormone (TH) receptor (TR)-mediated transcription. On the other hand, a hydroxylated-PCB5005 whose TEF was almost negligible, strongly suppressed the transcription. In this study, we performed a reporter gene assay with several PCBs and dioxins, and observed opposite tendency from TEF: many PCBs with little TEF showed a greater effect than dioxins. Furthermore the magnitude of suppression by PCBs and PCDF in neuroblastoma derived cell line was greater than that in fibroblast derived cell line. To analyze further the effect of these chemicals in TR-TH response element (TRE) binding, electrophoretic mobility shift assay (EMSA) was performed. PCB congeners that suppressed TR-mediated transcription dissociated TR from TRE, indicating that PCB action is exerted through this mechanism.These results suggest that TEF of dioxins and PCBs do not always correctly indicate their toxicity. The suppression of TR-mediated transcription by PCBs and dioxins should be incorporated to construct a novel index of those chemicals.

O117 (1O-03E1)

Mediatory roles for forebrain AMPA/kinate receptors in ADH secretion stimulated by hyperosmolality or bleeding

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The anteroventral third ventricular region (AV3V), a pivotal area for autonomic functions, contains synaptic boutons with Glu and three subtypes of glutamate receptors (Glu-Rs). Although our previous data suggest involvements of NMDA- and metabotropic Rs in ADH release triggered by several stimuli, roles of AMPA/kinate (non NMDA)-Rs related to Na^+ channels in hormone release and other functions have not been examined as yet. This study aimed to elucidate the issue through experiments in conscious rats with indwelling cerebral and vascular cannulae. Infusion sites in the brain were identified histologically after experiments. Topical AV3V infusion with a non NMDA-R agonist FWD augmented plasma ADH (pADH), glucose (Glc) and osmolality (Osm), and enhanced arterial pressure (AP) in a dose-dependent manner. All the responses of these variables were blocked by pre-administering NBQX, a selective non NMDA-R antagonist. When NBQX was applied to the AV3V structures such as the median preoptic nucleus, rises of pADH in response to systemic load of hypertonic saline or normo- or hypotensive bleeding were inhibited remarkably. The increases of AP, Osm or Na^+ provoked by the osmotic load and the responses of plasma angiotensin II, Osm, Glc or AP caused by the bleeding were not affected significantly. These results suggest that AV3V non NMDA-Rs, as well as NMDA-Rs, may contribute to both the hyperosmotic and hypovolemic ADH secretion.

O118 (1O-03E2)

Cross-talk between ACTH- and PAF-induced cortisol and aldosterone secretion by perfused guinea pig adrenals

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Platelet-activating factor (PAF) is a highly potent stimulator of the secretion of cortisol, corticosterone, and aldosterone. We previously reported that PAF acts mainly through PAF receptor accompanied by the activation of protein kinase (PK) C. While, ACTH acts through ACTH receptor accompanied by the activation of PK A. In the present study, we studied the cross-talk in adrenal steroidogenesis among PAF, ACTH, and angiotensin II (ANG II). 1) The administration of 1nM PAF or 10pg/ml ACTH significantly stimulated cortisol secretion. The rate of secretion peaked 2.5-5 or 10-12.5min after infusion of PAF or ACTH. When concurrently applied 1nM PAF with 10pg/ml ACTH evoked cortisol secretion less than additional and peaked 5-10min. 2) Aldosterone secretion in response to ANG II significantly stimulated at 1nM and peaked 15-20min after the infusion of ANG II. The administration of 10nM PAF did not induce significant aldosterone secretion. The concurrent administration of 1nM ANG II with 10nM PAF significantly inhibited the secretion of aldosterone to ANG II. 3) Aldosterone secretion in response to ACTH significantly increased at 1ng/ml and peaked 15-20min after the infusion of ACTH. The concurrent administration of 1ng/ml ACTH with 10nM PAF significantly evoked aldosterone secretion almost additional. The rate of secretion peaked 0-2.5min after infusion of the mixture. These results implicate that a cross-talk between the PK A system and the PK C system regulates the cortisol and aldosterone secretion.

O119 (2O-08E1)

Acyl-modification of ghrelin regulates its appetite-stimulating activity in mice

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Ghrelin is an acylated brain-gut hormone secreted primarily from stomach. The major active form of ghrelin is a 28-amino acid peptide with an n-octanoyl (C8) modification at Ser3 residue. There also exist other acylated forms of ghrelin, such as n-decanoyl (C10) ghrelin. The previous study demonstrated that intravenous administration of C8-ghrelin increases appetite and food consumption in rodents. To elucidate effects of acyl-modified ghrelins on appetite, we have therefore measured concentrations of acylated ghrelins in stomach and plasma of control and fasted mice. In control, the C8-ghrelin concentration in stomach was higher than the C10-ghrelin. In 48h-fasted mice, the C8-ghrelin significantly decreased whereas the C10-ghrelin significantly increased. Consequently, the C10-ghrelin was greater than the C8-ghrelin in plasma of 48h-fasted mice. Intraperitoneal (ip) injection and intracerebroventricular (icv) injection of C8- or C10-ghrelin significantly facilitated the food consumption 2h after the injection. Ip injection of either C8- or C10-ghrelin had a similar effect. On the other hand, 2h after icv injection of C8-ghrelin, the food consumption was greater than that 2h after icv injection of C10-ghrelin. These results suggest that the condition of energy metabolism influences the acyl-modification of ghrelin, and C10-ghrelin has a site-dependent activity for the stimulation of appetite in mice.

O120 (1O-02D1)

A study of religious beliefs and practices on menarche and menstruation among middle aged women and adolescent girls of Buddhist Hindu, Islamic and Catholic religions

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Objective- A qualitative descriptive study was conducted to determine the religious practices and beliefs related to menarche and menstruation among middle aged women and adolescent girls in the district of Colombo within Buddhist, Hindu, Islamic and Roman Catholic religions.Method-Information was gathered from the middle aged women and the adolescent girls by focus group discussion and by a self-administered questionnaire respectively. Pertinent religious scripture were identified through key informants of each religion. Results-The religious taboos observed during menstruation included not participating in religious activities, avoidance of sex and cooking. Restricted religious activities were observed among middle aged women of Buddhist, Hindu and Islam religions. Buddhist adolescent girls have given up this practice. Avoidance of sexual intercourse during menstruation was observed in all four religions. Hindu's observed unique practices such as restriction of water during menarche and reduced household activities during menstruation. Hindu and Islam women follow the scriptures strictly whereas the Buddhist practices seem to be influenced by the Hindu culture. Except Roman Catholics, others observe restrictions during menarche although there are no statements in scriptures of the four religions studied.Conclusion-Unsafe practices are still continuing among women and adolescents despite statements in religious scriptures.

ORAL
Reproductive physiology

ORAL
Development, growth & aging

O121 (2O-07D1)

Analysis of migrating neurons in adult brain using the antibodies specific to drebrin isoforms

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Although it is known that drebrin is expressed in the rostral migratory stream (RMS), a neurogenesis region in adult rat brain, the isoform of drebrin is unknown. In this study, a drebrin A specific antibody (DAS2) and another specific antibody (M2F6) recognizing drebrin E and A were used to identify the drebrin isoform that is specifically expressed in migrating neurons. To investigate the immunocytochemical characteristics of drebrin-positive cells, we carried out double-labeling with M2F6 and an antibody of PSA-NCAM, GFAP, or Ki-67. In addition, we performed double labeling of staining of RMS with DAS2 and M2F6 to identify the drebrin isoform. We also analyzed adult rat brains whose unilateral olfactory bulb (OB) had been removed. The migrating cells in subventricular zone of RMS were strongly stained with M2F6, but not with DAS2. This indicates that drebrin E but not A is expressed in these cells. These packed, bi-polar cells expressed PSA-NCAM but not GFAP. Some of them had been undergoing proliferation because their nuclei expressed Ki-67. These findings suggest that M2F6-positive and DAS2-negative cells are migrating neuronal precursors. Unilateral olfactory bulbectomy significantly increased the total area of M2F6-positive and DAS2-negative cells in the ipsilateral RMS comparing with the contralateral side, which is consistent with the previous report about the increase of migrating neuronal precursors by the bulbectomy

O122 (3O-13D6)

The aboral pore of Hydra and oral opening of higher organisms share common ancestral origin

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Oral opening of multicellular organisms is generally formed at the anterior end of the animal. Phylum Cnidaria is so far the only exception where oral opening is formed at the posterior end of the animal expressing Wnt-3a homologues (1), a typical gene which is expressed specifically at the posterior end. Why this opposite oral-aboral polarity relative to A-P polarity appeared only in this phylum remains unknown. Expression of Hox-1 homologues at the oral end has been proposed as evidence that oral end represents anterior end even in Cnidaria (2). Here we report that the aboral end of Hydra bears several similarities to the oral end of higher organisms.

O123 (3O-13D7)

Classification of proteomic trajectories of retinal proteins in mice during postnatal development

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Purpose: We attempt to classify the retinal proteins displayed on two-dimensional (2-D) gels based on their time-dependent expression patterns, which we designate "proteomic trajectory ", along the postnatal developmental axis. **Methods:** Retinas of C57/B6 mice were collected at postnatal (P) days 1, 3, 5, 7, 9, 14, 21 and at the adult stage. After separating the proteins by 2-D gel electrophoresis, the gel images were analyzed by Progenesis workstation. Protein spots were quantified and normalized, and the proteomic trajectory along the developmental axis was obtained. The results were averaged from four independent experiments. The proteomic trajectories were clustered by self-organizing mapping (SOM) using GeneCluster2. For protein identification each spot was excised and subjected to in-gel digestion by trypsin, followed by peptide mass fingerprinting (PMF). PMF search and confirmation were performed by MASCOT. **Results and Conclusions:** We identified ca 400 proteins. These proteins can be clustered by SOM into four major types, each exhibiting characteristic proteomic trajectory: Juvenile-type, showing abundant expression in the early postnatal stages and declining along the maturation process; Transient-type, showing transient expression during the development; Adult-type, showing increased expression in the later stages of development toward maturation; and Constitutive-Type, being expressed relatively constant during the entire developmental stages.

O124 (3O-13D8)

Role of Hox10 and 11 Paralogues in Congenital Kyphoscoliotic Rats

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The genetic background and key gene for congenital scoliosis has not yet been clarified. Ishibashi rats (IS) have congenital malformations of the lumbar vertebrae leading to kyphoscoliosis similar to that seen in human. This study investigated characteristics and gene expression of IS to provide insights into human congenital scoliosis.To characterize skeletal malformations of lumbar vertebrae in IS, radiographic and staining studies were performed.Then the gene expression profile of Hox10 and 11 paralogues, whose critical roles in determination of phenotypes of lumbar and sacral vertebrae are well known, between IS and Wistar strain rats by Real Time-PCR was studied.Significant differences on skeletal structures between IS and Wistar were found: (1) transitional vertebrae; (2) anterior wedged vertebra; (3) union of anterior vertebrae; (4) an additional vertebra (7th lumbar vertebra). Especially, transitional vertebra was frequently observed.Staining studies of IS fetuses revealed the fusion of primary ossification centers in the lumbar vertebral column, which was not observed in cervical and thoracic vertebral column.Regarding gene expression of Hox10 and 11 paralogues, the expression of some of these paralogues had low level in lumbar/ sacral region of vertebral column compared with that of Wistar.Our results indicate that Hox10 and 11 paralogues play critical roles in generating vertebrae of IS phenotype. Further work is in progress to elucidate the expression profile of Hox10 and 11 paralogues in the axial skeleton of IS.

O125 (1O-03E7)

Changes in anti-oxidant level in the blood and the brain corresponding to wide-ranging fluctuation of energy metabolism during Syrian hamster hibernation

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Arousal from hibernation with a rapid increase of the energy metabolism suggests being exposed to a strong oxidation stress whenever the animal awakes, therefore being considered to have an innate anti-oxidation mechanism to prevent pathological troubles. To elucidate the state of the oxidation stress, endogenous anti-oxidants were quantified along the time course of hibernation. Very slow flow (3.5 μL/h) brain microdialysis enabled temperature independent sampling of the brain extracellular fluid (ECF) during hibernation, arousal and cenothermia in Syrian hamsters (Mesocricetus auratus). Brain tissue and dialysates were analyzed to provide the first profile of ECF changes in levels of ascorbic acid (AA), glutathione (GSH) and uric acid (UA) during hibernation and the transition to cenothermia. Brain tissue content of AA and GSH were unchanged between hibernation and cenothermia, however arousal was associated with substantial oxidation of AA from the brain ECF and plasma compartments. ECF-GSH increased during arousal. Brain tissue UA content was decreased 50% during hibernation. ECF-UA levels were unchanged in hibernation and cenothermia, however transiently increased 100% during arousal. The results suggest that arousal from hibernation is a suitable experimental model for examination of the mechanisms by which non-pathological tissue integrity is maintained in the face of the generation of free radicals during increasing metabolism, temperature and cerebral reperfusion.

ORAL
Nutrition, energy metabolism & body temperature

O126 (1O-03E8)

Sex difference in thermoregulation-impact of estrogen on thermoregulation-

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Body temperature (T_b) is different between male and female, e.g. daily change in T_b is fluctuated with menstruation cycle in female rats. We hypothesized that estrogen plays a crucial role in the sex difference in T_b. **Methods** (1) Daily change of T_b was measured after gonadectomy in male and female rats. After the measurement, silicon tubes containing 17-beta estradiol (E_2) crystalline, aimed to maintain blood estrogen constant, were subcutaneously placed in the rats. Then T_b measurement was repeated. (2) Thermoregulation during 2-h heat exposure at 34°C or cold exposure at 5°C was assessed in gonadectomized female rats, and the same protocol was conducted in those with E_2 tubes. **Results** (1) Compared with male rats, T_b rhythm in female gonadectomized rats became unstable, showing 2-4 h irregular oscillations. T_b rhythm remained unchanged in male gonadectomized rats. In female gonadectomized rats with E_2 tubes, T_b rhythm returned to the normal level. However, there was no influence of E_2 on T_b rhythm in the male rats. (2) Both in the heat and cold, gonadectomized female rats could not maintain their T_b as those with E_2 tubes. Histological analysis for the rat brain showed that Fos-immunoreactive cells in the hypothalamus were smaller in the rats without E_2 tubes. **Conclusion** These results show that estrogen is involved in the thermoregulation in female rats. Estrogen may modulate thermal sensitivity to the environment at the level of the hypothalamus

ORAL
Environmental physiology

O127 (1O-03E4)

EPIDERMIOLOGIC STUDIES OF THE PREVALENCE OF ARTERIAL HYPERTENSION AMONG COMMERCIAL MOTOR BIKE RIDERS IN BENIN CITY, NIGERIA.

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An Epidermiologic study was carried out in the dry season on 250 commercial motor bike riders from five different parks. 69% of the bike riders were in the 31-40 and 41-50 age range while 31% were in 21-30, 51-60 and 61-70 age range. Half of the population studied were normotensive.Arterial hypertension was found in 25%of the examined workers ($p<0.05$), borderline hypertension was found in 26%of the workers ($p<0.05$). The severity of the hypertension increased with the age of the workers and the 31-40 age range had the highest incidence of hypertension accounting for 24(38%) of the total 63 frank cases of hypertension. The severity of the hypertension increased linearly with their duration of exposure to commercial motor bike riding($r=0.6$, $P<0.05$). heart rate showed a progressive increase with age but a drop was observed in the 51-60 age range. The characteristics of the hypertension structure and it's interesting relation to age, number of years of commercial bike riding, heart rate and body weight is discussed. Of particular interest is the significant number of young adult bike riders found to be hypertensive.

O128 (1O-03E5)

Pattern biology for an ideal cellular habitat created by micromechanical technology

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[Background] The frame pattern of substance for cell seeding can act not only on clustering of cells but also on cellular functions. However, the cellular kinetics depending on the frame pattern of habitat is not fully elucidated. We have established the technique for molding polymer resin with submicron accuracy, and have constructed many kinds of micro-frame patterns of substances. In this research, we explored micro-frame patterns suitable for neural network construction using PC12 cell line and evaluated the cellular functions on each micro-frame pattern. **[Method]** Micro-frame patterns were fabricated on the polymer resin of which the optical transparency was sufficient for the microscopic observation. **[Results]** One micro-domain was populated by 1-3 PC12 cells and a cellular network was formed by connecting one another through the open windows of the micro-domain. In addition, alterations in cellular growth and network formation occurred when the micro-domain structure was changed.**[Conclusion]** The results suggest that micro-frame patterns of substances play a critical role in cellular configuration.

O129 (1O-03E6)

Winter body temperature in the black-lipped pikas, Ochotona curzoniae, in their natural habitat

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The pikas, Ochotona, living in cold zone or in high mountains prefer cold and are weak to heat. They were reported to be diurnal, most active at dawn and dusk, or active in day and night after the field observations. We have previously studied pikas' body temperature rhythm in their natural habitat using bio-telemetry devices and showed that the pikas are essentially diurnal and may vary their activity rhythm from diurnal in the relatively cool environment to a crepuscular (dawn and dusk) pattern in the relatively hot environment to avoid the heat during midday. In this study, we monitored body temperature in wild black-lipped pikas, Ochotona cruzoniae, in their natural habitat in Qinghai, China during mid-winter season.

ORAL
Pathophysiology

O131 (2O-07D6)

Functional roles of the spontaneous calcium oscillations for the development of ischemic tolerance in neuron/astrocyte co-culture

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Spontaneous oscillations in the intracellular concentration of calcium (Ca^{2+} oscillations) contribute to the regulation of gene expression. Here we investigated whether and how the dynamics of Ca^{2+} oscillations changed after sublethal preconditioning (PC) for PC-induced ischemic tolerance in neuron/astrocyte co-cultures. Ischemia was simulated by depriving co-cultures of both oxygen and glucose (OGD). The frequency of spontaneous Ca^{2+} oscillations decreased significantly between 4 and 8 h after the end of PC in both neurons and astrocytes. The reduction in oscillatory frequency caused by treatment with 2-APB, an inhibitor of IP3 receptors, resulted in the development of ischemic tolerance, in a suppression of the rise in the extracellular concentration of glutamate during OGD, and in a down-regulation of the expression of the glutamate transporter GLT-1. The expression of GLT-1 is known to be up-regulated by treatment with PACAP. Treatment with PACAP6-38, an inhibitor of PACAP receptors, decreased the oscillatory frequency and GLT-1 protein levels, and induced ischemic tolerance. In contrast, treatment with PACAP38 increased the oscillatory frequency, and antagonized both the PC-induced down-regulation of GLT-1 expression and ischemic tolerance. These results suggested that the sublethal PC insult suppressed the spontaneous Ca^{2+} oscillations regulating various gene expressions for the development of the PC-induced ischemic tolerance.

O130 (2O-07D5)

Pathophysiological roles of ischemia-induced reverse mode operation of glutamate transporters in astrocytes.

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During brain ischemia, the excessive influx of Na^+ is caused, resulted in the reversal of neuronal/astrocytic glutamate transporters; that is, glutamate and Na^+ are co-transported to the extracellular space. Previous studies have revealed that this reversed uptake of glutamate occurs mainly via astrocytic GLT-1 and is the possibility cause of neuronal death. The present study aims at elucidating whether this reverse mode operation of GLT-1 has any functional meanings for astrocytes themselves. Analyses of the oxygen/glucose deprivation (OGD)-induced changes in the concentration of intracellular Na^+ and Ca^{2+} have revealed that OGD produced Na^+ overload, resulting in the reversal of Na^+/Ca^{2+}-exchanger (NCX). The reversed NCX then caused Ca^{2+} overload leading to the damage of astrocytes. When the cultures were treated with PACAP-38, a neuron-delivered peptide, to express GLT-1, the OGD-induced reversed GLT-1 released Na^+ out of the cell, and significantly reduced the rise in intracellular Na^+ and Ca^{2+} during OGD and the astrocytic cell damage. In contrast, however, OGD resulted in the co-transport of Na^+ and glutamate out of astrocytes via reversed GLT-1, and the marked rise in the extracellular glutamate in neuron/astrocyte co-cultures produced excitotoxic neuronal death. These results suggested that ischemia-induced reverse mode operation of GLT-1 was toxic to neurons but beneficial to astrocytes by maintaining their Na^+ gradient across cell membranes.

O132 (2O-07D7)

Brain monoamine levels in viral injection model rat produced by poly I:C: in vivo brain microdialysis study

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We have recently found that an intraperitoneal (i.p.) injection of a synthetic double-stranded RNA, polyriboinosinic: polyribocytidylic acid (poly I:C), which mimics viral infection, induces interferon-α (IFN-α) and serotonin (5-HT) transporter (5-HTT) in the brain. To explore the functional significance of their expression, we determined extracellular concentrations of 5-HT and other monoamines such as noradrenaline (NA) and dopamine (DA) in the medial prefrontal cortex (mPFC) of freely moving rats using in vivo microdialysis method. Following an i.p. injection of poly I:C (3 mg/kg), NE levels in the mPFC transiently increased but returned to the basal level within 6 hrs after the injection. DA levels were not affected by poly I:C. On the other hand, 5-HT concentration in the mPFC decreased to 60-70% of the basal level until 8 hrs after poly I:C, while levels of a 5-HT metabolite, 5-hydroxyindole acetic acid, did not alter. The poly I:C-induced decrease in 5-HT was significantly attenuated by local perfusion with a selective 5-HT reuptake inhibitor (fluoxetine) in the mPFC. Microinjection of IFN-α into the mPFC also decreased 5-HT levels, which was again attenuated by perfusion with fluoxetine. It is considered that the poly I:C-induced 5-HTT, which is shown to be induced by IFN-α in astrocytes or endothelial cells, may scavenge extracellular 5-HT into the blood or cerebrospinal fluid, thereby decreasing 5-HT levels. We have reported that the decrease in 5-HT in the brain is closely related to the central mechanisms of fatigue.

O133 (2O-07D8)

Decrease of connexin 43 expression in ventricular myocytes and prolongation of QRS duration on electrocardiogram in type II diabetes mellitus model OLETF rats

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Diabetes mellitus (DM) frequently accompanies with contractile dysfunction and arrhythmia. L-type Ca^{2+} channel current ($I_{Ca(L)}$), transient outward current (I_{to}), delayed rectifier outward K^+ current ($I_{K(delay)}$) and Na/Ca exchanger current (I_{NCX}) in ventricular myocytes were compared between control (LETO) and a type II DM model (OLETF) rats using a patch-clamp technique at 50 weeks of age to clarify electrophysiologic changes in diabetic heart. Blood pressure (BP) was measured at caudal artery by noninvasive tail-cuff method. After rats were anesthetized by sodium pentobarbital, electrocardiogram (ECG) was recorded by apex-base lead, and then hearts were excised and perfused with collagenase solution to isolate myocytes. Fibrosis of ventricles was histologically evaluated using Azan stain and connexin 43 protein (Cx43) expression was quantitated by western blot. Systolic and diastolic BPs were significantly elevated in OLETF rats. PQ interval and QRS duration were significantly prolonged and the cell sizes of myocytes were enlarged remarkably in OLETF rats. Current densities of ($I_{Ca(L)}$, I_{to}, $I_{K(delay)}$ and I_{NCX} were not changed in OLETF rats as compared with those in LETO rats. Although fibrosis was not seen in OLETF rat ventricles, Cx43 expression significantly decreased. We thought that the QRS duration was prolonged due to the delay in conduction of excitation in OLETF rat ventricles, which might be related with the decrease in Cx43 expression.

O134 (1O-06H1)

Evidence of microglial activation in the brain in acute stress

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Microglial cell has been demonstrated to be involved in various diseases, such as Alzheimer and Parkinson diseases, HIV encephalitis and multiple sclerosis. In spite of the facts that stress plays crucial roles in the progression of clinical diseases, the involvement of stress on the microglial activity remains to be elucidated. Based on finding that stress induced the elevation of proinflammatory cytokines, we hypothesized; (1) physical/emotional stress may have some effect on the microglial activation, (2) IL-18, a proinflammatory cytokine and demonstrated to be increased in stress from the adrenal gland, may participate in the microglial activation. We employed restraint combined with water immersion stress for 2 hours as acute stress. Immediately after release from stress, rats were sacrificed for experiments. Immunohistochemistry with OX-42 revealed that acute stress provoked morphological microglial activation in the thalamus, hypothalamus, hippocampus and central grey. Semi-quantitative real time PCR and immunohistochemistry showed that stress significantly induced IL-6 and iNOS from microglia. In addition, intraperitoneal IL-18 administration (5 μg/rat) caused robust microglial activation in the brain in a similar fashion observed in stress. Furthermore, in-vitro studies using microglia cell line (MG6-1) demonstrated that IL-18 administration (up to 500 ng/ml) significantly induced iNOS, IL-6, and IL-18 in a dose dependent manner. Thus, the present study suggests that stress may stimulate microglial cells to produce several pro-inflammatory cytokines and iNOS at least through stress-induced circulating IL-18.

ORAL
Miscellaneous—modeling & simulation, methodology, history, etc.

O135 (2O-07D2)

Role of Spikar in the maintenance of dendritic spines

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Dendritic spines are multiple functional units that receive most excitatory inputs in central nervous system. Modification of dendritic spine number is associated with several neurological diseases and synaptic plasticity. Spikar is a novel molecule which was isolated as a drebrin-binding protein using yeast two hybrid screening. In rat primary cultured hippocampal neurons, GFP-Spikar was localized primarily in nucleus and dendritic spines, and lesser amounts in soma, dendritic shafts, and axons. In this study, we investigated the role of Spikar in cultured hippocampal neurons during development. Hippocampal neurons were transfected with a Spikar-shRNA expression vector or an empty vector as a control at several developmental stages. The Spikar-shRNA expression vector caused 60-90% knock down (KD) of endogenous Spikar. In early stage of development, Spikar KD did not affect the density of dendritic protrusions. In contrast, at a stage of synapse formation, Spikar KD reduced spine density without changing filopodia density. In more mature stage when majority of dendritic protrusions are dendritic spines, Spikar KD reduced spine density as well. These results suggest that Spikar plays a role in the maintenance of dendritic spines without affecting the filopodia formation.

Poster Presentations

POSTERS
Cellular & molecular physiology

P1 (3P1-001)

Enhancement of Ca^{2+}-regulated exocytosis by indomethacin-induced arachidonic acid accumulation in guinea pig antral mucous cells

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Ca^{2+}-regulated exocytosis is enhanced by the PGE_2/cAMP pathway in antral mucous cells of guinea pigs. The inhibition of the PGE_2/cAMP pathway by a PKA inhibitor (H-89) or aspirin (ASA) decreased the frequency of ACh-stimulated exocytotic events by 60%. Indomethacin (IDM), however, decreased the ACh-stimulated exocytotic events only by 30%. Moreover, IDM increased the ACh-stimulated exocytotic events by 50% in H-89-treated or ASA-treated cells. IDM inhibits the synthesis of PGG/H and 15R-HPETE, while ASA inhibits only PGG/H synthesis. Thus, IDM accumulates arachidonic acid (AA). $AACOCF_3$ or ACA (PLA_2 inhibitors), which inhibits AA synthesis, decreased the ACh-stimulated exocytotic events by 60%. IDM, however, did not increase the frequency in $AACOCF_3$-treated cells. AA increased the frequency of ACh-stimulated exocytotic events in $AACOCF_3$- or ASA-treated cells, similar to IDM in ASA-treated cells. Moreover, in the presence of AA, IDM did not further increase the ACh-stimulated exocytotic events in ASA-treated cells. The PGE_2 release from antral mucosa indicates that inhibition of PLA_2 by ACA decreases AA accumulation in unstimulated and ACh-stimulated antral mucosa. The dose-response study of AA and IDM demonstrated that the concentration of intracellular AA accumulated by IDM is less than 100 nM. In conclusion, IDM modulates ACh-stimulated exocytosis via AA accumulation in antral mucous cells.

P2 (3P1-002)

FK506-induced Ca^{2+} release from microsomal vesicles of rat pancreatic acinar cells is biphasic

Ozawa, Terutaka (*Dept. Physiol. Tohoku Univ. Grad. Sch. Med., Sendai, Japan*)

The effect of the immunosuppressant drug FK506 on microsomal Ca^{2+} release was investigated in rat pancreatic acinar cells. When FK506 (0.1-200 μM) was added to the microsomal vesicles at a steady state of ATP-dependent $^{45}Ca^{2+}$ uptake, FK506 caused a dose-dependent and a biphasic release of $^{45}Ca^{2+}$. Almost 10% of total $^{45}Ca^{2+}$ uptake was released at concentrations of FK506 up to 10 μM (K_m = 0.47 μM), and 60% of total $^{45}Ca^{2+}$ uptake was released at concentrations of FK506 over 10 μM (K_m = 55 μM). Preincubation of the vesicles with cyclic ADP-ribose (cADPR: 0.5 μM), which is known to modulate the ryanodine receptor, increased the FK506 (< 10 μM)-induced $^{45}Ca^{2+}$ release (V_{max} value of the release: 8.1% without cADPR vs. 14.4% with cADPR). Preincubation with 200 μg/ml of heparin, an inhibitor of inositol 1,4,5-trisphosphate (IP_3) receptor, resulted in significant inhibition of the FK506 (30 μM)-induced $^{45}Ca^{2+}$ release. Subsequent addition of IP_3 (5 μM) after FK506 (100 μM)-induced $^{45}Ca^{2+}$ release did not cause any release of $^{45}Ca^{2+}$. These results indicate that there are two different types of FK506-induced Ca^{2+} release mechanisms in the endoplasmic reticulum of rat pancreatic acinar cells: a high-affinity mechanism of Ca^{2+} release, which is activation of the ryanodine receptor, and a low-affinity mechanism of Ca^{2+} release, which is activation of the IP_3 receptor.

P3 (3P1-003)

Properties of store-operated Ca^{2+} entry in rat chromaffin cells

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The application of thapsigargin (TG) or cyclopiazonic acid (CPA) to Fura-2-loaded chromaffin cells in the perfused rat adrenal medulla abolished a transient $[Ca^{2+}]_c$ rise induced by muscarine in Ca^{2+}-free medium due to the depletion of Ca^{2+} stores. Either TG or CPA induced a sustained increase of $[Ca^{2+}]_c$ in Ca^{2+}-containing medium, indicating that store-operated Ca^{2+} entry (SOCE) mechanism exists in this cell type. The TG-induced $[Ca^{2+}]_c$ increase was inhibited completely with 2 mM Ni^{2+} but only by 18% with 100 μM D600, whereas maintained $[Ca^{2+}]_c$ increases during prolonged stimulations with muscarine (100 μM) and high-K^+ (40 mM) were inhibited by 100% and 51% with Ni2+, by 53% and 80% with D600, respectively. In cells to which muscarine and Ni^{2+} had been co-applied, Ca^{2+} stores remained depleted to induce a sustained SOCE after the two agents were washed out. In isolated chromaffin cells, CPA induced a much smaller extent of elevation in $[Ca^{2+}]_c$, compared with that induced in cells being in the adrenal medulla, which possibly suggests that the mechanism involved in SOCE may be fragile and was impaired in dissociation. TG or CPA applied alone to the adrenal medulla did not elicit a detectable amount of catecholamine secretion despite the elevation of $[Ca^{2+}]_c$, nor promoted secretory responses to a significant extent when applied during stimulation with high-K^+. These results suggest that SOCE in rat chromaffin cells may not produce a sufficient increase in $[Ca^{2+}]_c$ near the secretory vesicles to trigger exocytosis.

P4 (3P1-004)

Expression and characterization of Calcium-sensitive myosin II

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Purpose: Myosin II is one of the typical motor proteins and is classified as non-regulated, phosphorylatable and Ca-binding myosins. Physarum myosin II belongs to Ca-binding one. Myosin II regulated by Ca-binding has not yet expressed as a recombinant protein. Here, we report the expression of heavy mero-myosin of physarum myosin II together with preliminary characterizations. Method: We used baculovirus expression system. Sf9 cells were infected with the virus constructs. Result: When baculovirus of heavy chain(HC) fragments was infected together with those of phosphorylated light chain (PLC) and Ca-binding light chain (CaLC), Sf9 cells produced soluble HMM, which were recovered in the supernatant together with PLC and CaLC. The HMM showed Mg-ATPase activity of 0.21 ($s^{-1}head^{-1}$), and actin-activated ATPase activity with Vmax=1.27 ($s^{-1}head^{-1}$), and Km=1.8μM. The movement of actin filaments on the HMM-coated glass surface was sensitive to Ca^{2+}. We will show the effect of Ca^{2+} on the movement of HMM associated with various kinds of light chains.

P5 (3P1-005)

Analysis of IP_3 dynamics during the intracellular Ca^{2+} oscillations in mammalian eggs

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At fertilization of mammalian eggs, the repetitive Ca^{2+} releases from intracellular stores through inositol 1,4,5-trisphosphate (IP_3) receptors are thought to be induced by the soluble factor originated from the sperm. Although phospholipase C zeta (PLCζ) is the possible candidate of such 'sperm factor', there are no evidence to show the elevation of intracellular IP_3 concentration at fertilization. We measured the changes in IP_3 concentration during Ca^{2+} oscillations in mouse eggs, using a novel FRET-based IP_3 probe, fretino. Eggs expressing fretino showed a transient decrease in FRET signal in response to the microinjection of IP_3, but not to inositol 1,3,4,5-tetrakisphosphate (IP_4). The signal decayed exponentially with a time constant of 100-180 s. When Ca^{2+} oscillations were induced in the eggs by insemination, the signal of fretino showed no detectable changes to indicate the increase in IP_3 concentration. On the other hand, eggs expressing a large amount of PLCζ showed significant decrease in the FRET signal from fretino. Furthermore, the FRET signal oscillated with Ca^{2+} in such eggs, suggesting the enhancement of PLCζ activity by cytoplasmic Ca^{2+}. The magnitudes of Ca^{2+}-induced IP_3 production in the fertilized eggs and in the eggs expressing PLCζ or PLCδ1, were also compared.

P6 (3P1-006)

CaMKI-induced Phosphorylation Regulates Drp-1 Dynamics and Mitochondrial Morphology in Hippocampal Neurons

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Mitochondrial morphology is regulated by balance of fission and fusion events. Certain dynamin family members such as dynamin-related protein 1 (Drp-1) are involved in the regulation of mitochondrial fission. Drp-1 specifically controls mitochondrial outer membrane fission. However, very little is known about the mechanism that initiates mitochondrial fission by Drp-1. In the present study, we detected Drp-1 was phosphorylated by CaMKI in vitro. In primary cultured hippocampal neurons, high K^+ stimulation induced phosphorylated Drp-1 increment and Drp-1 transition from cytoplasm to mitochondria and mitochondrial fragmentation. The effect of high K^+ was inhibited by KN93 (CaMK inhibitor). In vitro experiment, we found phosporylation promoted the Drp-1 complexes formation. Although overexpression of GFP-hFis1-C did not alter mitochondrial morphology, it inhibited high K^+ induced mitochondrial fission in neurons. These results suggest that Drp-1 dynamics and mitochondrial morphology may be regulated by CaMKI-induced Drp-1 phosphorylation.

P7 (3P1-007)

Na^+ entry via store-operated Ca^{2+} channels in mouse submandibular acinar cells.

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We reported previously that two store-operated Ca^{2+} channels (SOCs) could be separated by using Zn^{2+} in the submadibular acinar cells. In short, after depleting the Ca^{2+} stores with thapsigargin, SOC signals during readmission of external Ca^{2+} were detected. The signal showed two phases; the initial large transient and subsequent sustained phase. External Zn^{2+} inhibited the former but not the latter. External Ni^{2+} or excess of outside K^+ markedly reduced both. Based on this observation, we studied Na^+ entry through SOC. Loading benzofrane isophthalate (SBFI)-AM for 2 h at 37°C to the cells, internal $[Na^+]_i$ was monitored with digital imaging methods. Prior elimination of external Na^+ (replaced with an impermeable cation, $NMDG^+$) induced a substantial increase in Na^+ entry by Na^+ readmission, and it was strengthened by a simultaneous elimination of external Ca^{2+}. When Ca^{2+} stores were actively depleted with thapsigargin under Ca^{2+}- and Na^+-free condition, the largest Na^+ signals were counted by the Na^+ readmission. In contrast to the pattern of Ca^{2+} entry, that of Na^+ was monophasic and inhibited by external Zn^{2+}, Ni^{2+} and Ca^{2+} as well. The finding that external Ca^{2+} reduced Na^+ signals suggests that Ca^{2+} store depletion induces Na^+ entry through SOCs in a competitive manner with Ca^{2+}. Collectively, after depletion of Ca^{2+} stores, Na^+ may enter into the cells through the divalent cation-sensitive Ca^{2+}-entry pathway in mouse submandibular acinar cells.

P8 (3P1-008)

Q268X binds with wild-type HNF4α or its repressor SHP and accumulates in the nucleolus in cultured cells

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Although the HNF4 α protein is known to function as a dimer and Q268X heterozygotes carrying mutations in its allele cause MODY1, it is still unclear whether Q268X and wild type HNF4 α can dimerize, and what causes such a distinct phenotype. We visualized the practical and mutual interactions of HNF4 α and Q268X HNF4 α using Fluorescence Resonance Energy Transfer (FRET). A transition in cellular localization was seen in Q268X-HNF4 α complexes from the nucleoplasm to the nucleolus, where wild type HNF4α is normally localized in COS7 and CHO cells. Furthermore, FRET microscopy showed that Q268X-HNF4 α bound to wild type HNF4α and accumulated in the nucleolus. SHP, which is a repressor of HNF4 α, also bound to Q268X and translocated to the nucleolus. The cellular localization of a deletion mutant of HNF4 α showed that the site contributing to nucleolar accumulation is P333 to I338. Some proteins displayed an altered cellular function after localization to the nucleolus. According to these results, transfer to the nucleolus of the heterodimer Q268X-HNF4 α must affect the function of HNF4 α. Consequently, this study showed that Q268X-HNF4 α dimerizes with wild type HNF4 α and also binds with the repressor and changes its localization to the nucleolus. These effects, together with transcription function, may lead to the distinct phenotype of MODY.

P9 (3P1-009)

Effects of a time-varying magnetic field on intracellular organelles of bovine adrenal chromaffin cells in culture.

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We tested the effects of exposure to a switched 1.5 Tesla magnetic field on transient increase in intracellular Ca^{2+} stimulated by neurotransmitters in bovine adrenal chromaffin cells. $[Ca^{2+}]i$ was increased transiently by addition of acetylcholine (ACh) in Ca^{2+}-free medium and the ACh-induced increase was inhibited significantly by 2 hr-exposure to the magnetic field. The exposure caused not only to decrease the peak value but also to slow the decay phase of $[Ca^{2+}]i$ after peak. Delay of the decay phase was also caused by addition of KCN in the presence of ACh. The intracellular ATP content and oxygen consumption were influenced by the exposure in glucose-free medium. Measurement of mitochondrial membrane potential by using fluorescent probe, JC-1 (Molecular Probe), showed depolarization of the membrane in both cells exposed to antimycin and the magnetic field. The cellular content of F-actin stained with fluorescent probe (Alexa fluor 488 phalloidin) was also decreased by the exposure. These effects of magnetic field would be related to the eddy current.

P10 (3P1-010)

Enhancement of the priming step of exocytotic events caused by Cl^--free solution

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The final steps of exocytosis are consisted of three steps, docking, priming, and fusion, in which the priming step is maintained by ATP. We examined the effects of Cl^--free solution on the priming step.The isolated antral mucous cells were obtained by a collagenase treatment, and observed using video-microscopy. In Cl^--free solution, Cl^- was replaced with NO_3^-.Acetylcholine (ACh 1 μM) increases the frequency of exocytosis ; an initial phase followed by a sustained phase. The Cl^--free solution enhanced the initial peak frequency of ACh-evoked exocytosis approximately 4 fold respectively.To examine effects of Cl^--free solution on the priming step, intracellular ATP was depleted by anoxia (aerated with N_2 100%) or dinitrophenol (DNP 100 μM). Depletion of ATP eliminated the initial phase of ACh-evoked exocytotic events. After ATP depletion, Cl^--free solution did not evoke any initial phase.However, when cells were first perfused with Cl^--free solution, and then ATP was depleted, ACh induced an initial phase. Moreover, cells were first stimulated with ACh, which depelets the primed granules, and after a short interval (9min), cells were stimulated with ACh again. The initial phase was induced by the second ACh stimulation during perfusion with Cl^--free solution, while it was not during perfusion with the control solution.Based on the observation, Cl^--free solution increases number of the primed granules, which enhances ACh-evoked exocytotic events in antral mucous cells.

P11 (3P1-011)

Change in expression and distribution of tight junction-related proteins in primary cultured parotid acinar cells

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Tight junction is the essential structure for salivary epithelial cells to keep polarity and to secrete fluid unidirectionally. Previously, we established a system for primary culture of parotid acinar cells. Acinar cells isolated from the rat parotid glands formed large colonies and attached to the basement of dishes at 24 h after the dispersion. After 2 days, most cells spread as a monolayer whereas a part of cells formed hemispherical lumps. Analysis with electron microscopy suggests that cells in the lumps retained original tight junctions and lumens. On the other hand, cells in monolayer also formed tight junctions. Immunofluorescence microscopy showed claudin-1 was observed in the lumps, while claudin-4 was detected at the tight junctions in monolayer. Most cells in the monolayer that had claudin-4-positive tight junctions retained secretory granules containing amylase. Therefore, the claudin-4-positive cells were probably derived from acinar cells, but not from ductal cells. Immunoblotting analysis showed that claudin-4 was expressed at 24 h and its expression increased time-dependently during the culture, although it was not detected just after the dispersion. These results suggest that the expression of claudins changed from isotype 1 to 4 while the morphology of the acinar cells changed to monolayer. Claudins possibly have a correlation with the formation and maintenance of culture configuration in parotid acinar cells.

P12 (3P1-012)

Effects of ELF magnetic fields on differentiation of cultured osteoblast-like cells

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In this study, the effects of extremely low frequency (ELF) region on the osteoblast-like cells (MC3T3-E1) for the differentiation was examined. Sinusoidal (60Hz) magnetic fields were about 3 mT. Collagen protein contents of cultures were measured microscopically by using ImSpector system. Using insulin-like growth factor I (IGF-I), the difference between the effect by exposure was examined with these during effects for the collagen content of these cells. From these results, the effects of exposure and IGF-I treatment caused significant increase on collagen synthesis of osteoblasts. It is supposed that the effects of magnetic fields go through the intercellular signaling pathway. Therefore, we experiment by the use of some inhibitors which block the intercellular signal transduction and examine which route the exposure passes in order to influence on differentiation of osteoblasts. As a results, it is suggested that ELF magnetic fields stimulate collagen synthesis because of activation of p38 MAPK and induce the cell differentiation. These results indicate that the mechanisms of differentiation related to IGF in the osteoblasts were altered by the magnetic fields of extremely low frequency.

P13 (3P1-013)

Rapid recruitment of Na,K-ATPase to the cell surface

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Na,K-ATPase is a membrane-bound protein that maintains intracellular ionic concentrations, i.e., low Na+ and high K+, using the energy from hydrolysis of ATP. We have now observed a rapid recruitment of Na,K-ATPase to the cell surface in responce to extracellular low calcium concentration in the parathyroid cell that releases PTH in responce to low calcium. Using isotope-labelled ouabain that is a specific ligand for Na,K-ATPase, we observed that the recruitment occurs in a few minutes. Biotinylation of the cell-surface proteins revealed that a considerable amount of Na,K-ATPase is present in intracellular region and bound to a specific protein. In analysis of the protein-deficient mice, the molecular association is required for a novel mechanism of rapid recruitment of Na,K-ATPase. This rapid recruitment is essential for PTH release that is the first step of calcium regulation of the whole body. This indicates the importance of NA,K-ATPase in the calcium homeostasis.

P14 (3P1-014)

Cyclic GMP modulates ACh-stimulated exocytosis in guinea pig antral mucous cells

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In guinea pig antral mucous cells, acetylcholine (ACh) induces a biphasic increase in the Ca^{2+}-regulated exocytosis: an initial transient phase followed by a sustained one. We studied the effects of cGMP on ACh-stimulated exocytosis in guinea pig antral mucous cells using video microscopy. Cyclic GMP enhanced the frequency of ACh-stimulated exocytotic events, while cGMP alone induced no exocytotic events under the ACh-unstimulated condition. Cyclic GMP did not affect either Ca^{2+} mobilization or cAMP accumulation. cGMP shifted the Ca^{2+} dose-response curve upward with no shift to the lower-concentration, indicating that cGMP increases responsibility of the Ca^{2+}-regulated exocytosis, but not the Ca^{2+} sensitivity. When cGMP was added after ATP depletion by dinitrophenol (DNP) or anoxia (N_2 bubbling), ACh evoked only a sustained phase in the exocytosis without any initial transient phase. In contrast, when cells were pretreated with cGMP before ATP depletion, ACh evoked the biphasic exocytotic events. These observations indicate that cGMP modulates ATP dependent priming of Ca^{2+}-regulated exocytotic events. In conclusion, cGMP increases the number of primed granules via acceleration of the ATP-dependent priming step, which enhances the Ca^{2+}-regulated exocytotic events stimulated by ACh.

P15 (3P1-015)

Vesicle disruption and plasma membrane bleb formation caused by illumination with blue light in acridine orange-loaded cells

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Acridine orange (AO), a weakly basic fluorescent dye, is permeable to plasma and vesicle membranes and preferentially remains in intracellular acidic regions. Using fluorescence microscopy, we observed dynamic changes in AO-loaded cultured mouse cells during illumination with blue light. Immediately after the start of illumination, the successive disruption of vesicle membrane was observed as a flash of fluorescence, and shortly after that, blebs were formed on the plasma membrane regardless of the occurrence of vesicle disruption. Vesicle disruption was almost completely inhibited when cells were treated with the vacuolar H^+-ATPase inhibitor bafilomycin A1 followed by staining with AO, but not when bafilomycin A1 was treated after AO staining. Thus, the filling of AO in the vesicle, which is driven by vacuolar H^+-ATPase, is initially required for vesicle disruption. In contrast, bafilomycin A1 did not prevent plasma membrane blebbing, indicating that the blebs are formed independently of the vesicle disruption. Both the vesicle disruption and the formation of plasma membrane blebs were partially inhibited by removal of oxygen from the cell environment and by singlet oxygen scavengers, sodium azide, ascorbic acid, and L-histidine, but not by the hydroxyl radical scavenger dimethyl thiourea. Thus, both phenomena are likely caused at least in part by the generation of singlet oxygen. These photosensitive features of plasma and vesicle membranes may be based on the use of the photodynamic effect, such as cancer therapy.

P16 (3P1-016)

Live cell tracking of GLUT4 molecule in 3T3L1 adipocyte using Qdot nano-crystals

Fujita, Hideaki; Watanabe, Tomonobu; Nedachi, Taku; Higuchi, Hideo; Kanzaki, Makoto (*TUBERO, Tohoku Univ., Tokyo, Japan*)

Insulin stimulates glucose transport into adipocyte and muscle cells by inducing the translocation of the insulin responsive glucose transporter GLUT4 from intracellular storage compartments to the plasma membrane. GLUT4 translocation is a complicated process involving budding and fission at the storage compartment, trafficking to the plasma membrane, and fusion at the plasma membrane. Here, we have established a new method to visualize the movement of single GLUT4 molecule in living cells. 3T3L1 adipocyte expressing exofacial-myc-GLUT4-eCFP was labeled with Qdot-conjugated Myc antibody in the presence of insulin. Qdot-GLUT4 complex was then endocytosed by washing out of insulin. Observation was performed under video-rate confocal microscope equipped with high sensitivity EMCCD camera so that movement of GLUT4 molecule can be tracked for ~10sec. Movement of GLUT4 molecules was obtained before or after the 2nd insulin stimulation for 5, 15, and 30 min. Analysis of the diffusion of GLUT4 molecules showed that movements can be classified in either 1) free diffusion, 2) confined diffusion, or 3) transport and all these movement existed both at basal state and after insulin stimulation. Overall movement tended to be in confined diffusion at the basal state, but fraction of transported GLUT4 increased by insulin stimulation. Diffusion coefficient of GLUT4 was higher after insulin stimulation than basal state. Together, insulin increases the mobility of GLUT4 and enhances its translocation to the plasma membrane.

P17 (3P1-017)

Selective collection of catecholaminergic (CA) neurons in the brain and its application to functional analyses using tyrosine hydoroxylase (TH) - green fluorescent protein (GFP) transgenic mice

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CA neurons are involved in a wide spectrum of physiological functions in the brain including sensory, motor, emotional, autonomic and endocrine regulation. Most CA neurons are localized in the brainstem and hypothalamic regions and typically make clusters of cells, among which the noradrenergic (A1, A2, A6) and dopaminergic (A9, A10, A12) neurons predominate. In order to explore functional roles of these neurons, we sought to collect CA neurons selectively using TH-GFP transgenic mice in which GFP expression was driven under TH-promoter. Fetal (E14.5, E16.5, or E18.5) brain was extracted, and neurons were dispersed after treating with trypsin, then GFP-positive cells were sorted out by flow-cytometry (FACS). RNA was extracted from the GFP-positive (TH) neurons, reverse-transcribed, and analyzed by PCR.

P18 (3P1-018)

Electrophysiological analysis of electrogenic Na^+-HCO_3^- cotransporter activity in bovine parotid acinar cells

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Electrogenic Na^+-HCO_3^- cotransporter (NBCe) plays an important role in mediating HCO_3^- efflux and influx across the basolateral membrane in various HCO_3^--transporting epithelia, including kidney proximal tubules and pancreatic ducts. Its activity has been generally assessed by monitoring intracellular pH, Na^+ concentration, or membrane potential, but electrophysiological properties of the cotransporter at the native state still remain largely unknown. Using the whole-cell patch clamp technique, we have recently, for the first time, identified and characterized membrane currents attributable to the activity of a NBCe expressed in acutely dissociated acinar cells (BPA cells) from bovine parotid that secretes large volumes of a HCO_3^--rich fluid. Under voltage-clamp conditions, the currents were dependent upon extracellular Na^+ and HCO_3^- and DIDS-sensitive. Further analysis of the currents indicated that the stoichiometry of the native cotransporter is most likely to be 2 HCO_3^- : 1 Na^+. We could also demonstrate that BPA cells express transcripts of NBCe1-B (bNBCe1-B) and that recombinant bNBCe1-B currents in HEK293 cells shares common electrophysiological and pharmacological properties with those of the native currents. This study represents an initial attempt to provide electrophysiological characterization of a NBCe expressed in a native HCO_3^--secreting exocrine gland.

P19 (3P1-019)

Concentration-sensitive Na^+ channel (Na_C) is involved in the regulation of proliferation in rat C6 glioma cells

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The concentration-sensitive Na^+ channel (Na_C; c:concentration) works as a Na^+ sensor and it opens when $[Na^+]_o$ changes. The present study was carried out to clarify the function of the Na_C as one of the regulating factors in cell growth, using rat C6 glioma cells, since they have Na_C in quantity. The image analysis of Na^+ dynamics, using a Na^+ indicator (SBFI) and ARGUS-50 (Hamamatsu Photonics), revealed an elevation of $[Na^+]_i$ when $[Na^+]_o$ was raised from normal (140 mM) to 190 mM. This increase was augmented when Na^+ efflux was suppressed by inhibitors of the Na^+ pump (ouabain) or of the $Na^+/K^+/Cl^-$ cotransporter (bumetanide). Osmolarity alteration was not important because addition of mannitol to the external solution did not introduce any changes in $[Na^+]_i$. The expression of immediate early gene *egr-1*, measured by the real-time PCR method, was reduced when $[Na^+]_o$ was raised or when $[Na^+]_i$ was elevated by a Na^+ ionophore, monensin (Cell Biol Int 29:261-268, 2005). These procedures suppressed the rate of cell proliferation. When the expression of Na_C was selectively inhibited by RNA interference (RNAi) techniques, both $[Na^+]_i$ and the growth rate of C6 cells were less affected by $[Na^+]_o$ changes, indicating that Na_C was involved in cell growth (by controlling gene expression through introduction of Na^+ into the cell). It is concluded that Na^+ ions enter C6 glioma cells mainly through Na_C, and Na^+ ions regulate cell growth by controlling expression of proliferation-related genes.

P20 (3P1-020)

Voltage- and pH-dependence of proton flux through the plasmalemmal vacuolar-type H+-ATPase in osteoclasts

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The vacuolar-type H^+-ATPase (V-ATPase) is an electrogenic H^+ pump that is distributed in living organisms. By carrying out uphill H^+ transport, V-ATPases acidify lysozomes and energize intracellular membranes. In osteoclasts, the V-ATPases are enriched in the plasma membrane faced to the bone surface (ruffled membrane) and serve as a major acid-secretion pathway required for bone resorption. In this study, we attempted to identify the pump currents of osteoclasts electrophysiologically and investigated their dependence on the membrane potential and/or H^+ gradient, which may change widely under different functional states. Outward H^+ currents were increased by intracellular dialysis with ATP up to 10 mM in dose-dependent manner. The V-ATPase current was evaluated by blockers for the V-ATPase, bafilomycin A_1 and N,N'-dicyclohexylcarbodiimide, in the presence of 5 mM ATP. The V-ATPase currents were decreased by hyperpolarization, but were still outward at -80 mV under pH_o/pH_i of 7.3/5.5. The current amplitude was decreased by either intracellular alkalization or extracellular acidification, but did not show current reversal. Significant outward H^+ currents were seen at 0 mV even under pH_o/pH_i of 5.5/7.3. The data showed that the V-ATPase-medaited currents depends on both voltage- and pH gradients across the plasma membrane. The pump, however, could secrete H^+ upon exposure to strong acids as far as energy is supplied sufficiently.

P21 (3P1-021)

Effects of K^+ and Cl^- on Na^+-dependent Mg^{2+} efflux from rat ventricular myocytes

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We measured intracellular free Mg^{2+} concentration ($[Mg^{2+}]_i$) with fluorescent Mg^{2+} indicator furaptra (mag-fura-2) in Ca^{2+}-free condition (0.1 mM EGTA) at 25°C. After the cells loaded with Mg^{2+} in 24 mM-Mg^{2+} solution for 3 h, reduction of $[Mg^{2+}]_o$ to 1 mM caused a decrease in $[Mg^{2+}]_i$ in the presence of extracellular Na^+ (Na^+-dependent Mg^{2+} efflux). To study the effects of K^+ on Na^+-dependent Mg^{2+} efflux, the initial rate of decrease in $[Mg^{2+}]_i$ (initial $\delta[Mg^{2+}]_i$) /δt) was compared at high extracellular [K^+] (75 mM) and K^+-free (replaced by N-methyl-D-glucamine) conditions. $[Na^+]_o$ was kept constant at 70 mM, and membrane potential was set at -13 mV with amphotericin-B-perforated patch clamp technique. With the K^+-based pipette solution, the initial $\delta[Mg^{2+}]_i/\delta t$ values obtained in the presence of 75 mM K^+ and 0 mM K^+ in the perfusate were not significantly different (79.0±6.0% and 65.6±5.0%, respectively, of the control values measured at 140 mM $[Na^+]_o$ without any modification of extracellular and intracellular K^+ and Cl^-). Intracellular perfusion with K^+-free (Cs^+-substituted) solution from the patch pipette in combination with removal of extracellular K^+ did not significantly change the initial $\delta[Mg^{2+}]_i$) /δt (77.7±8.2% of the control). Finally, the initial $\delta[Mg^{2+}]_i/\delta t$ was unchanged by extracellular and intracellular perfusion with K^+-free and Cl^--free solutions (71.6±5.1% of the control). These results suggest that K^+ and Cl^- are not involved in the Na^+-dependent Mg^{2+} efflux.

P22 (3P1-022)

Volume-sensitive chloride channel involved in necrotic neuronal death by excitotoxicity

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Excitotoxicity is associated with stroke, brain trauma and neurodegenerative disorders. Focal swellings along dendrites called varicosities are a hallmark of acute excitotoxic neuronal injury. We previously reported that cultured mouse cortical neurons express the volume-sensitive outwardly rectifying (VSOR) chloride channel, which is involved in volume regulation after osmotic swelling. Here we studied a role of the VSOR chloride channel in excitotoxic neuronal injury in cultured mouse cortical neurons. The blockade of the VSOR chloride channel activity by NPPB (40 μM), phloretin (100 μM) or IAA-94 (1 mM) during excitotoxic stimulation inhibited varicosity formation and necrotic neuronal death. On the other hand, a $GABA_A$ receptor/chloride channel blocker, bicuculline (10 μM) or picrotoxin (100 μM), failed to inhibit neuronal necrosis induced by excitotoxicity. On-cell patch-clamp studies revealed robust VSOR chloride channel activity on varicosities during exposure to NMDA. These results suggest that the VSOR chloride channel is involved in aggravation of excitotoxicity by serving as the pathway for chloride influx, which induces varicosity formation and cell swelling leading to necrotic cell death.

P23 (3P1-023)

Inhibition of a glial K channel by various tricyclic antidepressants

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Around 70% of brain tissue is composed of glial cell, which regulates the homeostasis of various neurotransmitters, ions and water in the brain. However, little studies have been performed on the effects of CNS-acting drugs on glial function. We have examined the effect of various tricyclic antidepressant agents: amitriptyline, imipramine, nortriptyline, and desipramine, on a K^+channel responsible for the glial K^+-buffering action. The glial K^+ -buffering channels are composed either of homomeric assembly of Kir4.1 or of heteromeric assembly of Kir4.1 and Kir5.1. In this study, Kir4.1 homomeric channels were exogeneously expressed in tsA201 cells and whole-cell currents were recorded using a patch-clamp technique. Application of each of the various tricyclic antidepressants immediately and reversibly caused a reduction of inward and outward currents through this channel. The inhibition was stronger as the membrane was more depolarized. Development of the current blockage was well fitted with a single exponential function. These results indicate that the block of Kir4.1 channels by these antidepressants was clearly in a voltage- and time-dependent fashion. Thus, various tricyclic antidepressants may act as inhibitors at the glial Kir4.1 channels. We conclude that the inhibition of the glial Kir4.1 channels by these drugs underlies the therapeutic effects and some of the side effects, particularly seizures in overdose.

P24 (3P1-024)

Inhibition of hypertonicity-induced cation channel sensitizes HeLa cells to shrinkage-induced apoptosis

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Cell shrinkage is a hallmark of apoptosis. We previously demonstrated that the apoptotic volume decrease, which represents an early-phase event of apoptosis, is induced by K^+ and Cl^- efflux. On the other hand, it is known that osmotic cell shrinkage directly leads to apoptotic death in cells that lack the ability of volume regulation, called regulatory volume increase (RVI). In HeLa cells that can exhibit RVI, however, strong hypertonic stimulation failed to induce cell death. Since we have recently showed that the hypertonicity-induced non-selective cation channel (HICC) plays an important role in the RVI process in HeLa cells, we used flufenamate, a HICC blocker, to induce persistent cell shrinkage. Hypertonicity-induced cell death and activation of caspase-3 was enhanced by flufenamate in a concentration-dependent manner. The concentration dependency was in good accord with that for HICC current inhibition. These results suggest that HeLa cells are sensitized by inhibition of HICC to shrinkage-induced apoptosis.

P25 (3P1-025)

Iptakalim hydrochrolide inhibits ATP-sensitive potassium channel activity of rat pancreatic B-cells

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Iptakalim hydrochloride (IPT) is a novel ATP-sensitive potassium channel (K(ATP)) opener which has a different chemical structure from any other known K(ATP) opener, and produces vasodilution. In this study, we examined the effect of IPT on rat pancreatic beta-cell functions. In the perifusion experiment for islets, an application of IPT increased insulin secretion, tested with 5.5 mM glucose in the extracellular solution. Examined in isolated beta-cells loaded with fura-2, IPT elevated intracellular calcium concentration, which was restored by diazoxide. Under the patch-clamp whole-cell configuration, IPT induced depolarization in isolated beta-cells followed by action potential firing. The depolarization was associated with a decrease in membrane conductance resulting from a decrease in K(ATP) activity. Further, IPT applied into the bath solution inhibited K(ATP), recorded in the cell-attached mode. IPT applied to the intracellular surface of the membrane also inhibited K(ATP), recorded in the inside-out mode. These results indicate that IPT acts on pancreatic beta-cells as a K(ATP) blocker, which in turn causes electrical excitation of the cell and insulin secretion.

P26 (3P1-026)

Gene deletion and silencing refutes the long held hypothesis that maxi-anion channel is a plasmalemmal VDAC

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We have recently demonstrated that maxi-anion channels constitute a major pathway for the regulated release of ATP. It is widely held that a voltage-dependent anion channel (VDAC) located in the plasmalemma that normally functions in the mitochondrial outer membrane is the most likely candidate protein of this channel. This hypothesis was based on the similarity of shared biophysical properties, such as the large unitary conductance and bell-shaped voltage dependency of the maxi-anion channel and mitochondrial VDAC. In the present study, we deleted each of the three genes encoding the VDAC isoforms individually and collectively. We have demonstrated that maxi-anion channel (around 400 pS) activity in VDAC-deficient mouse fibroblasts was unaltered. The channel activity was similar in VDAC1/VDAC3 double-deficient cells and in double-deficient cells with VDAC2 protein depleted by RNA interference. VDAC deletion slightly down-regulated, but never abolished, the swelling-induced ATP release. The lack of correlation between VDAC protein expression and maxi-anion channel activity strongly argues against the long held hypothesis of plasmalemmal VDAC being the maxi-anion channel. Details of the biophysical profile, such as the different potassium-to-chloride and glutamate-to-chloride selectivity and a different pattern of the voltage-dependent gating provide independent support for our conclusion.

P27 (3P1-027)

Functional characterization of Cl-/HCO3- exchanger by using Cl- indicator dye

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Chloride ions subserve many physiological functions, including regulation of cell volume, intracellular pH, fluid secretion, and stabilization of the resting membrane potential. Cl^- is absorbed from the gastrointestinal tract is mediated by Cl^-/HCO_3^- exchanger. Recent studies have suggested that a major Cl^-/HCO_3^- exchanger is SLC26A3. Since multiple isoforms of the Cl^-/HCO_3^- exchanger are co-expressed in an intact colonic cell, complicating the functional analysis of an individual isoform, we generated an N-terminal hemagglutinin epitope-tagged human SLC26A3 construct and expressed transiently in CHO cells by using inducible gene expression systems. Using this system, we have previously characterized SLC26A3 by measuring of its activity with fluorescent pH-sensitive indicators, BCECF. To assess the validity of pH measurements, we measured the Cl^-/HCO_3^- exchange activity by using chloride-sensitive dye, MQAE. We first measured Cl^-/HCO_3^- exchange activity by MQAE and then measured its activity by using pH sensitive dye in the same cells. Cl^-/HCO_3^- exchange rate measured by using MQAE was 10-20-fold greater than the rate measured by using BCECF. In addition, a carbonic anhydrase inhibitor acetazolamide partially inhibited Cl^-/HCO_3^- exchange activity. These results suggest that even in the presence of a carbonic anhydrase, its reaction rate is not enough for intracellular pH measurements to assess the Cl^-/HCO_3^- exchange activity in CHO cells.

P28 (3P1-028)

Src kinase-dependent osmoregulation of Na+ reabsorption in renal A6 cells

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We have previously reported that hypotonic shock stimulates Na+ reabsorption by activating a PTK-dependent pathway and that hypotonic shock causes a decrease in cytosolic Cl- concentration ([Cl-]c) through regulatory volume decrease (RVD) in renal epithelial A6 cells. In this report, we found that hypotonic shock increased tyrosine phosphorylation of src kinase at pY416 (an active site of src kinase) in a manner dependent on the RVD-induced decrease in [Cl-]c. We further found that a decreasse in [Cl-]c caused a significant increase in tyrosine phosphorylation of src kinase at pY416 under an isotonic condition without any effect on tyrosine phosphorylation state of src kinase at pY527 (an inactive site of src kinase). Furthermore, pretreatment with PP2 (a specific inhibitor of src kinase) abolished the hypotonic shock-induced stimulation of Na+ rebasorption and alpha-subunit of epithelial Na+ channel (ENaC) mRNA expression. Taken together these results, it is suggested that hypotonic shock stimulates Na+ rebasorption through induction of alpha-ENaC gene expression by activating src kinase through the RVD-dependent decrease in [Cl-]c. Supported by Grants-in-Aids from JSPS (17590191 and 17390057).

P29 (3P1-029)

Regulation of maxi-anion channel by calcium, magnesium and ATP

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The maxi-anion channel is widely expressed in animal cells. We have recently demonstrated that this channel fulfils a general physiological function as an ATP-conductive gate for cell-to-cell purinergic signaling. However, the regulatory mechanisms of the maxi-anion channel remain poorly understood. We studied the activation mechanism of the maxi-anion channel in inside-out patches excised from mammary C127 cells. The channels activated upon excision in Ca-free solution in the presence of 1 mM Mg2+ and absence of ATP. Increasing both Ca^{2+} and Mg2+ ion concentration led to a dramatic increase in the rate of channel activation. Half-maximal activation occurred at the concentration of 0.0012 mM for Ca^{2+} ions and 2.8 mM for Mg2+ ions. MgATP added to bath (intracellular) solution greatly suppressed the channel activation with half-maximal inhibition at 0.037 mM. A non-hydrolysable analogue of ATP, AMP-PNP, did not suppress the channel activation suggesting that ATP hydrolysis (presumably, the channel phosphorylation) is necessary for the channel inactivation. When all Mg2+ ions were washed out, the free ATP still suppressed the channel, indicating that binding of free ATP can also close the pre-activated maxi-anion channel. Thus, the regulatory control mechanism of the maxi-anion channel involves divalent cation-dependent steps and possibly phosphorylation.

P30 (3P1-030)

Hypoxia activates maxi-anion channels and thereby induces ATP release from astrocytes

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Recent studies have shown that permeability of some chloride channels to organic anions, such as glutamate and ATP, is involved in cell-to-cell communication mediated by released organic anions. Previous our studies demonstrated that a maxi-anion channel serves as a conductive pathway for ATP release in a mouse mammary cell line (Sabirov et al., 2001), rabbit kidney macula densa cells (Bell et al., 2003) and rat cardiomyocytes (Dutta et al, 2004). In the present study, the possible relation between expression of maxi-anion channel and ATP release was tested in mouse astrocytes in primary culture. In response to hypoxia stress, astrocytes exhibited both activation of maxi-anion channel and massive release of ATP. Hypoxia-induced ATP release was inhibited by blockers of maxi-anion channel, but not by those of other candidate pathways for ATP release, such as gap junction hemi-channel, CFTR channel, exocytosis and volume-sensitive outwardly rectifying (VSOR) anion channel. Using a biosensor technique based on ATP responses of P2X2 receptors expressed in HEK293 cells, the local ATP concentration on a single astrocyte surface was found to increase to about 5 μM during hypoxia. Therefore, it is concluded that the maxi-anion channel serves as a major pathway for ATP release from astrocytes under hypoxia.

P31 (3P1-031)

Restoration of volume-sensitive chloride current in cisplatin-resistant human epidermoid cancer KB cells decreases their cisplatin resistance

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The platinum-based drug cisplatin is a widely used anticancer drug which acts by causing the induction of apoptosis. Some types of cancer have intrinsic or acquired resistance to cisplatin, however. A model of cisplatin resistance is provided by the cisplatin-resistant KB/CP4 human epidermoid cancer cell line. It was found previously in our laboratory that activity of the volume-sensitive, outwardly rectifying chloride channel (VSOR-ClC) is virtually absent in KB/CP4 cells. We hypothesized that the lack of VSOR-ClC current may contribute to cisplatin resistance in these cells. An attempt was made to restore the current in KB/CP4 cells so that the effect of its expression on cisplatin resistance could be tested. Treatment of KB/CP4 cells with trichostatin A (TSA), a histone deacetylase inhibitor, caused VSOR-ClC current to be partially restored. A cell viability assay showed that in response to cisplatin, viability of cells treated with TSA for 48 h decreased significantly compared to control cells. Moreover, a caspase-3 activity assay showed that TSA-treated cells underwent significantly increased apoptosis induced by cisplatin. These effects were blocked by simultaneous treatment of the cells with a VSOR-ClC blocker. From these results, we conclude that restoration of VSOR-ClC functional expression by TSA treatment leads to a decrease in cisplatin resistance and an increase in cisplatin-induced apoptosis in KB/CP4 cells.

P32 (3P1-032)

Electrophysiological properties of acid-activated anion channels in HeLa cells

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It has recently been reported that extracellular acid pH activates anion channels in several cell types. In the present study, we also found functional expression of proton-activated Cl^- channel in human epithelial HeLa cells. Whole-cell currents were rapidly activated by extracellular acidic solution (pH < 5.0) in a reversible manner. This current exhibited prominent outwardly rectification under symmetrical Cl^- conditions, time-dependent activation at positive potentials, and low-field anion permeability sequence of $I^- > Br^- > Cl^- >$ aspartate$^-$. A Cl^- channel blocker, 4, 4'-diisothiocyanostilbene-2,2'-disulphonic acid (DIDS) or phloretin, could inhibit the current in a concentration-dependent manner with the IC_{50} value of 0.12 or 18.6 μM, respectively. Anion selectivity and sensitivity to Cl^- channel blockers of this proton-activated current are similar to those of the volume-sensitive outwardly rectifying (VSOR) Cl^- channel current in HeLa cells. However, the other properties are distinct from those of the VSOR Cl^- channel which is inhibited by acid and exhibits modest outward rectification and inactivation kinetics at positive potentials.

P33 (3P1-033)

The importance of Fyn tyrosine kinase in Ca^{2+}-sensitization of vascular smooth muscle contraction induced by a sphingosylphosphorylcholine and Rho-kinase pathway.

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Whereas the Ca^{2+}-dependent contraction of vascular smooth muscle (VSM) which regulates physiological vascular tone, the Rho-kinase (ROK)-mediated Ca^{2+}-sensitization of VSM contraction contributes to abnormal VSM contraction such as vasospasm. We previously found that sphingosylphosphorylcholine (SPC) is an upstream messenger for the ROK-mediated Ca^{2+} sensitization and that inhibitors of Src family tyrosine kinase (Src-TK) blocked the SPC-induced contraction and activation of ROK. In the present study, we attempted to determine the enzyme molecule in a family of Src-TK which contributes to the Ca^{2+}-sensitization mediated by a SPC/ROK pathway. In order to accomplish this purpose, we performed knockdown of the target molecule by using siRNA which was transfected into the human coronary artery smooth muscle cells (CASMCs) with the efficiency of about 100%. The siRNA-mediated knockdown of Fyn inhibited the SPC-induced contraction of CASMC, whereas non-silencing control siRNA lacked any effect. These results provide the first direct evidence that Fyn mediates the Ca^{2+}-sensitization of VSM contraction induced by a SPC/ROK pathway. In addition, Fyn constructs (wild, constitutively active, and dominant negative types) were transfected to CASMCs with high efficiency (> 50%), although CASMCs were well-differentiated contractile cells. In poster presentation, the effects of transient overexpression of Fyn constructs on the contraction of CASMCs will be also discussed.

P34 (3P1-034)

Molecular structure responsible for nuclear translocation of phospholipase C-zeta

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Phospholipase C-zeta (PLCζ) is a strong candidate of the mammalian sperm factor that induces IP_3-mediated Ca^{2+} oscillations and subsequent embryonic development. PLCζ consists of 4 EF-hand domains (EF1-4) in the N terminus, X and Y catalytic domains, and C2 domain in the C terminus. PLCζ expressed by injection of cRNA into mouse eggs causes fertilization-like Ca^{2+} oscillations, and then it is accumulated into the formed pronucleus as the sperm factor is. The nuclear translocation ability (NTA) was investigated by expressing PLCζ mutants tagged with a fluorescent protein by RNA injection into eggs or 1-cell embryos. Point mutation analysis revealed a lysine-rich nuclear localization signal (NLS) sequence between Lys^{374} and Lys^{381} in the X-Y linker region. Truncation of EF1 resulted in the loss of NTA, and point mutation revealed a responsible sequence in the N terminus of EF1. However, even if EF1 was present, NTA was lost when EF2-4 or C2 domain was deleted. Both NTA and Ca^{2+} oscillation-inducing ability are lost in these truncation or deletion mutants. Similar results were obtained in cultured COS cells after transfection with cDNA of mutants. It is predicted from the 3-D structure of PLCδ1 that PLCζ is folded at the hinge region in the X-Y linker and that EF-hand domains and C2 domain make extensive contact. Besides NLS, highly coordinated overall structure of PLCζ is responsible for NTA as well as Ca^{2+} oscillation-inducing activity.

P35 (3P1-035)

PACAP/VIP receptors in the guinea pig gallbladder

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PACAP and VIP are closely related neuropeptides and act primarily as inhibitory transmitters on most of the gastrointestinal and vascular smooth muscle cells. However, their actions are opposite in the guinea pig gallbladder. In this study we have tried to identify PACAP/VIP receptor subtypes expressed in the guinea pig gallbladder. Total RNA was extracted from the guinea pig gallbladder. RT-PCR was conducted using the primers with high sequence homology among human, mouse, and rat PAC1, VPAC1, VPAC2 receptors. PAC1, VPAC1 and VPAC2 receptor mRNAs were abundantly expressed in the gallbladder. Sequence analysis of guinea pig PAC1 receptor revealed a high homology (91% in nucleotide sequence and 99% in amino acid) to human PAC1 receptor. There was an isoform of PAC1 receptor that contained an additional 84 nucleotides encoding 28 amino acids in the third intracellular loop. The amino acid sequence was identical to that of the hop variant reported in rats and humans. The nucleotide and amino acid sequences of guinea pig VPAC1 and VPAC2 receptors also had high homologies to the respective human (90% and 95%), rat (91% and 93%), and mouse (93% and 91%) sequences. The guinea pig gallbladder express PAC1, hop variant, VPAC1 and VPAC2 receptor mRNAs. The expression of the hop variant of PAC1 receptor may be related to the contractile response observed in the gallbladder.

P36 (3P1-036)

Signal mechanisms of regulatory volume increase (RVI) in HeLa cells and of RVI inhibition under apoptotic stimulation.

Takahashi, Nobuyuki; Muthangi, Subramanian; Okada, Yasunobu (*Dep. of Cell Physiology, Nat. Inst. Physiol. Sci., Nat. Inst. Natural Sci.*)

Signal mechanisms of regulatory volume increase (RVI) in HeLa cells and of RVI inhibition under apoptotic stimulationTakahashi, Nobuyuki; Subramanian, Muthangi; Okada, Yasunobu (Dept. Cell Physiol., Natl. Inst. Physiol. Sci., Okazaki, Japan)Most cells show cell volume recovery, called regulatory volume increase (RVI), after osmotic shrinkage. However, under apoptotic conditions, cell volume persistently decreases without exhibiting RVI. In human epithelial HeLa cells exposed to hypertonic solution, RVI was significantly inhibited by an Akt blocker. Moreover, exogenous expression of the dominant negative form of Akt inhibited RVI under hypertonic conditions. Akt was phosphorylated by hypertonicity, and this phosphorylation was inhibited by apoptotic stimulation by staurosporine, H_2O_2, or TNF-α. Either of these apoptotic stimuli suppressed RVI and then induced apoptotic cell death. Apoptosis signal-regulating kinase 1 (ASK1) was found to be activated by either apoptosis inducer. Overexpression of the kinase dead mutant of ASK1 restored both shrinkage-induced Akt phosphorylation and RVI under apoptotic conditions. Thus, it is concluded that Akt activation induced by hypertonicity is involved in the RVI mechanism in HeLa cells and that shrinkage-induced Akt activation is inhibited by ASK1 activated by various apoptotic stimuli thereby leading to persistent cell shrinkage in apoptotic cells.

P37 (3P1-037)

Effect of CXCL12 stimulation on matrix metalloproteinases-1 (MMP-1) expression of NK cells

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NK (Natural killer) cells have the ability to migrate and eliminate tumor cells. We evaluated the role of matrix metalloproteinases-1 (MMP-1) on regulating chemokine-dependent invasion of NK cells into type I collagen. We revealed that CXCL12 promoted the invasion program on freshly isolated human NK cells in a MAP kinase dependent manner, because CXCL12 enhanced NK cells invasion was significantly inhibited by p38MAP inhibitor SB203580 and MEK 1/2 inhibitor U0126. Next we examind whether CXCL12 especifically enhanced the production of MMP-1 from NK cells. This production wes significantly inhibited by SB203580 and U0126. Immunofluorescence confocal microscopic studies suggested that MMP-1 was co-localized with alpha2 integrin on the NK cell surface stimulated by CXCL12. The co-localization of MMP-1 and alpha2 integrin was significantly inhibited by SB203580 and U0126. Immunoprecipitation assy showd that productional MMP-1 associated with alpha2 beta1 integrin on NK cells stimulated by CXCL12. This association was significantly inhibited by SB203580 and U0126. These results suggested that MMP-1 associated with the cell surface was involved in NK cell invasion into type I collagen, and MMP-1 associated with alpha2 integrin on the cell surface may be a critical step in facilitating pericellular matrix degradation during cell invasion.

P38 (3P1-038)

Functional analysis of signal molecules of abnormal vascular contraction in lipid raft membrane

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Hypercholesterolemia is a major risk factor of cardiovascular events. A Rho-kinase-mediated Ca^{2+} sensitization of vascular smooth muscle (VSM) plays a critical role in abnormal vascular contraction such as vasospasm. We found that sphingosylphosphorylcholine (SPC) sequentially activated Fyn and Rho-kinase to induce the Ca^{2+} sensitization. We observed the strong link between the SPC-induced contraction and the tissue and cellular cholesterol in VSM, suggesting the involvement of the cholesterol-enriched membrane microdomains, membrane lipid rafts. In membrane-permeabilized VSM, SPC induced contraction in the absence of cytosolic GTP which is required for the activation of G-proteins and thus of GPCRs. Taken together with the localization of Fyn in the membrane lipid rafts, these findings suggest the importance of cholesterol and are compatible with the interaction of SPC with the other membrane components than GPCRs and/or the direct interaction between SPC and lipid membrane, which may in turn affects the function of membrane proteins. Therefore, we examined the interaction of SPC with raft model membranes. The surface plasmon resonance measurement (BIACORE system) revealed that SPC highly associates with the model membrane microdomains, lipid rafts and that cholesterol in the model membrane enhances the incorporation of SPC into the membrane. We propose that cholesterol and its enriched membrane lipid rafts may play a role in Ca^{2+} sensitization mediated by a SPC-Fyn-Rho kinase pathway.

P39 (3P1-039)

Serum-dependence of AMPA receptor-mediated proliferation in glioma cells

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Glutamate may cause Ca^{2+} entry through Ca^{2+}-permeable glutamate receptors, which in turn stimulates the anti-apoptotic signaling cascade in glioma cells. Here, we found that a human glioma cell line, U-87 MG, expressed GluR1, GluR2 and GluR3 subunits of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate acid-type glutamate receptors (AMPARs). Approximately 20% of GluR2 was expressed in the unedited form, which is known to assemble Ca^{2+}-permeable AMPARs. Ca^{2+} entry through the activation of these receptors by AMPA was detected clearly in approximately 20% of U-87 MG glioma cells. Cell proliferation assays revealed that the application of AMPA or glutamate facilitated cell proliferation by activating AMPARs in low-serum medium containing 0.5% fetal calf serum (FCS). Unexpectedly, cell proliferation by the activation of AMPARs was not detected in serum-rich medium containing 10% FCS. Overexpression of the unedited form of GluR2 (GluR2Q) by adenoviral-mediated gene transfer markedly increased the Ca^{2+} entry into U-87 MG cells. This treatment in the presence of glutamate facilitated proliferation and migration of U-87 MG cells in the low-serum condition, whereas it had again no effect in the serum-rich condition. It is therefore likely that cell proliferation and migration of U-87 MG cells are under the regulation of growth factors contained in the serum as well as Ca^{2+} entry through AMPARs, and that the latter regulation becomes evident only when serum factors are deprived of culture medium.

P40 (3P1-040)

Na^+ deprivation induces persistent cell shrinkage and apoptotic cell death

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Although cell shrinkage is one of the phenotypical features of apoptosis, it has been controversial whether it is a prerequisite to apoptosis induction. In this study, we examined whether a persistent decrease in cell volume could per se initiate apoptotic cell death without any apoptotic stimulus. When HeLa cells were incubated in isotonic Na^+-free solution, the mean cell volume immediately began to decrease and reached 84% of the original value within 30 min. After persistent shrinkage, activation of caspase-3 and reduction of cell viability were observed. Application of a blocker of the Na^+-K^+-$2Cl^-$ cotransporter (NKCC), bumetanide (100 μM) or furosemide (1 mM), or that of the Na^+/Ca^{2+} exchanger (NCX), KB-R7943 (25 μM), inhibited Na^+ deprivation-induced shrinkage and attenuated apoptotic cell death. These results suggest that shrinkage of HeLa cells exposed to Na^+-free solution is induced by efflux of Na^+, K^+ and Cl^- mediated by operation of NKCC and reversed operation of NCX, and that an isotonic volume decrease per se triggers the apoptotic process.

P41 (3P1-041)

The role of sphingosine-1-phosphate-1 receptors in pancratic B-cell functions

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Sphingosine-1-phosphate (S1P) receptor is known to show a variety of actions including endothelial permeability regulation. Multiple S1P receptors including S1P-1 are expressed on pancreatic islets, and S1P, a S1P-1 receptor agonist, was shown to potentiate insulin secretion. However, a precise mechanism of the receptor action is not clear at moment. Using Cre-LoxP system, we made mice specifically lacking S1P-1 receptor gene in pancreatic islet B-cells. The blood glucose levels in fasting state are the same in knockout and control mice. After intraperitoneal glucose challenge, knockout mice were significantly less able to normalize blood glucose levels than were the control mice. In the perifusion experiment for isolated islets, glucose stimulation increased insulin secretion in the control islets, whereas it failed in islets from knockout mice. In isolated pancreatic B-cells from both control and knockout mice, glucose stimulation caused depolarization followed by action potential firing. The membrane capacitance measurement revealed that calcium pulse stimulation could not increase the exocytosis of insulin granules. These results indicate that S1P-1 receptors are essential for B-cell insulin secretion in the exocytotic process at least distal to calcium signals.

P42 (3P1-042)

Gq/PLC-coupled receptor-induced transient reduction in plasma membrane phosphatidylinositol-4,5-bisphosphate (PIP_2)

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Phosphatidylinositol-4,5-bisphosphate (PIP_2) is well known to be a source of the important second messengers inositol-triphosphate (IP_3) and diacylglycerol (DAG). To investigate the intracellular localization of PIP_2, it has been commonly utilized pleckstrin homology (PH) domain fused with green fluorescent protein (PH-GFP) as a marker. However this method is not suitable for cells difficult to be transfected. In the present study, we established the assay method of receptor-induced transient dissociation of PIP_2 from the plasma membrane in rat brown adipocytes using anti-PIP_2 antibody. Cells incubated with or without stimuli for 0-120 sec were fixed immediately, blocked with BSA and incubated with anti-PIP_2 antibody. After washing, the cells were incubated with Alexa Fluor 546-labeled IgG and fluorescent signals were observed using confocal laser scanning microscope. In control cells, PIP_2 displayed staining which outlined the cells periphery. When the cells were stimulated with 1μM noradrenaline (NA), plasma membrane PIP_2 was rapidly decreased within 2.5 sec. Dissociation of PIP_2 from the plasma membrane was transient and relocalized to the plasma membrane within 2 min. Stimulation of the cells with 50μM ATP showed similar response to NA. 5μM wortmannin inhibited relocalization of PIP_2 to the plasma membrane in NA- or ATP-stimulated cells, indicating that phosphatidylinositol-4-kinase (PI4K) plays an important role in PIP_2 reproduction.

P43 (3P1-043)

Prostaglandin E_2 release from antral mucosa of guinea pig: Differrent role COX-1 and COX-2

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Contributions of COX-1 and COX-2 in basal and ACh-stimulated prostaglandin E_2 (PGE_2) release were studied in antral mucosa of guinea pig. PGE_2 was released from antral mucosa spontaneously. Acetylcholine (ACh), which increases $[Ca^{2+}]_i$, increased the PGE_2 release from antral mucosa in a dose dependent manner. COX-1 or COX-2, which generates PGE_2 from arachidonic acid (AA), was regulated by $[Ca^{2+}]_i$. Basal and ACh-stimulated PGE_2 release were increased by the addition of AA, and was inhibited by a PLA_2 inhibitor and COX inhibitors. SC560 (100 nM, a selective inhibitor of COX-1) decreased ACh-stimulated PGE_2 release without any decrease in basal PGE_2 release, while ionomycin increased PGE_2 release. NS398 (20 μM, a selective inhibitor of COX-2) decreased basal PGE_2 release without any decrease in ACh-stimulated PGE_2 release. Moreover, in isolated antral epithelial cells, SC560 inhibited ACh-stimulated-PGE_2 releases, however, NS398 did not. Thus, in antral mucosa, basal PGE_2 release is maintained via COX-2 of interstitial cells and ACh-stimulated PGE_2 release is maintained via COX-1 of antral epithelial cells. These observations suggest that PGE_2 released via COX-2 in the interstitial cells maintains an integrity of the resting antral mucosa and that released via COX-1 in antral epithelial cells maintains an autocrine mechanism, which enhances Ca^{2+}-regulated exocytosis in ACh-stimulated antral mucosa, such as during meals.

P44 (3P1-044)

Germ cell apoptosis in rat testis is induced by oxidative stress via oral administration of di(2-ethylhexyl)phthalate, and is significantly prevented by treatment of antioxidant vitamins or rare sugars

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Phthalate esters have been used as plasticizers of synthetic polymers. Recent studies revealed that they induce atrophy of the testis, although its pathogenesis remains unknown. Testicular atrophy with aspermatogenesis was induced by feeding with 2% DEHP-containing diet. The biochemical and immunohistochemical analysis revealed that DEHP increased the reactive oxygen species generation, with concomitant decrease of glutathione and ascorbic acid, and selectively induced apoptosis of spermatocytes, thereby causing atrophy. Oxidative stress was selectively induced in germ cells, but not in Sertoli cells, treated with mono(2-ethylhexyl)phthalate (MEHP), a hydrolysed metabolite of DEHP. Furthermore, MEHP selectively induced the release of cytochrome c from mitochondria of the testis. These results indicate that oxidative stress elicited by MEHP principally injured mitochondrial function, and induced apoptosis of spermatocytes and atrophy of the testis. Using the 2% DEHP-dose, the effect of simultaneous administration of vitamins C and E was examined. The vitamin supplementation significantly prevented the testicular injury. Results suggest that antioxidant vitamins can protect the testes from DEHP-toxicity. Some of rare sugars (i.e. D-psicose and D-allose) are also effective in prevention of the testicular injury. Microarray analysis has been applied to elucidate the genes involved in the DEHP-toxicity and the protection mechanism.

P45 (3P1-045)

Lysophosphatidic acid (LPA)-induced cell migration inhibition is independent on ROCK-mediated reduction in PI3-kinase (PI3K) products

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PI3Ks produce 3'-phosphoinositides (3'-PIs) including PI(3,4,5)P_3 and PI(3,4)P_2, whereas PTEN dephosphorylates 3'-PIs to decrease the contents of PI(3,4,5)P_3 and PI(3,4)P_2. Elevation of PI(3,4,5)P_3 and PI(3,4)P_2 contents induces activation of PDK1 and Akt, resulting in cell migration and cell survival. Very recently, it has been shown that Rho-ROCK stimulates PTEN, resulting in inhibition of Akt and cell migration. These observations led us to investigate the effects of Rho-stimulating GPCR agonist LPA on Akt activation and cell migration. In CHO cells that endogenously express LPA_1 receptor, IGF-I stimulated Akt phosphorylation (P-Akt) and chemotaxis in a PI3K inhibitor-sensitive manner. In PTX-treaed cells, LPA inhibited IGF-I-induced P-Akt and chemotaxis. Y-27632, a ROCK inhibitor, prevented LPA-inhibition of IGF-I-induced P-Akt, indicating the ROCK mediates inhibition of P-Akt. However, Y-27632 failed to abrogate LPA-inhibition of IGF-I-induced chemotaxis. Thus, there was a discrepancy between LPA-induced inhibition of the cellular 3'-PIs amount, which is reflected by the extent of P-Akt, and inhibition of cell migration. These results suggest that LPA-induced inhibition of cell migration is not dependent on ROCK-mediated stimulation of PTEN and, thereby, reductions of cellular 3'-PIs contents

P46 (3P1-046)

Effects of various local anesthetics on axonal transport in cultured mouse dorsal root ganglion neurons

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We previously reported that intrathecal administration of various local anesthetics resulted in the dorsal root axonal degeneration near entry into the spinal cord in rats. The level of neurotoxicity of local anesthetics in the dorsal root depended on the kind of anesthetics and this was comparable to the level of side effects occurred in the clinical use. In the present study, we assessed the effects of various local anesthetics on axonal transport in cultured mouse dorsal root ganglion neurons. Lidocaine, bupivacaine, and ropivacaine (concentrations: 1-50 mM) all decreased axonal transport, but in different degree. The order of potency was lidocaine<bupivacaine<ropivacaine. At a concentration of 50 mM, lidocaine and bupivacaine but not ropivacaine caused the rupture of plasma membrane in some dorsal root ganglion neurons. Considering with the pharmacological potency, lidocaine:bupivacaine:ropivacaine = 1:4:4, the neurotoxocity of ropivacaine is likely much less than that of lidocaine. These in vitro experiments may be useful for determining the neurotoxicity of local anesthetics.

P47 (3P1-047)

Hippocalcin-mediated regulation of Mixed Lineage Kinase (MLK) 3 activity

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Hippocalcin is a member of the neuronal calcium sensors (NCS) family predominantly expressed in the hippocampal pyramidal cells. We have found that hippocalcin binds to C-terminal region of Mixed Lineage Kinase (MLK) 3. Here we analyzed activity of MLK3 in hippocalcin deficient mice. Immunoblot analysis using substrate specific anti-phospho-antibodies revealed that MKK3, one of the substrate of MLK3, in hippocalcin deficient mice was higher phosphorylated than that in wild type. Kinase activity of immunoprecipitated MLK3 was examined using bacterially expressed MKK4 as a substrate. The resting MLK3 activity in hippocalcin deficient mice was higher than that in wild type. By adding recombinant hippocalcin (100ng) to the assay condition, the activity of MLK3, which was immunoprecipitated from wild type mice, was inhibited. These results indicate that the binding of hippocalcin to C-terminal domain of MLK3 directly inhibits MLK3 kinase activity.

P48 (3P1-048)

Sphingosine-1-phosphate accelerates ischemia-induced angiogenesis in the mouse limb

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S1P has a critical role in vascular maturation during mammalian development. However, little is known about the role of S1P in ischemia-induced angiogenesis in adults. We investigated the effect of both exo- and endogenous S1P on angiogenesis in ischemic skeletal muscle in adult mice. Unilateral hindlimb ischemic model is a well-established *in vivo* angiogenesis assay system. We monitored post-ischemic angiogenesis by blood flow recovery with laser doppler imager and capillary density with anti-CD31 immunohistochemistry after surgery. First, we injected S1P into ischemic muscle everyday after surgery in C57BL6/J mice. Limb blood flow was 2.5 fold elevated in S1P(10^{-8}M)-injected mice at day 7. Capillary density was 1.5-fold increased in S1P group at day 10. These effects were comparable to bFGF administration. Trying to create long acting S1P, we prepared the slow-release microsphere containing S1P with polylactide-co-glycotide and intramuscularly administrated it just once immediately after surgery. Mice received microsphere containing S1P increased blood flow in ischemic limb. We also examined the effect of endogenous S1P overproduction by generating sphingosine kinase 1 transgenic (SphK1-Tg) mice, and blood flow was slightly increased compared with littermate wild type mice. $S1P_1$ receptor selective agonist SEW2871 also accelerated blood flow recovery. We showed S1P accelerates ischemia-induced angiogenesis, most likely via $S1P_1$ receptor in adult mice. S1P and SEW2871 is a potential therapeutic for ischemic diseases.

P49 (3P1-049)

Nitric oxide donors enhance alkylating cytotoxicity in rat C6 glioma cells

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1,3-Bis(2-chloroethyl)-1-nitrosourea (BCNU) is commonly used for adjuvant chemotherapy to treat malignant glioma including glioblastoma multiforme (GBM). BCNU kills tumor cells via multiple actions including carbamoylation and alkylation. Herein we test the effects of NO donors on alkylating cytotoxicity to rat C6 glioma cells. The alkylating agents tested included methyl methanesulfonate (MMS), N-methyl-N-nitrosourea (MNU), and N-ethyl-N-nitrosourea (ENU). The synergistic effects of three NO donors, namely S-nitrosoglutathione (GSNO), diethylamine NONOate (DEA/NO), and sodium nitroprusside (SNP) on alkylating agents were determined by colony-formation assay. We found that inclusion of NO donors substantially reduced the extents of colony formation following exposure of glioma cells to all three alkylating agents. Among the three NO donors, GSNO appeared to be the most potent one. GSNO also exerted similar synergistic actions reducing the extents of colony formation when co-administrated with 1,2-bis(methylsulfonyl)-1-(2-chloroethyl)-hydrazine, another alkylating agent that mimics the chloroethylating action of BCNU. O6-Methylguanine methyl-DNA transferase (MGMT) is a DNA repair enzyme that removes the cytotoxic O6-alkylguanine adducts induced by alkylating agents. Western analysis indicated that expression of MGMT was reduced in the presence of GSNO, suggesting the possibility that GSNO enhanced alkylating cytotoxicity via, at least in part, decreasing cellular MGMT contents.

P50 (3P1-050)

Granule localization of neurotrophin-3 in mouse alveolar macrophages

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Neurotrophins are growth factors that exert multiple actions on neuronal and nonneuronal cells. We have previously shown that neurotrophins, NT-3 and NT-4/5, are expressed in mouse alveolar macrophages. In the present study, we further attempted to clarify the intracellular localization of NT-3 and its co-localization with interleukin IL-1, fibroblast growth factor FGF, and histamine in alveolar macrophages. Immunocytochemical staining with antibodies against NT-3, lysosomal membrane protein LAMP-2, IL-1, FGF, and histamine was performed on alveolar and peritoneal macrophages obtained from the adult mouse. Approximately 80% of alveolar macrophages were immunoreactive for NT-3. In contrast, peritoneal macrophages were rarely immunoreactive for NT-3 (<5%). In these NT-3-immunoreactive macrophages, NT-3 was localized in intracellular granules. The granule localization of NT-3 was confirmed, since the NT-3-containing granules were immunoreactive for LAMP-2, which labels intracellular granule membrane. Almost all alveolar and peritoneal macrophages were immunoreactive for IL-1, FGF, and histamine. IL-1 and FGF were diffusely distributed in the cytoplasm. Histamine was localized in granules. In some granules, histamine was co-localized with NT-3. Since a large number of alveolar macrophages contain NT-3 in their granules and NT-3 is co-localized with histamine in some granules, they may be co-released in an exocytotic manner from alveolar macrophages and may play synergic roles in pulmonary pathophysiology.

P51 (3P1-051)

Microglia expressing NG2 chondroitin sulfate proteoglycan in normal and pathologic brains as multipotent neural progenitors

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Rat primary microglia (MG) acquired a multipotent property to give rise to neuroectodermal cells through two-step culture in 10 and 70% serum-supplemented media for 5 d (Yokoyama et al., Glia 2004; 45, 96-104). Such multipotent MG called promicroglioblasts (ProMGB) formed cell aggregates, which generated cells with neuroectodermal phenotypes shortly after transfer into serum-free medium. As revealed by immunohistochemistry, there were a few MG expressing NG2-chondroitin sulfate proteoglycan (NG2) in the neonatal rat brain. Primary culture from the neonatal brain contained NG2+ MG, which appeared being the source of NG2+-ProMGB aggregates. The aggregates were MG-markers+/GFAP+/NCAM+/S-100b- and possessed an alkaline phosphatase activity. Marked accumulation of NG2+ MG was observed in the close vicinity of stab wounds made in mature rat brain. The NG2+ MG in the wounds separated with trypsin-EDTA formed NG2+ aggregates in 70% serum-supplemented medium and then turned into cells with neuroectodermal phenotypes in serum-free medium. Although it is quite difficult to separate viable neurons from mature brains, cells from the stab wounds generated process bearing b-tubulin III+ cells easily. These data suggest that NG2+ MG in normal developing or pathologic brains are involved in genesis or regeneration of the brain.

P52 (3P1-052)

Microglial cells generate osteoclast-like multinucleated giant cells and cells with neuroectodermal phenotypes, depending on culture conditions.

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Although microglial cells are currently considered mesodermal cells, it has not been completely determined whether they are hematopoietic or mesenchymal origins. To obtain some insights in this issue, we induced dedifferentiation of microglial cells in culture by incubating in 70% serum-supplemented medium for 2 d. Microglial cells were separated from primary mixed glial culture that was started from neonatal rat forebrains or from ischemic brain lesions of rats whose right middle cerebral artery was transiently occluded. Cells cultured in 70% serum-medium gradually exhibited amoeboid shape and often formed cell aggregates, while increasing expression of Id genes and getting highly proliferative. Such cell aggregates differentiated into cells with neuroectodermal phenotypes after they were transferred into serum-free medium on poly-L-lysine-coated substrate. By contrast, cells that had been cultured in 70% serum-medium gradually fused resulting in formation of multinuclear giant cells, after they were transferred into 10% fetal calf serum-supplemented medium containing M-CSF and RANKL. As revealed by RT-PCR, such giant cells elevated expressions of mRNAs encoding DC-stamp, TRAP and Cathepsin K that are specific markers of osteoclasts. Taken that osteoclasts are derived from hematopoietic stem cells (HSCs) and HSCs are known to generate neuroectodermal cells, microglial cells may be of hematopoietic origin.

P53 (3P1-053)

Multipotent amoeboid microglia appear in brain lesions may come from blood: immunohistochemical comparison of microglial reaction in lesions with and without breakdown of blood brain barrier

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We found that amoeboid-shaped microglia expressing NG2 proteoglycan accumulated in stab wounds in the brain. Some of the NG2-positive microglia expressing nestin and GFAP turned into cells with neuroectodermal phenotypes in vitro. To elucidate whether such amoeboid NG2-positive microglia are blood-borne or the activated form of resident microglia, we compared the nature of cells expressing microglia markers using three kinds of brain pathology models using Wistar rats; stab-wound, middle cerebral artery occlusion (MCAO), and facial nerve axotomy models. The former two models accompanies breakdown of blood brain barrier (BBB), while the axotomy model does not. A huge number of NG2-positive amoeboid shaped cells expressing Iba1, a marker of microglia/macrophages, accumulated in the stab wounds and the core lesions of MCAO. The majority of the amoeboid cells were proliferating as revealed by Ki67-immunostaining. In contrast, microglial cells in the axotomied facial nerve nucleus enlarged somata but still kept ramified shapes, and none of them were Ki67-negative. Most of resident microglial cells died within 2 days after the stab-lesioning or MCAO, while none of microglia died in the facial nerve nucleus. These observations suggest that multipotent NG2-positive microglia in the brain lesions are blood-borne and distinct from resident ramified microglial cells.

P54 (3P1-054)

Reaction of electron-transferring flavoprotein with D-lactate dehydrogenase and enoyl-CoA reductase

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The anaerobic bacterium *Megasphaera elsdenii* uses lactate as the carbon source and produces lower fatty acids in the rumen of cattle and sheep. Electron-transferring flavoprotein (ETF) is a key enzyme in the intracellular redox system of *M. elsdenii*. ETF receives electrons from flavoprotein D-lactate dehydrogenase (D-LDH), which oxidizes D-lactate to pyruvate. The received electrons are then transferred to flavoprotein enoyl-CoA reductase (ECR), which reduces enoyl-CoA to acyl-CoA. The acyl-CoAs are eventually changed to fatty acids by CoA elimination. ETF also receives electrons from NADH, which is reduced by many other redox reactions. ETF contains two FAD molecules as the cofactor. The functions of the two FAD molecules are presently unlcear. In this study, we found the followings by spectrophotometric experiments using purified flavoproteins. Here the two FAD molecules in ETF are designated FAD-1 and FAD-2. (1) NADH reduces both FAD-1 and FAD-2. (2) D-LDH interacts with only FAD-1. (3) ECR interacts with both FAD-1 and FAD-2. (4) Electron transfer between FAD-1 and FAD-2 can occur without NADH/NAD^+, D-LDH, and ECR.

P55 (3P1-055)

Isolation of a receptor cDNA on macrophages for allogeneic MHC (H-2K^d)

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[Background] Previously, we found that allograft-induced macrophages (AIM; H-2D^bK^b) were the major effector cells responsible for allograft (e.g., BALB/c skin and Meth A tumor; H-2D^dK^d) rejection. In the last meeting, we reported isolation of a cDNA, which encoded a novel receptor on AIM for allogeneic MHC (H-2D^d), by using anti-AIM monoclonal antibody (mAb; R15) and H-2D^d tetramer. We named this receptor "macrophage MHC receptor (MMR)". In the present study, we obtained a cDNA encoding a novel receptor on AIM for allogeneic MHC (H-2K^d).

[Method] cDNA fragments were isolated by the T7 phage expression cloning method using R12 mAb and H-2K^d tetramer. Full length of the cDNA was obtained by the RACE method. mRNA expression was estimated by RT-PCR. cDNAs fused to GFP cDNA were transfected to HEK293T cells; and the binding of H-2 molecules to the transfectants was explored under a confocal microscope. The dissociation constant (K_d) of AIM toward H-2 molecules was assessed by flow cytometry.

[Results] We isolated the full length (ξχ5 2.4kb) of cDNA, which encoded a receptor on AIM for allogeneic MHC (H-2K^d), and named the receptor 'MMR2'. The MMR2 mRNA was expressed exclusively in AIM but not in other cells infiltrating into allograft. HEK293 cells transfected with MMR2 cDNA reacted with H-2K^d, but not with other H-2, molecules. The H-2K^d binding was completely suppressed by R12 or anti-H-2K^d mAb. The K_d value of AIM toward H-2K^d was 2.8×10^{-9} M.

P56 (3P1-056)

Expression of the tight junction protein claudins in salivary gland cells

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Tight junction (TJ) is an important adhesion system in epithelial cells, and also plays a role for regulation of paracellular flux across epithelial sheets. Claudins, a component of TJ, comprise a large family consisting of more than 20 members in mammals, and contribute to regulation of barrier function. We have compared expression of claudins in culture and intact duct cells of the rat submandibular gland with that in MDCK cells, a typical epithelial cell line. SMIE cells derived from rat submandibular duct were kindly provided by Dr. B. J. Baum (NIDCR). Duct cells were isolated from rat submandibular gland. TJ proteins were detected by western blotting. Immunofluorecence strain of TJ proteins was observed by a confocal laser scanning microscope. SMIE cells formed TJ as well as MDCK cells, which was confirmed by immunofluorecence strain of the TJ proteins occludin and ZO-1. Claudin-3 protein was detected in both cells. Claudin-1 and claudin-4 proteins were detected in MDCK cells, but not in SMIE cells, which was confirmed by immunofluorecence strain of claudins. However, in rat submandibular duct cells, claudin-4 was detected by western blotting and immunofluorecence strain. In SMIE cells, the transepithelial electrical resistance was lower and the flux of FITC-dextran was higher than in MDCK cells, indicating that SMIE cells are more permeable than MDCK cells. These results suggest that claudin-4 expression contributes to barrier function of intact salivary gland duct cells.

P57 (3P1-057)

Highly reduced status of lumber cerebrospinal fluid monitored by the redox state of the sulfhydryl in albumin

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In order to examine the redox state of cerebrospinal fluid (CSF) albumin together with serum albumin from orthopedic patients, we have analyzed the percentage of reduced and oxidized albumin fractions in CSF and serum by HPLC. Forty-two patients had no detectable evidences of systemic diseases such as hepatic and renal dysfunctions, and they were divided into two groups by their age (young group, average age = 31.3 years (n = 10); elder group, 64.8 years (n = 32)). Informed consent was obtained in all cases. Albumin is the mixture of reduced and oxidized albumins. Oxidized albumin is composed of two type of albumin, i.e., mixed disulfide with cysteine (tentatively called HNA-1) and oxidation product higher than mixed disulfide with reactive oxygen species (called HNA-2). In the elder patients, mean values for the fraction of HMA, HNA-1 and HNA-2 were 93.1, 5.8 and 1.1% for CSF, and 69.5, 28.6 and 1.9% for serum, respectively. In the young patients, those values were 93.0, 6.7 and 0.3% for CSF, and 76.4, 22.0 and 1.6% for serum, respectively. CSF is believed to maintain the homeostasis of brain functions, especially defense against oxidative stress. From our HPLC results, in both groups, significant difference between the values for CSF and serum indicates the function of blood-brain barrier is maintained. Significant high value for reduced albumin level in CSF in both groups indicates the redox state of CSF is kept highly reduced status.

P58 (3P1-058)

Proteome analysis of livers from LEC rats using proteins separated by two-dimensional electrophoresis

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Long-Evans Cinnamon (LEC) rats have been used as an animal model for human Wilson's disease. LEC rats developed hepatic abnormalities due to accumulation of copper in the liver. The aim of this study was to search for liver proteins related to Wilson's disease. The livers from LEC and WKAH/Hkm rats (12 and 16 weeks of age) were homogenized and performed using two-dimensional electrophoresis (2-DE). Their 2-DE spots were analyzed by an image analysis software for comparison of protein quantity. Although no significant difference was observed between the two strain rats of 12 weeks, 17 spots were different between the two of 16 weeks. One spot of 36.5 kDa was positive in the WKAH rat, but was negative in the LEC rat. Four spots were denser by 1.5 times in the LEC than in the WKAH, Two of them were denser by around 7 times. On the other hand, 8 of 12 spots were much thinner by 2 times in the LEC than in WKAH. These results suggest that two-dimensional electrophoresis may be useful for the study of Wilson's disease.

P59 (3P1-059)

In vivo stable transduction of protein into the rat brainstem using the Hemagglutinating virus of Japan-envelope vector

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We have demonstrated for the first time that the Hemagglutinating virus of Japan-envelope (HVJ-E) vector allows the efficient transduction of protein into the rat brainstem using the technique of microinjection in vivo. Successful transduction of an exogenous protein, β-galactosidase (β-gal), was performed via the direct injection of only 100 nl HVJ-E vector solution into the specific restricted brain area (nucleus tractus solitarius, NTS). To examine whether the β-gal activity was maintained in the rat brainstem, samples were collected 3, 6 and 24 hours after transduction and a colorimetric assay was utilized to detect and quantify β-gal activity. A constant and high transduction level of β-gal activity was maintained in the rat brainstem, that was not significantly reduced within 24 hours following transduction compaired with β-gal without HVJ-E vector. This kind of targeted delivery system using the HVJ-E vector should have wide applications of various therapeutic proteins to the central nervous system in vivo.

POSTERS
Transport across cell membrane

P60 (2P1-001)

Expressions of Na/K-ATPase αsubunit isoforms in rat salivary glands

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There are three isoforms in Na/K-ATPase α subunit, α1, α2 and α3. It is known that α1 is expressed in wide range of cells/tissues and that α2 and α3 localize abundantly in neuronal tissues. We examined the expression of Na/K-ATPase α isoforms in salivary glands by RT-PCR. Alpha1 was expressed in three major salivary glands. Alpha2 was expressed in submandibular gland (SMG) and sublingual gland (SLG) and faintly parotid gland (PG). Alpha3 was expressed in SLG alone. The DNA sequence of α2 and α3 PCR products from salivary glands were identical with the corresponding portion of brain. We examined mRNA levels of these α isoforms by comparing the quantity of the PCR products. Alpha1 levels in 3 salivary glands were same as that in brain. Alpha2 levels in SMG, SLG and PG were determined to be 1/32, 1/16 and 1/256 of that in brain, respectively. Alpha3 level in SLG was at 1/8. Since α3 is abundant in nerve tissues, we examined whether α3 expression is induced when pheochromocytoma PC12 cells are differentiated into neuron-like cells by nerve growth factor (NGF). PC12 cells expressed α1 and α3, however α3 was not changed by the NGF-treatment of the cells. The α isoforms of HSY cells, a cell line of human parotid gland adenocarcinoma, were also examined. HSY cells expressed α1 alone. The expression pattern of Na/K-ATPase α isoforms in cells/tissues seemed to be rather stable and it was not easily altered with differentiation factors or carcinogenesis.

P61 (2P1-002)

The role of apical Na^+-H^+ exchanger in HCO_3^- secretion in mouse pancreatic duct cells

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In the present study, to investigate the role of apical Na^+-H^+ exchangers (NHE) in HCO_3^- secretion from pancreatic duct cells, we examined the activity of apical Na^+-H^+ exchange in interlobular pancreatic duct segments isolated from normal mice and δF508 mice where function of CFTR (cystic fibrosis transmembrane conductance regulator) is disrupted.Interlobular duct segments were isolated by microdissection.The ducts were superfused with HCO_3^--free Hepes-buffered solutions and the lumen was microperfused separately.Intracellular pH (pH_i) was measured by microfluorometry in ducts loaded with pH-sensitive fluoroprobe BCECF.The duct cells were acid-loaded with 20 mM NH_4^+,which was followed by a Na^+-free solution in both the bath and lumen.The rate of pH_i recovery after re-addition of Na^+ to the luminal solution was calculated as a measure of the activity of apical Na^+-H^+ exchange.The rate of pHi recovery (dpH/dt) was 0.12 ± 0.01 pH unit/min (mean ± SD, n = 8) in wild type ducts, which was completely inhibited by 100 μM HOE642,an inhibitor of NHE. Forskolin reduced the apical NHE activity to 0.05 ± 0.01 pH unit/min (n = 9, $p < 0.01$).The apical NHE activity in cystic fibrosis ducts was 0.20 ± 0.01 pH unit/min (n = 6),which was significantly ($p < 0.01$) higher than that in wild type ducts and was accelerated to 0.66 ± 0.11 pH unit/min (n = 6, $p < 0.01$) by application of forskolin. In mice pancreatic duct cells,the activity of apical NHE was suppressed by functional CFTR and it was stimulated by cAMP in the absence of functional CFTR.

P62 (2P1-003)

Characterization of Cl^--dependent bicarbonate secretion in mouse ileum

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Bicarbonate secretion in mouse ileum has at least two components, one being Cl^--dependent, and the other being Cl^--independent and activated by cAMP. We have previously demonstrated and characterized Cl^-/HCO_3^- exchange activity in the mouse ileal villous cell by microfluorometric intracellular pH (pH_i) measurements. The purpose of this study is to determine if the Cl^-/HCO_3^- exchanger that we characterized is responsible for the ileal Cl^--dependent HCO_3^- secretion. To this end isolated mouse ileum was mounted in Ussing chamber and the alkaline secretion rates ($J_{OH(sm)}$) were determined by continuously titrating the luminal buffer-free solution to pH 7.2 with 1mM H_2SO_4 using a pH stat device. The serosal side was always bathed with HCO_3^-/CO_2-buffered solution. The Cl^--dependent $J_{OH(sm)}$ was inhibited by 30μM niflumic acid (by 25%) and by 100μM acetazolamide by (40%) both being added to the mucosal side, whereas cAMP-induced $J_{OH(sm)}$ was not inhibited by these compounds. Glibenclamide (100μM) added to the mucosal side had no effect on either component. Br^-, I^-, NO_3^-, and SO_4^{2-} added to the mucosal side induced $J_{OH(sm)}$ of 80%, 50%, 50%, and 65%, respectively, of the $J_{OH(sm)}$ induced by Cl^-. These inhibitor sensitivity and ion selectivity profiles of Cl^--dependent bicarbonate secretion generally agreed with those of the Cl^-/HCO_3^- exchanger we have previously shown in the enterocytes by pH_i measurement, suggesting that the Cl^-/HCO_3^- exchanger is responsible for Cl^--dependent bicarbonate secretion. The molecular identity of the exchanger remains to be determined.

P63 (2P1-004)

Activation of neuron-specific K^+-Cl^- cotransporter KCC2 by brtain-type creatine kinase

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GABA, a major inhibitory neurotransmitter in the adult CNS, is excitatory at early developmental stages due to the elevated intracellular Cl^- concentration ($[Cl^-]_i$). This functional switch is primarily attributable to the K^+-Cl^- cotransporter KCC2, the expression of which is developmentally regulated in neurons. Previously we reported that KCC2 interacts with brain-type creatine kinase (CKB). To elucidate the functional significance of this interaction, HEK293 cells were transfected with KCC2 and glycine receptor α2 subunit, and gramicidin-perforated patch-clamp recordings were performed to measure the glycine reversal potential (E_{gly}), giving an estimate of $[Cl^-]_i$. KCC2-expressing cells displayed the expected changes in E_{gly} following alterations in the extracellular K^+ concentration ($[K^+]_o$) or administration of an inhibitor of KCCs, suggesting that the KCC2 function was being properly assessed. When added into KCC2-expressing cells, dominant-negative CKB induced a depolarizing shift in E_{gly} and reduced the hyperpolarizing shift in E_{gly} seen in response to a lowering of $[K^+]_o$) compared to wild-type CKB. Moreover, 2,4-dinitrofluorobenzene (DNFB), an inhibitor of CKs, shifted E_{gly} in the depolarizing direction. In primary cortical neurons expressing CKB, the GABA reversal potential was also shifted in the depolarizing direction by DNFB. Our findings suggest that in the cellular microenvironment, CKB activates the KCC2 function.

P64 (1P1-001)

Rapid electrical stimulation of H9c2 cells induces apoptosis via both the death receptor and mitochondrial pathways.

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Background: Recent studies have revealed that tachyarrhythmias cause electrical and structural remodeling of cardiomyocytes, and that apoptosis contributes to myocardial remodeling in certain arrhythmias. We suspected that tachyarrhythmias lead to arrhythmogenic substrates through the induction of apoptosis, and then may tend to initiate and perpetuate themselves. In this study, we tested the hypothesis that rapid electrical stimulation of cardiomyocytes in culture causes apoptosis. **Methods and Results:** The cultured H9c2 cardiac cells were subjected to rapid electrical stimulation for 72 hours at a pacing frequency of 5 Hz. To determine the degree of apoptosis, the percentage of hypodiploid cells, mitochondrial transmembrane potential ($\Delta\Psi_m$), and activities of caspases-3, -8, and -9 were measured quantitatively. Compared with the cells in the absence of stimulation, the cells subjected to rapid stimulation showed apoptosis in terms of the appearance of hypodiploid cells (4.7±0.5% vs 31.0±8.5%), loss of $\Delta\Psi_m$ (5.3±0.5% vs 19.5±2.7%), and activation of all caspases tested (caspase-3, 0.050±0.015 vs 0.117±0.030; caspase-8, 0.038±0.004 vs 0.056±0.002; caspase-9, 0.029±0.003 vs 0.046±0.010) with statistically significant differences. **Conclusions:** Rapid electrical stimulation of H9c2 cells induced apoptosis via both the death receptor and mitochondrial pathways. The present in vitro model may be useful to elucidate mechanisms of tachycardia-induced myocardial remodeling via apoptosis, and to lead to therapies for preventing the arrhythmogenic substrate development.

POSTERS
Heart & circulation

P65 (1P1-002)

Molecular basis of pacemaker activity in the rabbit sinoatrial node

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The ionic mechanisms underlying pacemaker activity have been extensively studied in the sinoatrial (SA) node, but little is known about the molecular basis of ionic currents. The aim of this study was to evaluate mRNA expression for ion channels, connexins and Ca^{2+}-handling proteins using quantitative PCR and to visualize the distribution of these transcripts using *in situ* hybridization in and around the rabbit SA node. Quantitative PCR showed that there were significant differences in the abundance of 60% of the 30 mRNAs studied in different tissues (SA node center, SA node periphery and atrial muscle). Grouping analysis of the PCR data identified two significantly different clusters: mRNAs tended to increase (Cx45, Nav1.1, Cav1.3, HCN1, HCN4, Kv4.2, ERG, KvLQT1, Kir2.2 and Kir3.1) and those tended to decrease (Cx43, Cx40, RYR2, SERCA2a, Nav1.5, Cav1.2, Kv1.4, Kv4.3, KChIP2, Kv1.5, minK, Kir2.1 and Kir6.2) from the atrial muscle to the center of the SA node. The mRNA expression profiles of the center and the periphery of the SA node were similar but there were significant differences for some transcripts (e.g. Cav1.3, HCN1 and HCN4). *In situ* hybridization confirmed these regional differences in the mRNA expression pattern. This study shows a complex variation in the expression of ion channel mRNAs from the atrial muscle to the SA node, which may be important in the functional organization of the SA node as a physiological and dependable pacemaker of the heart.

P66 (1P1-003)

Properties of Ca^{2+} handling in sarcoplasmic reticulum of saponin-treated sarcolipin transgenic mouse myocardium

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We investigated whether the alteration of Ca^{2+} uptake rate in sarcoplasmic reticulum (SR) would influence the Ca^{2+} content and the leakage of Ca^{2+} in SR. In this study, we used saponin-treated thin trabeculae obtained from the hearts which overexpresses sarcolipin (SLN). We reported that SLN reduced the activity of SR Ca^{2+}-ATPase. The advantage of saponin-treated preparation is that integrated structure around SR and Ca^{2+} handling functions are well preserved. Thus, the functions of SR to release and uptake Ca^{2+} can be measured under more physiological conditions compared to isolated vesicular SR. Ca^{2+} content was measured by releasing all Ca^{2+} from SR by caffeine (50 mM) after loading Ca^{2+} (pCa 6.2) into SR in the presence of ATP (4 mM) for different periods. Leaked Ca^{2+} was estimated by measuring the remaining Ca^{2+} in SR after washing for various durations, following Ca^{2+} loading. Ca^{2+} uptake rate was slower in SLN transgenic (SLN-TG) than that in non-transgenic (SLN-NTG) myocardium. The maximal Ca^{2+} content and leakage of Ca^{2+} in SLN-TG and SLN-NTG were almost identical. The results suggest that the modulation of Ca^{2+} uptake rate could not affect maximal Ca^{2+} content and Ca^{2+} leakage measured at steady state. The modulation of Ca^{2+} uptake rate influences Ca^{2+} handling in each heartbeat but does not alter the maximal Ca^{2+} content and leakage at steady state.

P67 (1P1-004)

Phosphorylation status of contractile proteins and functional characteristics in myocardium of dilated cardiomyopathy of Syrian hamster (TO-2 strain)

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To understand the pathophysiology of hereditary cardiomyopathy, we measured the phosphorylation status of contractile proteins, ventricular light chain 2 (VLC2), troponin I (TnI), troponin T (TnT) and myosin-binding protein C (MyBP-C), and the Ca^{2+}-dependence of tension and ATPase activity in skinned trabeculae obtained from normal control (F1B) and cardiomyopathic hamsters (TO-2). To change the phosphorylation status of contractile proteins, isolated trabeculae were exposed to Tyrode's solution containing 30 mM BDM for 30 minutes before skinning. In the BDM-untreated preparations, all the contractile protein phosphorylation levels were higher in F1B than in TO-2, while the Ca^{2+} sensitivities of tension and ATPase activity were substantially lower in F1B than in TO-2. The BDM treatment did not alter the contractile protein phosphorylation levels as well as the Ca^{2+} sensitivities of tension and ATPase activity in TO-2 preparations. However, the BDM treatment decreased the contractile protein phosphorylation levels as well as increased the Ca^{2+} sensitivities of tension and ATPase activity in F1B preparations to the levels similar to those in TO-2 preparations. These results suggest that the increase in Ca^{2+} sensitivities of tension and ATPase activity in TO-2 hamster hearts results from the decreased basal level of TnI and TnT phosphorylation, since the dephosphorylation of VLC2 and MyBP-C has been reported to decrease Ca^{2+} sensitivities.

P68 (1P1-005)

Abnormal cardiac function is associated with a mutation of the SERCA2a gene

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Background: Recently, mutations of the phospholamban gene, a peculiar inhibitor of sarcoplasmic reticulum calcium ATPase (SERCA2a), have been found in patients with familial cardiomyopathy. This result suggests that mutations of SERCA2a gene also cause cardiomyopathy. In our previous study, we found one patient with hypertrophic cardiomyopathy who harbored a missense mutation in the SERCA2a gene. The mutation changed the amino acid sequence from valine (540) to alanine in the cytoplasmic region close to the ATP binding site. **Objective:** We examined whether the V540A mutation of SERCA2a could cause the abnormal cardiac function or not. **Methods:** Mouse SERCA2a cDNA was cloned and the V540A mutation was inserted into it. Transgenic mice overexpressing the V540A mutation of SERCA2a in the heart was generated. Cardiac phenotypes were observed in SERCA2a transgenic mice (TG) using in vivo echocardiography and hemodynamic analyses. **Results:** By Western blot analysis, we found that the expression of SERCA2a protein was increased by 1.7-fold in the ventricles of TG when compared with those of non-transgenic mice. The weight of the left ventricle was slightly, but significantly increased in TG. The maximal first derivate of left ventricular pressure in TG was significantly lower by 16%, when compared with non-transgenic mice. **Conclusion:** We concluded that the mutation of the SERCA2a gene resulted in the abnormal cardiac function in mice. Our results imply that the V540A mutation of the SERCA2a gene is a causal mutation in human cardiomyopathy.

P69 (1P1-006)

Developmental changes in nitric oxide production during hypoxia and reoxygenation in the rat striatum

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In the brain nitric oxide (NO) is implicated in blood flow regulation, neurotransmitter release, learning and memory, neuronal plasticity, and neuronal degeneration and survival. To understand the role of NO in physiological as well as pathological conditions, it is essential to observe the dynamics of NO production itself. Using an NO-selective electrode system that allows the real-time in vivo measurement of NO, we examined changes in NO production during hypoxia and reoxygenation in the striatum of rats from late prenatal to adult stages. Under urethane anesthesia, an NO-selective electrode and laser Doppler flow probe was inserted into the striatum for measuring changes in NO concentration and tissue blood flow, respectively. In adult rats, spontaneous rhythmic production of NO ranging from about 0.2 - 1.5 Hz was observed, which was not related directly to cardiac or respiratory cycles. The rhythmicity in NO production was reduced after systemic administration of the NMDA channel blocker ketamine. In adult rats, brain hypoxia abolished the rhythmicity, but caused a large amount of NO production in the striatum. This overproduction of NO during hypoxia was also observed in the fetal and neonatal rat brain, although the basal rhythmic NO production was not detected. The immature rat brain may have less capacity to produce NO than the matured rat brain. The appearance of the rhythmic NO production in the brain may indicate the maturity of the brain NO-producing system. Further study is needed to clarify the origin of this rhythmic NO production in the rat brain.

P70 (1P1-007)

Cytochalasin D as the uncoupler of E-C coupling for the optical monitoring of action potentials in the isolated rat atrium

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Multiple-site optical recording methods, using a fast voltage-sensitive merocyanine-rhodanine dye (NK2761) and a multi-element (16 X 16) photodiode array, were employed to simultaneously monitor action potentials from many sites in the isolated rat atrium preparation. Cytochalasin D was used to reduce contraction-related optical artefacts by suppressing muscle contraction without affecting electrical action potential. The suppression effect of this chemical (40μM) on the contraction-related optical artefacts is time-dependent for the first 30 min. and become steady state within 40 - 60 min. after the application to the bath. The suppression of the artefact of this chemical is generally stronger than that of 2,3-butanedione monoxime (BDM: 20 mM). Using this chemical, we successively evoked tachycardia-like excitation (TE) in the isolated rat right atrium preparation and mapped optically the excitation spread pattern during TE.

P71 (1P1-008)

RhoB-geranylgeranylation is involved in Na^+/Ca^{2+} exchanger mRNA increase by lisophosphatidylcholine in H9c2 cells.

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Cardiac Na^+/Ca^{2+} exchanger1 (NCX1) expression levels change under various pathophysiological conditions, e.g. heart failure. However, its mechanism is unknown. We previously showed that fluvastatin (Flv), an HMG-CoA reductase (HMGR) inhibitor, decreased NCX1 mRNA and protein expression via inhibiting a small G-protein, RhoB in H9c2 cardiomyoblasts. Flv-induced down-regulation of NCX1 mRNA was reversed by mevalonate, farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP), suggesting an involvement of isoprenylation of RhoB. Conversely, we also found that lisophosphatidylcholine (LPC) increased NCX1 mRNA and protein by activating RhoB. RhoB requires isoprenylation for its activation by either GGPP or FPP. Here, we investigated which isoprenoid is involved in NCX1 increasing effect of LPC. Treatment of H9c2 cells with Flv for 24 hours decreased NCX1 mRNA to about 60% of control. Addition of GGPP or FPP restored NCX1 mRNA, which had been decreased by Flv, to a control level within 24 hours. No significant difference was observed between GGPP and FPP. When LPC was applied with Flv, NCX1 mRNA remained decreased. However, when LPC and GGPP were applied simultaneously, NCX1 mRNA was increased to a level significantly higher than the control. Unlike GGPP, FPP did not induce this increase. These results suggest that an isoprenoid involved in the effect of LPC increasing NCX1 mRNA is GGPP but not FPP.

P72 (1P1-009)

Ca^{2+} entry through the T-type Ca^{2+} channels causes apoptosis via the mitochondrial pathway

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Background: It is postulated that T-type Ca^{2+} channels play important roles not only in physiological condition of various organs and tissues, but also during the progression of various diseases. In this study, we tested the hypothesis that Ca^{2+} entry through the T-type Ca^{2+} channels causes apoptosis using recombinant $Ca_V3.2$ (α1H) T-type Ca^{2+} channels expressed in HEK293 (HEK-α1H) cells. **Methods and Results**: Cultured HEK293 cells and HEK-α1H cells were incubated for 12 hours in serum-free medium containing different concentrations of Ca^{2+} (1.8-7.2 mM). To determine the degree of apoptosis, mitochondrial transmembrane potential ($\delta\Psi_m$) and activities of caspase-3, -8, -9 were measured quantitatively. Intracellular free Ca^{2+} measured by flow cytometry using Fluo-3 was elevated depending on Ca^{2+} concentration in the medium ($[Ca^{2+}]o$) in HEK-α1H cells but not in HEK293 cells. In HEK-α1H cells, apoptosis in terms of loss of $\delta\Psi_m$ and activation of caspase-3 and -9 was observed at $[Ca^{2+}]o$ of 5.4 mM or more, while caspase-8 was not activated. In contrast, apoptosis was not induced at any $[Ca^{2+}]o$ in HEK293 cells. **Conclusion**: We demonstrated that Ca^{2+} entry through the T-type Ca^{2+} channels causes apoptosis via the mitochondrial pathway. Thus, T-type Ca^{2+} channels may be therapeutic targets for certain pathophysiological conditions related to apoptosis.

P73 (1P1-010)

A human ventricular action potential model for the analysis of arrhythmias elicited by "intracellular Calcium handling dysfunction".

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Dysfunctions in the gating of ryanodine-receptors (RyR) and intracellular Ca^{2+} handling play critical roles to evoke lethal arrhythmias not only in inherited arrhythmgenic syndromes such as CPVT, but also in more common cases including heart failure. To develop a human ventricular action model which allows analyses of the effects of altered RyR properties on arrhythmogenesis, we incorporated a calcium release system with 4-state Markovian RyR gating (Stern, *J.Gen.Physiol.*1999) into a simplified human action potential model (TenTusscher, *Am.J.Physiol.*2004). Introduction of Ca concentration-dependence on RyR opening rate was a first step to model Ca-induced Ca-release. To obtain reasonable RyR gating in the control, it was necessary to add a new Ca compartment (cleft), where Ca concentration increased and decayed much faster than cytosolic Ca transient. Under these settings, augmented sensitivity of RyR on cleft Ca easily evoked spontaneous oscillatory Ca releases during the decay phase of Ca transient. Induction of DADs leading to triggered activity, however, could not be reproduced straightforwardly in spite of further inclusion of SR Ca-load dependence of RyR gating. Generation of triggered action potential after repolarization required additional manipulation of sarcolemmal current systems including a reduction of I_{K1}, a situation found in patients with terminal heart failure. Our model provides basis to elucidate mechanisms of Ca-mediated arrhythmias, and further to find out RyR-targeted anti-arrhythmic therapy.

P74 (1P1-011)

The relationship betweeen action potential duration and the rate of Ca^{2+} replenishment to the junctional sarcoplasmic reticulum in heart muscle of hypertensive rat

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To characterize Ca^{2+}handling by the junctional sarcoplasmic reticulum (JSR) in heart muscle of hypertensive rat(SHR), we examined the time course of short-term mechanical restitution(STMR) after varying magnitude of twitch contraction with the papillary muscle of adult(20 weeks-old)SHR and age-matched control(WKY) and action potential duration at various stimulus frequencies. The slope of the STMR in WKY was independent of the magnitude of the preceding twitch and similar to that of SHR unless the preceding twitch was not so large as the rested state twitch. The slope decreased after the rested state twitch in SHR. The functions G(t) and H(t), representing the time courses of the JSR Ca^{2+} replenishment and release, respectively, were derived graphically from a family of the mechanical restitution curves for the both strains. The action potential duration as measured at its half width was significantly greater in SHR than WKY($p < 0.005$). Both a time to peak tension and a half relaxation time were significantly longer in SHR than WKY($p < 0.0001$). The result suggests slower Ca^{2+} replenishment to the JSR in SHR than WKY irrespective of the longer action potential in the former than the latter.

P75 (1P1-012)

A Simulation Study of Ca^{2+} Regulation of Cardiac Mitochondria

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To clarify complex interactions between cardiac excitation-contraction (E-C) coupling and energy metabolism, a cardiac mitochondria model is developed, which consists of oxidative phosphorylation, TCA cycle, pyruvate metabolism, fatty acid β oxidation, malate shuttle and Ca^{2+} dynamics. Using this model, we studied regulation of mitochondrial function by Ca^{2+}, and investigated contribution of each functional element of oxidative phosphorylation to mitochondrial oxygen consumption (mVO2), mitochondrial membrane potential and NADH. Calculation of the membrane potential was improved by integrating charge flux across the membrane via proton pumps and transporters. The model simulation revealed that the activation of F1F0-ATPase, adenine nucleotide translocator enhanced mVO2 by about 60%, This result is supported by experimental data demonstrating a large increase in mVO2 of state 3 mitochondria by Ca^{2+} (Territo *et al. Am J Physiol.* **278**:C423-35, 2000). The expanded computer model, which combines the mitochondria model with an E-C coupling model of cardiomyocyte (Kyoto model), suggested that Ca^{2+}-dependent activation of both oxidative phosphorylation and Ca^{2+}-dependent dehydrogenases plays pivotal roles in regulating cardiac mitochondrial function by stabilizing metabolite concentrations during an increase in workload induced by changing beating frequency.

P76 (1P1-013)

Effects of redox potential on acetaldehyde-induced activation of the type 2 ryanodine receptor

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Acetaldehyde (AcA), an oxidized product of alcohol, may contribute to alcohol-induced cardiac muscle dysfunction. Recently, we have demonstrated that even at 1-3 μM, AcA activates the ryanodine receptor type 2 (RyR2) channel and promotes Ca^{2+} release from the cardiac muscle SR (Oba et al., JJP 55:S90, 2005). AcA produces ROS and oxidizes GSH to GSSG to lead to oxidative stress in tissues. We studied whether cytoplasmic redox potential (RP) affects AcA-induced activation of the RyR2 channel and mediates cardiac dysfunction. In experiments under RP control using glutathione buffers, fixation of cytoplasmic (*cis*) RP at the oxidative state activated the channel incorporated into bilayers, whereas definition of luminal (*trans*) potential did not. Exposure of the RyR2 to AcA without defining RP stimulated markedly the channel in a dose-dependent manner (1-100 μM). When RP in *cis/trans* sides of the channel was fixed at -220/-180 mV, AcA at concentrations less than 3 μM caused no longer the channel activation, but higher concentrations increased 2 fold the open probability. In condition under *cis/trans* potential at -250/-180mV, AcA failed to activate the RyR2 channel. These results indicate that the activation of RyR2 channels by AcA exposure is elicited even at reductive states and suggest that cytoplasmic RP may have a protective effect against pathophysiological changes by AcA in cardiac myocytes.

P77 (1P1-014)

Two different subtypes of α_1-adrenoceptor modulate L-type Ca^{2+} current via different intracellular signal transduction pathways in rat ventricular myocytes

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Purpose: We have recently reported that the effects of α_1-adrenoceptor stimulation (α_1ARS) on L-type Ca^{2+} current (I_{Ca}) can be classified in two opposite effects (negative and positive effects) and the positive effect is protein kinase C (PKC)-dependent. We postulate that these two effects simultaneously occur through different subtypes of α_1-adrenoceptor and different intracellular signal transduction pathways. In this study, we investigated the effects of α_{1A}ARS and α_{1B}ARS on I_{Ca}. **Methods:** Perforated patch-clamp was used for recording of I_{Ca} from isolated adult rat ventricular myocytes. Holding potential was set at -40 mV and depolarization pulse to 0 mV was applied every 10 sec. **Results:** Biphasic change in I_{Ca} (a transient decrease followed by a sustained increase) was induced by a non-selective α_1-adrenoceptor agonist, phenylephrine (Phe). However, a selective α_{1A} agonist, A61603 showed only positive effect on I_{Ca}, and the application of Phe with a selective α_{1A} antagonist, WB4101, caused only sustained negative effect. After pertussis toxin treatment, a transient decrease in I_{Ca} induced by Phe disappeared and only sustained increase was observed. **Conclusion** α_{1A}ARS showed positive effect on I_{Ca}, which was PKC-dependent. On the other hand, α_{1B}ARS showed negative effect mediated through $G_{i/o}$ protein. Thus, α_{1A}ARS and α_{1B}ARS produce opposite effects on I_{Ca} via different intracellular signal transduction pathways.

P78 (1P1-015)

Formation of functional multiheteromeric channels by KCNQ1, KCNE1 and KCNE2

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The KCNE proteins (KCNE1-5) are single transmembrane peptides that function as ancillary β-subunits of voltage-gated K^+ channels. Functional coupling of KCNE1 (E1) with KCNQ1 (Q1) to form cardiac I_{Ks} channel is well documented. However, Q1 is also found to have an affinity for all other members of KCNEs. The present study tested the possibility for the formation of functional channels consisting of Q1, E1 and KCNE2 (E2). We constructed a plasmid encoding a fusion protein in which the N terminus of Q1 was fused to the C terminus of either E1 or E2, and characterized the function of heteromeric channels by measuring whole-cell currents from COS7 cells transiently transfected with the plasmids. It was comfirmed that E1-Q1 fusion channels produced voltage- and time-dependent currents, similar to those obtained by coexpression of E1 together with Q1, while E2-Q1 fusion channels evoked constitutively active time-independent currents, again similar to those induced by coexpression of E2 and Q1. Coexpression of the E1-Q1 with E2 results in time-dependent currents which had a faster deactivation time course and a more depolarized half-activation voltage, compared to the E1-Q1 current. On the other hand, coexpression of E2-Q1 and E1 exclusively produced voltage- and time-dependent current without any constitutively active component, providing a functional evidence to show that all E2-Q1 coassembles with E1. These results suggest that E1 and E2 can be couple to a Q1 channel molecule forming complexes with unique current properties.

P79 (1P1-016)

Intracellular and extracellular magnesium concentration-dependent alteration of electrical excitability in cardiac myocytes

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[Background] Regulation of intracellular and extracellular free magnesium (Mg^{2+}) concentration is important to control the excitability in cardiac myocytes. [Objective] To assess the role of intracellular and extracellular Mg^{2+} imbalance in cardiac myocytes associated with membrane potentials and ionic currents derangement. [Methods and Results] Serum and intracellular Mg^{2+} concentrations were measured from normal (control) and the Mg^{2+} -deficient food feeded rats. The serum $[Mg^{2+}]e$ and intracellular Mg^{2+} concentrations $[Mg^{2+}]i$ were significantly decreased from 6.9 ± 0.3 mg/dl and 2.3 ± 0.2 mg/dl to 4.4 ± 0.2 mg/dl and 0.7 ± 0.1 mg/dl on Mg^{2+} -deficient diet on day 28, respectively. We obtained ECG signals by a radio transmitter from control and the Mg^{2+}-deficient rats. Low $[Mg^{2+}]e$ significantly prolonged RR duration (control, 181 ± 17 ms versus low $[Mg^{2+}]e$, 158 ± 18 ms, $p < 0.05$) in accordance with various types of cardiac arrhythmias such as supraventricular extrasystole, atrioventricular blocks and so on. Action potentials (AP) from rat ventricle myocytes were recorded by using intracellular microelectrode technique. AP durations (APD_{90}) in myocytes obtained from Mg^{2+} -deficient rats were significantly prolonged (control, 101.2 ± 3.2 ms versus low-Mg^{2+}, 314.0 ± 11.8 ms, $p < 0.01$). [Conclusion] Decreases in intracellular and extracellular Mg^{2+} concentrations cooperatively contribute toward arrhythmogenecity presumably affecting intracellular Ca^{2+} concentrations.

P80 (1P1-017)

Regulation of muscarinic K^+ channel by extracellular lysophosphotidylcholine via G2A membrane receptor

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In recent years, some of biological actions of lysophosphotidylcholine (LPC) have been found to be mediated through a class of G protein-coupled receptors, termed G2A. The present study was designed to examine the possible regulation of the muscarinic K^+ current ($I_{K,ACh}$) by LPC and its associated signaling pathways in guinea-pig atrial myocytes, using whole-cell patch-clamp method. Bath application of LPC (2 μM) reversibly and almost completely (93.3%; n = 9) inhibited $I_{K,ACh}$ preactivated by acetylcholine (ACh). On the other hand, LPC almost irreversibly inhibited $I_{K,ACh}$ preactivated by intracellular loading of non-hydrolyzable GTP analogue GTPγS (84.2%; n = 6), suggesting an involvement of G protein. The inhibitory action of LPC was partially, but significantly, attenuated by pretreating myocytes with an anti-G2A antibody and phospholipase C (PLC) blockers (compound 48/80 and neomycin). Furthermore, the inhibitory effect of LPC was still significantly reduced by exogenously adding phosphatidylinositol 4,5-bisphosphate (PIP_2, 50 μM) to the cell inside, and became nearly irreversible when atrial myocytes were continuously exposed to wortmannin (10 μM), which suppresses the resynthesis of PIP_2. As expected, LPC greatly reversed the shortening of action potential duration evoked by ACh. Based on these results, we conclude that LPC markedly inhibits $I_{K,ACh}$ through a mechanism involving an activation of G protein-coupled G2A receptor causing a depletion of membrane PIP_2 via PLC activation.

P81 (1P1-018)

Quantitative analysis of cardiac cell volume regulation by experiments and simulation

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In general, animal cells tend to swell if Na/K pump is blocked. However, the volume of guinea-pig ventricular myocytes remained almost constant during 3 hours under 40 μM ouabain. The Na/K pump block simulation using the Kyoto model showed that the cell swelling is retarded in parallel to a decrease in the background membrane conductances for Cl^- and Na^+, suggesting very low conductances for these ions in actual ventricular cells. In addition, it is suggested by the simulation that the active Ca^{2+} extrusion by sarcomlemmal Ca^{2+} pump (PMCA) has an important role in the delayed cell swelling. The transmembrane $[Ca^{2+}]$ gradient is partially maintained by PMCA, and this makes the Na/Ca exchanger extrudes Na^+ in reverse mode, compensating for Na/K pump. In the experiment where the Cl^- leak was increased beyond these compensatory mechanisms by applying 5 μM isopreterenol, cell swelling could be induced, but only after a delay of about 55 min after ouabain application. The simulation revealed a gradual membrane depolarization during the delay, and finally it reached a voltage range of continuous opening of a significant fraction of L-type Ca^{2+} channels (window current). A rapid accumulation of Ca^{2+} subsequently activates Ca^{2+}-dependent Na^+ conductance, which finally initiates the rapid cell swelling.

P82 (1P1-019)

Termination of Spiral-wave Reentry by Regional Cooling in 2-dimensional Rabbit Ventricular Myocardium

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Spiral-wave reentry is a principal mechanism of life-threatening ventricular tachyarrhythmias. We investigated the effects of regional cooling (RC) on the dynamics of spiral-waves. Methods and Results: Optical action potential signals were recorded from 2-dimensional ventricular myocardium (1 mm thick) of Langendorff-perfused rabbit hearts. Ventricular tachycardias (VTs, >2 min) were induced by burst stimulation. RC (by 5-7°C) was applied to the left ventricular free wall using a transparent cooling device (diameter, 10 mm). RC caused a marked prolongation of action potential (by 25±4%) and a slight reduction of conduction velocity (by 16±5%) in the affected region under constant pacing (2.5Hz, n=10). Spiral-wave dynamics during VTs were transformed by RC from stationary to chaotic meandering types. During RC, the rotors moved irregularly around the cooled region, and often collided with the anatomical boundary (atrioventricular groove) resulting in spontaneous termination. The incidence of termination of VTs within 15 s was increased from 2/18 (11%) without RC to 14/18 (78%) with RC. Conclusion: RC facilitates spontaneous termination of spiral-wave reentry through the unpinning of rotors.

P83 (1P1-020)

Effects of Pacemaker Currents on Creation and Modulation of Pacemaker Activity in Human Ventricular Myocytes: a theoretical study with applications to engineering of biological pacemaker

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The cardiac biological pacemaker (BP) has been created by suppression of the inward-rectifier K^+ current (I_{K1}) or overexpression of the hyperpolarization-activated current (I_h) in ventricular (Purkinje) or atrial myocytes, suggesting possible development of the functional BP as a therapeutic alternative to the electronic pacemaker. In this study, we investigated the effects of incorporating regulatory inward currents (pacemaker currents) such as I_h, sustained inward current (I_{st}), and low voltage-activated L-type Ca^{2+} channel current ($I_{Ca,LD}$) on 1) creation of BP cells, 2) robustness of BP activity to electrotonic loads of adjacent non-pacemaker cells, and 3) BP cell ability to drive the surrounding non-pacemaker cells. Bifurcation structures of single BP cell and coupled-cell models for human ventricular myocytes (HVMs) were explored during changes in conductance of I_{K1}, the regulatory inward currents, and gap junction. Our findings suggest that 1) incorporating I_h and I_{st} (or I_h and $I_{Ca,LD}$) facilitates BP generation during I_{K1} suppression, although it does not lead to BP oscillation without I_{K1} inhibition, and that 2) incorporating I_{st} or $I_{Ca,LD}$ significantly improve the structural stability of BP cells to electrotonic loads and BP cell ability to drive adjacent HVMs.

P84 (1P1-021)

Activation of cardiac chloride current and the electrocardiographic properties in transverse aortic-banding mice

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Cardiac hypertrophy is an adaptive response to chronic cardiac overload, and causes an abnormal electrical activity leading to arrhythmia. It is important to understand the electrophysiological mechanisms underlying the development of this condition. Therefore we analyzed the electrocardiogram (ECG) in hypertrophic heart of mice persistently pressure-overloaded by transverse aortic constriction (TAC). In pre-operated or sham-operated (SO) mice, the first upstroke in QRS-complex (designed to as "a") is followed by a fast second upstroke ("b") and sometimes a slower downstroke ("c"). In TAC mice, "b" is often followed by another slow upstroke ("b'") before "c", and a prolongation of Qc interval was revealed. These results suggest that ECG is sensitive enough to confirm development of cardiac hypertrophy in TAC mice. In addition, the whole-cell patch clamp recordings showed a prolongation of the action potential duration (APD) and a basal activation of chloride (Cl^-) current in single myocytes freshly isolated from left ventricle of TAC mice. The Cl^- current may reflect a persistent activation of volume-regulated Cl^- current ($I_{Cl,vol}$) in isotonic condition, because of the time-dependent inactivation at higher positive potentials, the outwardly rectifier current-voltage relationships and the inhibitory effect of 4,4'-Diisothiocyanatostilbene-2,2'-disulphonic acid. The basal activation of $I_{Cl,vol}$ may be responsible for prolongation of APD and Qc interval in TAC mice.

P85 (1P1-022)

Role of I_{CFTR} in positive inotropic action of isoproterenol in cardiac myocyte: a simulation study

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β-adrenergic receptor agonists, such as isoproterenol, activate protein kinase A (PKA) and induce positive inotropy in the ventricular myocyte. Major mechanisms underlying the positive inotropy are enhanced activities of the sarcolemmal L-type Ca^{2+} channel (I_{CaL}) and the sarcoplasmic reticulum Ca^{2+}-ATPase via phosphorylation of phospholamban. However, role of activation of cystic fibrosis transmembrane conductance regulator channel (I_{CFTR}) in the inotropic effect has not been well understood.We have constructed a computer model of β-adrenergic signaling network including PKA activation of I_{CaL}, phospholamban and I_{CFTR} based on Saucerman et al., (2003, 2004) and implemented it into Kyoto model (Matsuoka et al., 2004). When 1 μM isoproterenol was applied, the amplitudes of I_{CaL}, Ca^{2+} transient, and cell shortening were increased, and the action potential duration was prolonged. Elimination of the activation of I_{CFTR} resulted in a slight rise of the plateau phase of action potential and a decrease in the amplitudes of I_{CaL} due to the decrease in driving force. Ca^{2+} transient was attenuated by about 20%. The results demonstrate that the activation of I_{CFTR} induces compensatory increase in I_{CaL} and facilitates indirectly the positive inotropic action of isoproterenol.

P86 (1P1-023)

Cardiac energetics calculated using a comprehensive cardiac myocyte model.

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The analytical method of cardiac energetics, developed by H.Suga, has been extensively used to evaluate the relationship between mechanical contractility and energy consumption by the left ventricle both in experimental and in clinical studies. The end-systolic maximum elastance (Emax) is measured to indicate contractility of the heart, and the mechanical energy is obtained by the pressure-volume area of a cardiac contraction cycle (PVA), which well correlates with the oxygen consumption. Here, we aim at interpreting the cardiac energetics in terms of the cellular mechanisms. We constructed a heart model of spherical shape to apply the Laplace law in converting the wall tension to pressure. The wall tension was calculated by the contraction model of Negroni and Lascano (1996), which is driven by the Ca transient of the comprehensive cardiac myocyte model, Kyoto Model. The 'Laplace heart' was connected with preload and afterload, and the pressure-volume loop was constructed. Our results of simulation under an aerobic condition show that the Negroni & Lascano model well reconstructs the linear PVA - oxygen consumption curve. The PVA-independent oxygen consumption could be well simulated by the ATP hydrolysis via the Na/K pump and the Ca pump on the sarcoplasmic reticulum.

P87 (1P1-024)

Contractiel properties of cardiac muscle of non-insulin-dependent daibetic mice: Effect of insulin and free fatty acid.

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Though non-insulin-dependent (type 2) diabetes which accounts for over 90% of all diabetic patients leads to congestive heart failure, contractile property of cardiac muscle in type 2 diabetes has not been well characterized. We studied the contractile property of left ventricular papillary muscle of model mouse of the type 2 diabetes, db/db mouse, and effect of insulin and palmitate on it at 20 °C. There was no difference in the time course of twitch between db/db mouse and the control except its magnitude; the rested-state twitch force (Fmax) in the Krebs solution (10 mM glucose) was 21.2 mg/mm^2 (n=6) in the db/bd mouse and 190.3 mg/mm^2 (n=6) in the control. Application of either insulin (80 i.u./l) or palmitate (0.4 mM) increased Fmax in both the db/db mouse and the control. Steady-state force decreased with increasing stimulus frequency. Insulin and palmitate increased the steady-state force at a frequency > 1 Hz in the bd/bd mouse and the control except that palmitate decreased it at a frequency > 1 Hz in the db/db mouse. The rate of short-term mechanical restitution (SMR) decreased with increasing magnitude of the preceding twitch. Palmitate delayed the SMR, while insulin had variable effect on it. There was no apparent difference in the characteristics of the SMR between the db/db mouse and the control. We characterized Ca^{2+} release/content in the cardiac sarcoplasmic reticulum from the SMR with or without insulin and palmitate in the db/db mouse and the control according to the rationale described previously (Tameyasu et al, 2004).

P88 (1P1-025)

A simple system for video-based measurement of heart cell contraction

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Excitation and contraction are the two main functions of heart cells. Therefore for studies of physiology and pathophysiology of heart cells, measurement of cell contraction is as important as that of membrane current and action potentials. In addition, measurement of cell contraction provides a simple way for assesing intracellular calcium transients. However, the measurement of single-cell contraction has not been a popular experimental technique, because it requires very specialized instruments, and because the measurement of cell contraction from patch-clamped cells was not quite trivial. In this report, we describe a simple system for video-based measurement of heart cell contraction, which can be used with patch-clamp instruments without difficulty. In this system, motion of the cell is recorded with a CCD video camera attached to the microscope, and the video images are digitized and analysed on a Windows-based PC using ScionImage public domain software. With the aid of software image manipulation using Sobel filter, cell shortening could easily be measured at 1/60 s time-resolution, from patch-clamped single heart cells. Values of the measurements are stored in text-based data files, which can easily be imported into pClamp or spreadsheets for further analysis. Contraction properties of patch-clamped mouse heart cells is also provided as an example of cell analysis using this system.

P89 (1P1-026)

Cardiovascular effects of a Chinese herbal medicine, Seabuckthorn, in the spontaneously hypertensive stroke-prone rat, SHRSP

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Cardiovascular effects of sea buckthorn (Hippophae rhamnoides L.) called simply "Saji" in China were studied in SHRSP. Saji is a spiky shrub with yellow or orange berries.It has nodule rooting capability of fixing nitrogen from the air. Chemical analyses of Saji berries revealed that it contained vitamins C, B1, B2, E, F, K, P, provitamin A. The average protein content was 22% of the crude material, including polyphenols having high SOD activity. Further, it contained serotonin, cytosterol and selenium as well as zinc. In the in vivo study 5 SHRSPs were fed with rat chow containing powdered Saji fruits at the dose of 30mg/400g for 60 days. As the control group 4 SHRSPs were fed with normal rat chow. Then the arterial blood pressure and heart rate were measured on the tail. The venous blood weas sampled under anesthesia and subjected to biochemical analyses, and the heart was removed and frozen quickly in liquid nitrogen for histochemical analyses. The left ventricle was sliced horizontally in a crosssectional plane and studied by the double staining methods as described previously for the analyses of the arteriolar, intermediate and venular capillary portions. The arterial blood pressure and heart rate decreased. HbA1c, total plasma cholesterol and triglyceride decreased significantly. The arteriolar capillary portion tended to decrease, indicating the release from the stress caused by the hypertension.Thus the herbal medicine, Saji, is beneficial for cardiovascular and metabolic functions.

P90 (1P1-027)

Propofol increases pulmonary vascular resistance during acutely elevated vasomotor tone

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We tested whether pulmonary vascular effects of propofol depend on the level of vasomotor tone. We used the isolated perfused normal rat lung and monocrotaline (MCT)-induced pulmonary hypertension (PH) rat model. Rats were subcutaneously given MCT (PH group). On day 21 after injection, the rats were anesthetized, and the lungs were isolated and perfused. Pulmonary perfusion pressure (PPP) was measured. Changes in PPP during administration of propofol were compared between both groups in the absence or presence of phenylephrine (PE). Propofol had no effect on PPP in the absence of PE. Propofol caused dose-dependent pulmonary vasoconstriction in the presence of PE. To study effects of propofol on endothelial function or myofilament Ca^{2+} sensitivity, N-nitro-L-arginine methyl ester and indomethacin or an inhibitor of PKC, bisindolylmaleimide I (BIS) and calphostin C were administrated before PE treatment. BIS and Calphostin C significantly decreased propofol-induced PPP elevation in MCT rats. In contrast, indomethacin significantly decreased propofol-induced PPP elevation in normal rats. Propofol may exert pulmonary vascular contractility through PKC activation in MCT rats and through inhibition of cyclooxygenase pathway in normal rats.

P91 (1P1-028)

Responses of arterial pressure to perturbations on a simulation model by a neural network algorithm

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We have been trying to calculate of arterial pressure (AP) on simulation model by a neural network (NN) algorithm without approximate equations, to develop a new approach to estimate the relationship between AP and other factors (renal sympathetic nerve activity (RSNA), heart rate (HR) and respiration rate). Previously, we confirmed the simulated AP consisted with measured AP by the NN model. In this study, we examined whether the simulated AP would consist with measured AP when measured values of hypertensive rats were input to the learned NN by those of normotensive rats. The AP simulation model was developed using Neural Network Toolbox of MATLAB (The Mathworks, Inc.). The back propagation was selected as a learning algorithm of the layered NN. AP, HR, RSNA and respiration rate were obtained from conscious chronically instrumented rats. Those were used for the learning of NN algorithm to establish the relationship between AP and other factors. When measured values obtained from hypertensive rats were input to the learned NN by those from normotensive rats, the simulated AP were lower than measured AP. We confirmed that the relationship between AP and other factors of hypertensive rats were different from those of normotensive rats by the NN model. These results suggest that AP of hypertensive rats was not only dependent on the relationship between HR, RSNA and respiration rate.

P92 (1P1-029)

Oxidative stress index at the cell level in hypertensive and/or hypercholesterolemic rat

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We investigated the effects of exercise on endothelial function in Dahl salt-sensitive rats with risk factors for atherosclerosis, i.e. hypertension and/or hypercholesterolemia. We randomly assigned 174 rats to four experimental diet groups: (MF) control diet; (S) high-salt diet; (C) high-cholesterol diet; and (SC) combination of high-salt and high-cholesterol diet. The rats took these diets ad libitum. Each group was further assigned to two exercise conditions: sedentary group (SD) and exercise group (EX), swimming for 1 hour/day, 5 days/week. Chronic swimming exercise and experimental diet were started at the same time and continued for 8 weeks. After physiological examination every 2 weeks, we observed the distribution of NO and superoxide production in endothelial cells around aorto-renal bifurcation by using confocal laser-scanning microscopy. We evaluated the endothelial function with the oxidative stress index, the ratio of superoxide production to NO production. Chronic swimming exercise decreased the physiological risk for atherosclerosis, growth rate of body weight in all diet groups significantly, blood pressure in MF and C significantly, tended to decrease trigriseride, and increased HDL in all diet groups significantly. There was no difference in the oxidative stress index between artery portions, risk factors, and sedentary and exercise, but, decrease of NO production was related to atheroma prone portion. Therefore NO production may more contribute to the localization of atherosclerosis than oxidative stress.

P93 (1P1-030)

Accumulation of colloidal particles in rabbit popliteal lymph nodes in vivo

Ikomi, Fumitaka; Nagai, Takashi; Suzuki, Shigeru; Mizuno, Risuke; Kawai, Yoshiko; Ohhashi, Toshio (*Dept. of Physiol., Shinshu Univ. Sch. of Med., Matsumoto, Japan*)

Colloidal particles are widely used as tracer and vehicle of drugs which target the lymph nodes. Little information, however, exists regarding mechanisms of colloidal accumulation in the lymph nodes in vivo. Thus, in this study, we have attempted to evaluate size-dependent mechanisms for accumulation of colloidal particles in the lymph nodes in vivo. Male Japan White rabbits were anesthetized with ketamine (20 mg/kg iv.) and pentobarbital (20 mg/kg iv.). Centripetal and retrograde cannulations were performed in one of the popliteal afferent and efferent lymph vessels, respectively. All other efferent lymphatics were ligated completely. Labeled latex with fluorescence microspheres with 0.5, 1.1, 1.7 and 1.9 μm in diameter were injected into the afferent lymph vessel. Two microgram of each particle was administered at one time and artificial lymph fluid was injected through the same route at constant rate of 1.47 ml/h. Then, changes in concentration of the microspheres in the efferent lymph vessel were determined by measuring the number of the particles under a fluorescent microscope. $11.0 \pm 6.4\%$ of the 0.5 μm-microsphere passed through the lymph node in 2.5 h. On the other hand, no microsphere with 1.9 μm in diameter was observed in the efferent lymph. The decreasing order of ability for accumulating in the lymph node was as follows: 1.9 μm > 1.7 μm >1.1 μm > 0.5 μm-microsphere. These results strongly suggest that size-dependent accumulating mechanisms exist in the lymph node.

P94 (1P1-031)

Uptake of micro- and nano-particles from interstitial space into lymphatic system

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Micro- and nano-particles are known to be taken up into initial lymphatics. The particles have been used for efficient delivery of drugs and diagnostic agents to lymphatic system. Little information, however, exists regarding size-dependent mechanisms for the particle uptake. Thus, in this study, we have attempted to evaluate effects of size on uptake of the particles. Male Japan White rabbits were anesthetized with ketamine chloride (20 mg/kg iv.) and pentobarbital sodium (20 mg/kg iv.). Retrograde cannulation was performed in one of the popliteal afferent lymph vessels. Labeled latex with fluorescence microspheres with 0.5, 1.1, 2.0, 5.6 and 10.0 μm in diameter were injected subcutaneously at dorsal portion of rabbit foot. Then, concentrations of the microspheres in the efferent lymph vessel were determined by measuring the number of the particles under a fluorescent microscope. The decreasing order of particle concentration in efferent lymph was as follows: 0.5 μm > 1.1 μm > 2.0 μm-particle. No particle with 5.6 and 10.0 μm in diameter was observed in the efferent lymph. When mechanical massage was administered on the injection site, both lymph flow rate and particle concentration were markedly increased. These results strongly suggest that size- and mechanical stimulation-dependent mechanisms exist in transport of micro- and nano-particles from subcutaneous tissue into the lymphatic system.

P95 (1P1-032)

Lymph flow in vivo-resistance through the lymphatic vessels with popliteal lymph node

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The lymphatic vessels and lymph nodes serve a one-way drainage system which return fluid and protein from the interstitial space to blood stream. Almost all of lymph passes through one or more lymph nodes in which water is exchanged freely between lymph and blood compartments. Little information, however, exists regarding lymphdynamics through the lymph nodes in vivo. Thus, in this study, we have attempted to evaluate pressure-flow relationships in rabbit leg lymphatic system and then estimate perfusion resistance through the lymph node. Male Japan White rabbits were anesthetized with ketamine chloride (20 mg/kg iv.) and pentobarbital sodium (20 mg/kg iv.). Retrograde cannulation was performed in one of the popliteal efferent lymph vessels at the groin. All other efferent lymphatics were ligated completely. Then, centripetal cannulation was performed in one of the popliteal afferent or efferent lymph vessels near to the lymph node. Lymph infusion pressure-outflow rate relationship was determined at the constant outflow pressure of -5 cmH_2O. Lymph flow rate increased monotonically with infusion pressure. Calculated resistance at infusion pressure of 25 cmH_2O was about 50 cmH_2O/ml/h with the lymph node and about 15 cmH_2O/ml/h without the lymph node. From these results, resistance of the lymph node was calculated as about 35 cmH_2O/ml/h. Effect of water exchange between lymph and blood compartments should be evaluated in future.

P96 (1P1-033)

Electrical muscle stimulation effectively improve popliteal venous flow on sitting position

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In sitting position, combined effects of movement restriction and hydrostatic pressure difference between the heart and the lower limb might enhance venous pooling/stasis, and then enhance development of deep venous thrombosis. However, our understanding of the actual hemodynamic effects generated by sitting position in the venous circulation of the lower limb remains surprisingly incomplete. Accordingly, the purpose of the present study was to examine this and the effect of electrical muscle stimulation (EMS) on the popliteal venous flow was further examined. Fifteen healthy adult volunteers, none with a history of lower limb surgery or thromboembolism, were recruited. One lower limb was randomly selected for stimulation with the other serving as a control. The sitting position significantly decreased maximum velocity (Vmax) from 30.2 ± 5.7 to 6.5 ± 0.5 cm/s at 30 min and continuously suppressed for 120 min. In contrast to the Vmax, cross sectional area was significantly increased from 9.6 ± 1.2 to 53.1 ± 6.7 mm^2. Due to the combined effect of the decreased Vmax and the increased cross sectional area, the flow volume was not affected by the sitting position (from 116.8 ± 20.7 to 115.9 ± 21.1 ml/min). The Vmax of the EMS leg was significantly higher than that in the non-EMS leg throughout the 120 min sitting period. There was no difference in the cross sectional area between the EMS leg and the non-EMS leg, while the flow volume was significantly higher in the EMS leg compared in the non-EMS leg. Thus, lower limb venous stasis elicited by sitting position was improved by the EMS.

P97 (1P1-034)

Effects of trypsin on effective permeability of 77KDa dextran in isolated lymph vessels

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One of the major functions of the lymphatic system is to return plasma proteins from the interstitial space to the blood stream. Previously, we have demonstrated that small molecular FITC-dextran are permeable from the intraluminal to extraluminal space of isolated lymph vessels and that the endothelial cell surface structure plays a barrier role in effective permeability of medium size of FITC-dexran through the wall of the lymph vessels (Am. J. Physiol. 289: H1676-H1682, 2005). In the present study, we further investigated the effects of trypsin, which is useful enzyme for removal of lymphatic endothelial cells in vitro, on effective permeability of large size of FITC-dextran (77 KDa) in isolated lymph vessels. Afferent lymph vessels were isolated from iliac lymph nodes of Wistar rats. The isolated lymph vessels were cannulated with glass-micropipettes and perfused with Krebs-bicarbonate solution with or without 77 KDa FITC-dextran. Changes in the intensity of 77 KDa FITC-dextran in an intraluminal space of the lymph vessels were measured by a video-microscope system and the concentration of 77 KDa FITC-dextran were calculated before and after treatment with trypsin. 77 KDa FITC-dextran did not permeate the wall of lymph vessels before and after the treatment with trypsin. These results suggest that the non-endothelial wall structure of lymph vessels may be a barrier for effective permeability of a large size of FITC-dextran in vitro.

P98 (1P1-035)

Development of in vitro assay system for evaluating macromolecular permeability through cultured rat lymphatic endothelial cells

Kawai, Yoshiko; Mizuno, Risuke; Ohhashi, Toshio (*Shinshu Univ. Sch. of Med., Matsumoto, Japan*)

One of the important functions of lymph vessels and lymph nodes is to return plasma proteins from the interstitial space in tissues. There exists few or no report, except for one (Ono, et al: AJP H1676-H1682, 2005), regarding for evaluation of hydrophilic permeability through the walls of lymph vessels. No experiment in vitro model using cultured lymphatic endothelial cells has been reported to elucidate macromolecular permeability through the cell layer. Therefore, we have attempted to develop firstly in vitro assay system to study hydrophilic permeability and then investigated the effects of TNF-α.Rat lymphatic endothelial cells cultured from the thoracic ducts or afferent lymphatic vessels were seeded onto the collagen-coated inserts of double chambers in-vitro vascular permeability assay kit (CHENICON). The cell monolayers were treated with or without TNF-α (10ng/ml) over night, and then fluorescent-labeled dextrans (4, 12, and 77KD) were added on the top of the cells coated on upper chamber to evaluate the hydrophilic permeability. The extent of permeability was determined by measuring fluorescent activity of the solution dropped down the lower chamber. The pretreatment with TNF-α caused a significant increase of the permeability of all FITC-dextran through the cultured endothelial cell layer.These findings suggest that the in vitro assay system may be suitable for hydrophilic permeability through cultured lymphatic endothelial cell layer. Inflammatory cytokine, TNF-α, caused a significant increase of permeability with FITC-dextran.

P99 (1P1-036)

Correlation between compliance and high frequency centroid of pressure waveform in the abdominal aorta.

Tanaka, kunihiko; Abe, Chikara; Morita, Hironobu (*Grad. Sch. Med. Gifu Univ. Dep. Physiology*)

According to Windkessel theory, diastolic pressure waveform is a function of the total peripheral resistance and the compliance of the artery. Higher compliance makes the decrescence gentler, and the waveform would be further different from the input pressure waveform made by the cardiac contraction. Frequency analysis of the arterial pressure waveform shows fundamental frequency (F) derived from the heart rate and harmonic frequency which is the integral multiple of the F. In the present study, we measured abdominal aortic pressure, abdominal aortic flow, and the diameter during infusion of Nitroprusside with the speed of 1, 0.6, 0.3 μg/kg/h. The compliance was calculated from the pulse pressure and volume change calculated by the diameter and the length of the aorta. The compliance was also calculated with first order of the Windkessel theory. High frequency centroid (HFC), which is amplitude-weighted mean frequency of the third to seventh peaks of the harmonic waveform was analyzed. HFC was normalized by F. Total harmonic distortion (THD), which is an index of distortion from a pure sine wave was also analyzed. Measured compliance and the calculated compliance of the aorta were significantly correlated. Normalized HFC and both measured compliance and the calculated compliance also showed significant linear correlation. THD did not show significant correlation between the compliance. These results suggest HFC can be an index of the compliance or the aorta, and the compliance may be analyzed from the aortic pressure waveform only.

P100 (1P1-037)

Vasodilator-induced spreading dilatation requires arterial hyperpolarization

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August Krogh first reported that the local application of vasodilators could stimulate extensive vasodilatation which spreads rapidly to distant sites. Conducted responses of this type are referred to as spreading dilatation, a response which cannot be explained simply on the basis of diffusion of the locally applied dilator agent. The present study was designed to test the hypothesis that local stimulation of hyperpolarization can be conducted longitudinally over significant distances to spread dilatation in small mesenteric arteries. A segment of mouse mesenteric artery was cannulated at each end with a glass pipette and then pressurized. In the presence of the NO synthase inhibitor, L-NAME, focal application of the PAR2 agonist, SLIGRL induced dilatation at the site of application. This local dilatation was associated with simultaneous dilatation along the entire artery segment. Measurement of smooth muscle membrane potential revealed local hyperpolarization to SLIGRL which was also observed at distant sites. In contrast, focal application of forskolin did not induced any hyperpolarization, and although it evoked local dilatation, this was not associated with any coordinated dilatation along the isolated mesenteric artery. These results indicate that spreading dilatation responses in mesenteric resistance arteries are only evoked with vasodilators which hyperpolarize the artery.

P101 (1P1-038)

Role of vestibular system in controlling arterial pressure during 60°head-up tilt in conscious rats

Abe, Chikara; Tanaka, Kunihiko; Morita, Hironobu (*Dep. Physiology, Grad. Sch. Med. Gifu Univ, Gifu, Japan*)

Previous studies from our laboratory demonstrated that the vestibular system has a significant role in controlling arterial pressure during gravitational stress. The vestibular system is thought to be stimulated not only by gravitational change but also by postural change. Thus, the vestibular system might have a significant role in controlling arterial pressure during postural change. To examine this, arterial pressure was measured during 60°head-up tilt in conscious rats with or without intact vestibular system. Rats were divided into 3 groups: intact, sinoaortic denervation (SAD), and SAD+vestibular lesion (VL). The posture change did not alter arterial pressure in intact group. In SAD group, however, arterial pressure was increased by the posture change. This increase in arterial pressure was completely abolished by additional VL. Thus, the vestibular system has a significant role in an increase in arterial pressure during posture change in SAD group. The increased arterial pressure induced by the vestibular system is buffered by baroreflex, thus arterial pressure is well maintained during posture change in intact group.

P102 (1P1-039)

Inhibitory effect of deep ocean water on mild hypertension in KHC rabbits

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We investigated effect of deep ocean water on hemodynamics in Kurosawa and Kusanagi-Hypercholesterolemic (KHC) rabbits. The refined deep ocean water (RDOW) at a degree of hardness of 1,000 was freely accessed to the KHC rabbits aged 4 months old for 6 months. Aortic pressure and flow waves were simultaneously recorded under pentobarbital anesthesia. There were no significant differences in body weight and food and water intakes. Systolic, diastolic, mean and pulse pressures, augmentation index of pressure waves and total peripheral vascular resistance were significantly lower in the RDOW group than in the control group, whereas no significant differences were observed in mean aortic flow and total percent lesioned area of the aorta between the two groups. Though serum lipid levels, plasma renin and ACE activities and angiotensin 1 and 2 levels tended to decrease after the feeding of RDOW in the two groups, the difference in these parameters before and after the feeding of RDOW was almost the same between the two groups. Serum electrolyte levels except Mg^{2+} changed little during the intake of RDOW. We can conclude that RDOW improved mild hypertension in KHC rabbits, which might be partly related to the slight but significant increase in serum Mg^{2+} due to the intake of RDOW.

P103 (1P1-040)

Long-term monitoring of pulmonary arterial pressure in conscious, unrestrained mice

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The ability to genetically engineer specific gene knock-out mice has provided a powerful tool for investigating the pathogenesis of pulmonary arterial hypertension (PAH). Yet, there have been no reports describing the measurement of pulmonary arterial pressure (PAP) in conscious mice; an essential requirement for monitoring dynamic changes associated with PAH. In this study we describe a new technique for long-term measurement of PAP in conscious mice using telemetry. In five male C57BL/6 mice (B.W. 25-30 g) the sensing catheter of a telemetric transmitter was inserted into the right ventricle and advanced into the pulmonary artery. The transmitter body was positioned within the abdominal cavity or subcutaneously on the back. During recovery from surgery mean PAP was recorded daily for one week. Subsequently, the PAP responses to acute hypoxia (8% O_2 for 10 min) and L-NAME (50 mg/kg, s.c.) were tested in three mice. By one-week post surgery, all mice had fully recovered from surgery and baseline MPAP was stable at 14.9 ± 0.7 mmHg. Additionally, acute hypoxia and L-NAME provoked a 63% and 86% increase MPAP, respectively. In summary, this study has demonstrated the ability to accurately measure PAP by telemetry in conscious mice. One important application of this technique for future studies is the possibility to assess the contribution of specific genes (i.e. knock-out mice) for the development of pulmonary pathological conditions.

P104 (1P1-041)

Role of nitric oxide in the ischemia-reperfusion injury in mouse livers

Shibamoto, Toshishige; Cui, Sen; Liu, Wei; Takano, Hiromichi; Kurata, Yasutaka (*Dept. Physiol., Kanazawa Med. Univ., Uchinada Ishikawa, Japan*)

We determined the changes in hepatic sinusoidal pressure and liver weight, and the effects of a NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME) in ischemia-reperfusion (I/R) injury of isolated mouse livers perfused portally with diluted blood (Hct 3%). Following pretreatment with L-NAME (100 μM) or D-NAME (100 μM), Ischemia was induced at room temperature by occlusion of the inflow line of the portal vein for 1 hour, followed by 1-hour reperfusion in a recirculating manner. The sinusoidal pressure was assessed by the double vascular occlusion pressure (Pdo). In the D-NAME group, immediately after reperfusion, the portal pressure increased by 2.8±0.1(SE) mmHg, which was accompanied by an increase in Pdo by 1.5±0.1 mmHg, suggesting increases in pre- and post-sinusoidal resistances in a similar degree. Liver weight increased 0.14±0.04 g after reperfusion followed by a gradual return towards baseline. Liver injury, assessed by perfusate levels of hepatic enzymes was observed at 60 min after reperfusion. There were no significant differences in changes in any variables between the D- and L-NAME groups. In conclusion, the increases in both the hepatic vascular resistances and the sinusoidal pressure were small in magnitude, resulting in absence of edematous changes in mouse hepatic I/R, and nitric oxide does not play any significant roles in this injury.

P105 (1P1-042)

Role of liver in anaphylactic hypotension of anesthetized mice

Liu, Wei; Shibamoto, Toshishige; Cui, Sen; Takakno, Hiromichi; Kurata, Yasutaka (*Dept. Physiol., Kanazawa Med. Univ., Uchinada Ishikawa, Japan*)

We determined the roles of liver and splanchnic vascular bed in anaphylactic hypotension in anesthetized mice. In anesthetized mice sensitized with ovalbumin (0.01 mg), an intravenous injection of 0.01 mg ovalbumin caused not only a decrease in systemic arterial pressure (Psa) from 92±1.6 (SE) to 39±2.6 mmHg but also an increase in portal venous pressure (Ppv) which persisted for 40 min after the antigen injection. The elimination of the splanchnic vascular beds, by the occlusions of the celiac and mesenteric arteries, combined with total hepatectomy attenuated anaphylactic hypotension. In addition, a head-down tilt maneuver, which could facilitate venous return in the splanchnic organs, significantly attenuated the decrease in Psa induced by an antigen injection. These results suggest that liver and splanchnic vascular beds are involved in anaphylactic hypotension presumably due to anaphylactic contraction-induced portal hypertension, which induced splanchnic congestion resulting in a decrease in circulating blood volume and thus systemic arterial hypotension.

P106 (1P1-043)

L-NAME potentiates anaphylactic presinusoidal venoconstriction in perfused rat livers

Cui, Sen; Shibamoto, Toshishige; Liu, Wei; Takano, Hiromichi; Kurata, Yasutaka (*Dept. Physiol., Kanazawa Med. Univ., Uchinada Ishikawa, Japan*)

Effects of a NO synthase inhibitor, N^G-nitro-L-arginine methyl ester (L-NAME), on anaphylaxis-induced venoconstriction were examined in isolated rat liver perfused with blood of various hematocrit (Hct) to clarify the role of nitric oxide (NO) in anaphylactic venoconstriction in rat livers.The rats were sensitized with ovalbumin (1 mg), and two weeks later, the liver was excised and perfused portally and recirculatingly at a constant flow with blood at Hct of 0, 5, 16, and 22%. We measured the viscosity of perfusing blood. Using the double occlusion technique to estimate the hepatic sinusoidal pressure (Pdo), presinusoidal resistance (Rpre) and postsinusoidal resistance (Rhv) were calculated. The antigen, ovalbumin (0.1 mg), was injected into the reservoir 10 min after pretreatment with L-NAME (100 μM) or D-NAME (100 μM). The viscosity, a determinant of vascular resistance and shear-stress, increased in a Hct-dependent manner. L-NAME pretreatment increased exclusively basal Rpre in liver perfused at Hct 22%. The antigen caused hepatic venoconstriction as characterized by predominant presinusoidal constriction in all antigen administered livers. L-NAME pretreatment potentiated the antigen-induced venoconstriction, as compared with the D-NAME pretreatment, by increasing Rpre, but not Rpost. These finding suggests that hepatic anaphylaxis increased production of NO, which consequently attenuated anaphylactic presinusoidal venoconstriction in isolated perfused rat livers.

P107 (1P1-044)

Autonomic control based on heart rate variability in congenital heart diseases with increased pulmonary blood flow

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Objective Ventriculoseptal defect;VSD and atrial septal defect;ASD exhibit increased pulmonary blood flow through the left-to-right shunt. In patients with these pathological states arising from volume and/or pressure overload in the right heart can result in sympathovagal imbalance. We investigated the correlation between the HRV and respiratory frequency, and shunt ratio (Qp/Qs) measured by the Doppler echocardiography.**Subjective and methods**We enrolled 66 patients. Diagnoses were: VSD (n=21) and ASD (n=45). Mean age was 5.6 ± 3.3 years. ECG and respiratory waves were recorded during the Doppler UCG test. We performed HRV analysis to calculate LF, HF, TF, respiratory frequency (RF), LF/HF, RF/TF, and LF/RF. Qp/Qs was also calculated and quantified by multivariate analysis with HRV parameters. **Results**1) Qp/Qs and LF/HF exhibited correlation in both ASD and VSD groups. 2) The ASD group showed a positive correlation between Qp/Qs and LF/RF. However, in the VSD group the correlation was negative. 3) Qp/Qs correlated negatively with RF/TF in the ASD group, however, the correlation was positive in the VSD group.**Conclusions**ASD and VSD showed different effects of respiratory vagal activity on HRV. We believe that a reason is that volume and low pressure overload to the right atrium in ASD inhibits respiratory vagal innervation of the sinus node.

P108 (1P1-045)

Sympathetic nerve response to endogenous hemeoxygenase inhibition in conscious rats

Hirakawa, Haruhisa; Kemuriyama, Takehito; Hiruma, Megumi; Nishida, Yasuhiro (*Dept. Physiol. II, Natl. Defense Med. Coll., Saitama, Japan*)

Carbon monoxide is formed in the process of degradating heme from biliverdin by heme oxygenase (HO) in the various tissues, including central nervous system (CNS). Previous studies suggested that inhibition of HO activity increased arterial pressure (AP) mediated by the autonomic nervous system. The present study was designed to investigate the sympathetic nerve response to inhibition of HO activity by a direct renal nerve recording in conscious rats. Zinc deuteroporphyrin 2, 4-bis glycol (ZnDPBG), an inhibitor of HO activity, was administered ip in chronically instrumented Wistar rats: 8 intact, 8 atropine-treated, and 7 sinoaortic denervated (SAD). ZnDPBG induced significant increases in mean AP (MAP) from 95.9 ± 1.6 to 116.9 ± 4.7 mmHg and renal sympathetic nerve activity (RSNA) from 100.0 to 186.7 ± 19.2%, but no significant change in heart rate (HR) in intact rats. In atropine-treated rats, ZnDPBG also induced significant increases in MAP from 96.7 ± 1.5 to 110.6 ± 2.0 mmHg and RSNA from 100.0 to 184.7 ± 22.5%, but induced no change in HR. In SAD rats, ZnDPBG induced significant increases in MAP from 91.4 ± 5.7 to 118.8 ± 8.5 mmHg, HR from 356.8 ± 10.4 to 409.1 ± 14.4 beats/min, and RSNA from 100.0 to 211.6 ± 18.9%. The present study suggested that inhibition of HO activity caused sympatho-excitation via a direct action on CNS, resulted in an increase in AP. Further study is required to investigate the underlying mechanism of HR response to HO inhibition.

P109 (1P1-046)

Spinally mediated articulo-cardiac sympathetic reflex in anesthetized rats

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It has been proven that noxious articular stimulation of hindlimb produce reflex increase in heart rate, in anesthetized animals, to be a reflex response whose reflex center is in the brain and efferent arc is a cardiac sympathetic nerve. Using central nervous system (CNS)-intact and acutely spinalized anesthetized rats, the present study aimed to examine the possibility of whether articular stimulation could influence heart rate via sympathetic nerve at the spinal level. In CNS-intact rats, noxious articular movement of both knee and elbow joint produced an increase in the cardiac sympathetic nerve activity and heart rate. In acutely spinalized rats, noxious movement of elbow joint produced large increases in the cardiac sympathetic nerve activity and heart rate, while noxious movement of knee joint induced no increase in the cardiac sympathetic nerve activity and only a marginal increase in heart rate. As the marginal heart rate response following knee joint stimulation in spinalized rats was abolished after adrenalectomy, the responses were suggested to be induced by catecholamine secreted from the adrenal grand. It is concluded that the spinal cord is capable of producing propriospinally the reflex increases in heart rate via reflex activation of the cardiac sympathetic efferent nerve following stimulation to the elbow joint stimulation whose afferent information enters the spinal cord at the same segments or segments overlapping the cardiac sympathetic outflow.

P110 (1P1-047)

Differencial effect of short term exercise training on the cardiovagal baroreflex sensitivity and carotid arterial compliance in older subject

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Previous studies have reported that carotid arterial compliance increased by habitual exercise, so that the cardiovagal baroreflex sensitivity increased. This concept is based on the anatomical fact that the arterial baroreceptor is a stretch sensitive receptor, a part of which is located in the carotid sinus. However, we previously reported that neural component of baroreflex sensitivity estimated by R-R interval corresponding end-systolic lumen diameter was greater in physically active young men than that in sedentary, but mechanical component of baroreflex sensitivity estimated by end-systolic lumen diameter corresponding systolic blood pressure was not different between active and sedentary group. We hypothesized that short term exercise training increase arterial baroreflex sensitivity due to the neural alteration but not increase arterial compliance because alteration in neural property will occur faster than that in mechanical property of blood vessel wall. To examine this hypothesis, we estimated baroreflex sensitivity and carotid arterial compliance in elderly subjects before exercise training, and at 2, 6, and 12 weeks after training. Arterial baroreflex sensitivity increased at 2 weeks after training but arterial compliance did not increase at this time point. This result suggests that arterial baroreflex sensitivity increase by short term exercise training, which is probably due to alteration in "neural" arc of the arterial baroreflex.

POSTERS
Respiration

P111 (3P1-060)

The carotid body of spontaneously hypertensive rats

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Morphological characteristics in the carotid bodies of spontaneously hypertensive rats (SHR) and those of age-matched normotensive Wistar rats (NWR) as well as age-matched genetically comparable Wistar rats (WKY) were examined. The distribution and abundance of four different regulatory neuropeptides, substance P (SP), calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), and neuropeptide Y (NPY) in the carotid bodies of three strains of rat were also examined. The carotid bodies of SHR were greater than those of NWR and WKY. The values in the long axis of the carotid bodies of SHR were significantly 1.3 times larger than those of NWR and WKY. In the carotid bodies of SHR, the percentage of relatively large vessels were similar to that in the carotid bodies of WKY, although the carotid bodies themselves were significantly larger than in WKY. In the carotid bodies of NWR and WKY, the density of NPY-immunoreactive varicose fibers were more numerous than that of VIP, SP, and CGRP fibers. These immunoreactive fibers were mainly associated with the vasculature and the clusters of glomus cells. The density of VIP varicose fibers in the carotid bodies of SHR were smaller than that in the carotid bodies of WKY, although that of SP, CGRP, and NPY fibers was similar to that in the carotid bodies of NWR and WKY. The present results suggest that the mechanisms of carotid body enlargement in hypertensive rats are different from those in hypoxic rats.

P112 (3P1-061)

Distribution and axonal projection of pontine respiratory neurons in the rat

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The area around the parabrachial nucleus (PB) including the Kolliker-Fuse (KF) nucles is deeply involved in respiratory control, but not enough information is available about the respiratory neurons of this area. In Nembutal-anesthetized, paralyzed, and artificially ventilated rats with intact vagal nerves, we recorded from more than 300 respiratory neurons in the PB and KF areas. The respiratory neurons were densely distributed in the KF nucleus and sparsely in the medial parabrachial nucleus (PBM) and its vicinity. Only few neurons were purely inspiratory (I) and most "inspiratory" neurons had more or less expiratory (E) activity, exhibiting the property of E-I phase-spanning neurons. By far the less number of I-E phase-spanning neurons were recorded, and their firing was labile and weak. The E neurons exhibited augmenting, decrementing or whole-phase expiratory firing patterns. Activity of the respiratory neurons was variously modulated by lung volume, but we could not find non-respiratory neurons whose activity was modulated thoroughly by lung volume. The majority of the respiratory neurons examined by antidromic stimulation had medullary axons, some having axons descending the midline medulla toward the spinal cord. Many E and "inspiratory" neurons projected to the nucleus tractus solitarii (NTS). Some "inspiratory" neurons projected to the hypoglossal nucleus. It was suggested that PB and KF neurons project to motor output nuclei, such as the nucleus ambiguus and the hypoglossal nucleus, as well as to the rhythm-related structures, such as the Botzinger complex and the NTS.

P113 (3P1-062)

Hypoxic ventilatory response in the light and dark periods in unanesthetized mice lacking histamine type-1 receptors

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The effects of the circadian light/dark cycle on ventilatory responses to chemical stimuli have rarely been studied in experimental animals, despite evidence that the cycle may be a factor in respiratory results. We measured the ventilatory response to hypoxia (HVR) in unanesthetized wild-type and histamine type-1 receptors knockout (H1RKO) mice in the light and dark periods with a whole-body, single-chamber plethysmograph. Animals were subjected to a 10-min hypoxic exposure (7% O_2 and 3% CO_2 in N_2) after acclimatization to the chamber for 90 min. In both groups of mice, minute ventilation increased in response to the hypoxia and declined gradually after the peak response regardless of when HVR was determined. However, we found differences in the HVR between wild-type and H1RKO mice. In wild-type mice, the minute ventilation response was higher in the dark period than in the light period, which was due to differences in the tidal volume response rather than the respiratory frequency response. Conversely, in H1RKO mice, minute ventilation responses did not differ between the two periods, which were similar to the response of wild-type mice determined in the dark period. In summary, the circadian light/dark cycle altered the HVR in wild-type mice, whereas, in H1RKO mice, the cycle difference in the HVR disappeared. These results suggest that H1R contributes to the circadian light/dark cycle differences in the HVR in unanesthetized mice.

P114 (3P1-063)

Momentary rise in breathing rate during light sleep in mice and humans

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Breathing activity during sleep is closely related to sleep stage. Indeed, breathing rate (BR) during rapid eye movement (REM) sleep show much larger fluctuation than that during deep sleep. In the present study, breathing movement of freely moving C57B6/J mice were analyzed during sleep by piezoelectric device placed on the floor of the cage in a non-invasive manner (PCT/2003/001109), while animal behavior was monitored by an infrared camera. We found that mice momentarily exhibited an extraordinary large rise in BR by up to 14 breaths/sec during sleep. Such rise in BR was detected in all four mice tested, especially after atonia and shortly before awakening (mean BR = 10.5±1.2 breaths/sec, which is 4.3±1.3 times the BR in stable deep sleep; mean duration = 0.4±0.1 sec). In humans, interestingly, a similar momentary increase in BR by up to 116 breaths/min (2.4-3.7 times the BR in deep sleep) was detected during REM sleep in normal volunteers tested. No such increase was detected in stages 3 and 4. In addition, more prolonged increase in BR also was observed during REM/light sleep in human subjects. Further studies may clarify the correlation between the central regulation mechanisms of respiration and sleep stages.

P115 (3P1-064)

Does cardiorespiratory synchronization show competitive aspect of cardiorespiratory modulation?

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Respiratory modulation of the heart rate (i.e.,respiratory sinus arrhythmia:RSA) and phase synchronization of respiratory and cardiac rhythms are manifestation of cardiorespiratory coordination. However, the question of whether they represent two competing entities of interaction remains unresolved. The present study was designed to characterize the synchronization and modulation between patterns of breathing and RSA during normoxia and hypoxia under resting condition. For this purpose, we recorded ventilation and electrocardiogram in 9 subjects while breathing was paced either 6,8,10, or 12 breaths/min for 5 min each. The instantaneous phases were calculated for RSA curve and respiratory patterns using Hilbert transform and then relative phase differences were obtained. The cardiorespiratory synchronization and modulation were quantified by the index based on conditional probability of the phase difference and by the amplitude of RSA power calculated using a fast Fourier transform, respectively. The synchronization index decreased significantly at 12 breath/min compared with that observed at lower breathing frequency, while the modulation index (i.e., RSA power) decreased almost linearly with increasing breathing frequency. There was a significant positive correlation between the indexes of the synchronization and modulation ($p<0.01$). The observed relation was not altered by hypoxia, suggesting that the coordination may not depend on functional modulations. Our analysis indicates that the cardiorespiratory synchronization can coexist with the modulation.

P116 (3P1-065)

Effects of hypercapnia on respiratory neurons of rat medullary raphe nuclei.

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Numerous studies have suggested that caudal raphe nuclei may play some roles in respiration. However, details of the role of the raphe nuclei in controlling of respiration have not been clarified. We have previously reported that respiratory neurons are present in medullary raphe nuclei. The present study was undertaken to examine the effects of hypercapnia on the respiratory neurons of medullary raphe nuclei. The experiments were performed on decerebrate, paralyzed, vagotomized and artificial ventirated rat. Extracellular recordings were made from neurons having firing patterns related to phrenic nerve discharge in the midline medullary tegmentum. We recorded mainly Inspiratory (I) throughout neurons which firerd throughout the I phase in the raphe obscurus and pallidus. They were tested changes in neuronal discharges when the ventilator was stopped (during 8-24 sec; end-tidal CO2 levels were raised from 5±0.4 to 8±0.3%). Most of the neurons examined responded to hypercapnea in the neuronal discharge. But some neurons examined did not respond. These results suggest that the midline caudal raphe nuclei are involved in central chemoreception.

P117 (3P1-066)

Proposal of the fine anatomical model for the central respiratory chemoreceptor

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We have studied the fine anatomical architecture of the central respiratory chemoreceptor using electrophysiological, optical and histological techniques. We have identified medullary chemosensitive sites in the surface parts of the rostral ventrolateral medulla, raphe pallidus and parapyramidal regions. Pontine regions (locus coeruleus and A5 region) were also chemosensitive. We found two types of CO2-excitable cells (Type I and II cells) in the superficial ventral medulla. A Type I cell is smaller in size, located within the marginal glial layer, and intrinsically CO2-excitable. A cluster of Type I cells surround fine penetrating vessels, and a large surface vessel covers this region. A Type II cell is larger, located at a depth of a few hundred micrometer from the medullary surface, and excited transsynaptically by CO2. A single Type II cell forms dendro-somatic synapses with multiple Type I cells within the marginal glial layer, and sends axonal branches to multiple neurons of the ventral respiratory group (VRG). We propose a cell-vessel architecture model for the central respiratory chemoreceptor. Type I cells are primary chemosensors. In response to increased CO2 in perivascular tissue, Type I cells secrete neurotransmitter, and excite Type II cells. Excited Type II cells relay and amplify this information by innervating multiple VRG neurons.

P118 (3P1-067)

Effects on hypoxic respiratory responses of 5-HT2 receptors in the dorsomedial medulla oblongata in mice

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We have reported that serotonin (5-HT) type 2 receptors in the dorsomedial medulla oblongata (DMM) influence ventilation and airway resistance in mice. In the present study, the role of 5-HT2 receptors in the DMM in respiration to hypoxia was investigated. Each male mouse was anesthetized with pentobarbital sodium i.p. for insertion of a microdialysis probe into the DMM. The mouse was placed into a double chamber plethysmograph to obtain two respiratory flow curves to calculate respiratory variables, while extracellular fluid was collected at 1.2 microL/minute every 5 minutes. Extracellular fluid via the microdialysis probe was analyzed with an ECD-HPLC. Artificial cerebrospinal fluid (aCSF) or 1 x 10^{-5}M LY-53857 (a 5-HT2 receptor antagonist) was perfused via the microdialysis probe in the DMM with air or hypoxic gas (7% O_2 in N_2) inhalation. Changes in respiratory variables and the 5-HT concentration in the DMM were analyzed every 5 minutes. Respiratory variables during hypoxia in a LY-53857-perfused group were not different from those in an aCSF-perfused group. Post-hypoxia frequency in the LY-53857-perfused group was decreased compared to that in the aCSF-perfused group, while the 5-HT concentration in the DMM was significantly increased under hypoxia in both the aCSF-perfused group and the LY-53857-perfussed group. We discuss whether or not the post-hypoxia frequency decline with LY-53857 perfusion in the DMM is a result of respiratory hypoxic excitation with a more detailed analysis of respiration variables.

P119 (3P1-068)

Characteristics of GABAergic respiratory neurons in the ventrolateral medulla: studies in GAD67-GFP knock-in neonatal mice

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We investigated electrophysiological and morphological characteristics of GABAergic neurons in the pre-Boetzinger complex (PBC, the caudal part of the rostral ventrolateral medulla), the putative kernel site for respiratory rhythm generation. We used GAD67-GFP knock-in neonatal mice, which enabled us to identify GABAergic neurons in a living condition. We recorded respiratory activity from the hypoglossal nerve in medullary transverse slices that contained the PBC and whole-cell recorded activities of GFP-positive neurons in the PBC. Six out of 32 GFP-positive neurons were inspiratory. All of the remaining neurons were non-respiratory. In addition, 6 GFP-negative inspiratory neurons were recorded in the PBC. The electrophysiological properties of GFP-positive inspiratory neurons included high membrane resistance and mild adaptation of spike frequency in response to depolarizing current pulses. The somata of GFP-positive inspiratory neurons were smaller than those of GFP-negative inspiratory neurons. These results indicate that GABAergic inhibition by inspiratory neurons with particular properties in the PBC is involved in neural respiratory control.

P120 (3P1-069)

Dopaminergic modulation on the respiratory neuronal network using isolated brainstem-spinal cord preparation from neonatal rat

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Here we report that unique 'switching' roles of dopamine in regulating the respiratory rhythm generation. We employed medulla-spinal cord block preparation which contains intact respiratory rhythm generator. In this preparation, respiratory rhythm generator consists mainly Pre-Inspiratory (Pre-I) neurons and inspiratory (I) neurons. I neurons are premotor neurons and Pre-I neurons trigger I neuronal firing as a pacemaker. Optical imaging with voltage-sensitive dye revealed that application of dopamine selectively disrupted phasic Pre-I neuronal firing and slightly enhanced I neuronal firing. This dopaminergic effect for Pre-I neuron was mimicked by dopamine D4 receptor agonist, PD168077 application and the enhanced effect of I neuronal firing was mediated with D1 receptor. Dopamine depolarized Pre-I neuronal membrane potential significantly but not on I neurons under TTX perfusion. Finally, dopamine depressed I neuronal PSPs which are probably originated from Pre-I neurons. Dopamine selectively disrupts synchronized phasic Pre-I neuronal firing through dopamine D4 receptor, and enhances I neuronal network through dopamine D1 receptor. Dopamine has opposite effects for respiratory network though different type of dopamine receptors. It seems to be the switching involuntary respiration to voluntary respiration.

P121 (3P1-070)

Deletion of histamine type I receptors affects circadian pattern of ventilation.

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Ventilation changes depending on circadian light-dark cycle. Histaminergic neurons are involved in the circadian regulation in the brain, but a role of circadian pattern of ventilation is unknown. We examined ventilation and metabolism for 24 h in histamine type 1 receptor knockout (H1RKO) and wild-type mice. Mice were measured for ventilation by a whole-body plethysmograph and metabolism by a magnetic-type mass spectrometry, and consciousness was characterized by electroencephalogram. In both genotypes, minute ventilation (V_E) and metabolic rate were greater during the dark period (20:00 to 8:00) than during the light period (8:00 to 20:00). H1RKO mice during the light period were greater in V_E with increased tidal volume and greater in VCO_2 without changes in VO_2 than wild-type mice. The increased V_E during the light period was responsible for the increased CO_2 production with an identical ratio of V_E to VCO_2. However, H1RKO mice decreased the ratio compared to that of wild-type mice during the dark period, which was responsible for an increase of delta/theta ratio calculated from power spectrum density of electroencephalogram. Thus, deletion of H1 receptor affects patterns of ventilation accompanied with changes in metabolic substrates and arousal state of the dark period. Results suggest that histamine modulates ventilatory pattern during the light and the dark periods via H1 receptors.

P122 (2P1-005)

Habutobin inhibits the tyrosine-phosphorylation of FAK at an early stage of collagen-induced platelet aggregation

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Habutobin, thrombin-like enzyme that converts rabbit fibrinogen to fibrin, was purified from *T. flavoviridis* . Habutobin bound to β_3 subunit of integrin $\alpha_{IIb}\beta_3$, and then habutobin inhibited the collagen-induced aggregation, in previously study. We investigated the effect of habutobin for intracellular signaling through tyrosine(Tyr)-phosphorylation of protein occurring in the collagen-induced aggregation. Rabbit platelet was washed with ACD-A and HEPES-Tyrode's sol (Ca^{2+}, Mg^{2+} free) and the washed platelet was suspended in the Tyrode's sol containing Mg^{2+} (1 mM). From results of Western blot with anti-phosphotyrosine antibody(4G10), Tyr-phosphorylation of several intracellular proteins increased dramatically during collagen-induced aggregation. Tyr-phosphorylated proteins were observed in 55-60, 72, 85 and 116 kD at 90 sec after the addition of collagen. In the presence of habutobin, although Tyr-phosphorylated proteins was not observed at 90 sec after the addition of collagen, they were observed in 60, 72 and 116 kD at 3 min after the addition of collagen. In addition, in the presence of habutobin, FAK Tyr-phosphorylation was inhibited at an early stage of collagen-induced aggregation, but it was not inhibited at a late stage of collagen-induced aggregation. From the present study, it was suggested that the binding of habutobin to β_3 subunit of integrin $\alpha_{IIb}\beta_3$ resulted to inhibit FAK Tyr-phosphorylation, and habutobin inhibits the β_3 signaling, that is, outside-in signaling.

POSTERS
Blood

POSTERS
Kidney & body fluids

P123 (2P1-006)

Effects of angiotensin II and nicotine on rat subfornical organ neurons

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The subfornical organ (SFO) plays important roles for drinking behavior and cardiovascular regulation. It is well known that excitation of SFO neurons by angiotensin II (ANG) induces the two behavioral responses, but that by nicotine (NIC) induces only cardiovascular responses. Therefore, there may be present subpopulations of SFO neurons showing different sensitivity between ANG and NIC. In the present study, we verified that intracerebroventricular (i.c.v.) injections of ANG at 400 ng induced large water intake in rats, but that of NIC at 50 μg induced small water intake. The i.c.v. injection of NIC induced cardiovascular responses increased *c*-Fos expression in the SFO. In slice preparation, we compared with the responses of ANG and NIC on 101 SFO neurons by using an extracellular recording system. ANG and NIC respectively increased spontaneous firing frequencies in 67 and 62 SFO neurons. Although 45 ANG-sensitive neurons had NIC-sensitivity, the remaining 22 neurons did not. Recently, we have reported cell classification of dissociated SFO neurons, F- and S-types, and that a half of F-type and all S-type neurons have ANG-sensitivity. A patch-clamp recording showed that although almost F-type had NIC-sensitivity (n = 19/21), S-type did not (n = 7/8). From these results, we hypothesize that classified S-type cells have ANG-sensitivity without NIC-sensitivity and are related to drinking behavior, but not to cardiovascular responses, and their function is different from that of F-type cells.

P124 (2P1-007)

Intracellular pH dependent regulation of neuronal nitric oxide synthase activity in cultured mouse macula densa cells

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Kidney macula densa cells sense the chloride concentration of the fluid in the adjacent lumen and control glomerular filtration rate. We have used a functionally intact macula densa cell line (NE-MD) established from immortalized renal cells in culture. NE-MD cells specifically express neuronal nitric oxide synthase (nNOS) regulated by either low NaCl intake or furosemide (an inhibitor of Na^+-K^+-$2Cl^-$ symporter). We have examined whether L-arginine-induced NO production is pH dependent or not in NE-MD cells, by using a NO-sensitive electrode. The NO production was low in control, but increased when NE-MD cells were treated with furosemide (12 μM) for 2 hrs. When furosemide-treated NE-MD cells were incubated in the presence of 100 μM amiloride (an inhibitor of Na^+/H^+ exchanger), L-arginine-induced NO production was unaffected. However, the NO production significantly decreased by 42% when the cells were placed in a solution containing amiloride. Similar results were obtained when NE-MD cells were incubated in the low pH solution (pH=7.1). These results strongly suggest that furosemide-induced expression of nNOS protein is not sensitive to acidosis, but its activity is pH sensitive. This may partly account for polyuria in abnormalities of electrolyte and acid-base balance.

P125 (2P1-008)

Effect of CO_2 on intracellular pH in perfused bullfrog proximal tubules

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We investigated the effect of basolateral CO_2 on the cytosolic pH (pH_i) regulation in the proximal tubule of perfused bullfrog kidney by using H^+-selective microelectrode. Furthermore, in sliced kidney, we monitored the changes in acridine orange fluorescence in acid vesicles of proximal tubule cells during the elevation of basolateral CO_2 with or without H^+-pump inhibitors. Elevating baolateral CO_2 from 1.5 to 5% at constant HCO_3^- concentration induced an initial slight decrease followed by a sustained increase in pH_i with 10 mV hyperpolarization of basolateral membrane. In the presence of 10^{-6} M bafilomycin A1 (BAFA) or 10^{-7} M concanamycin A (CNCA), a specific H^+-pump inhibitor, elevating basolateral CO_2 produced no increase in pH_i, but a decrease in pH_i with a depolarization of basolateral membrane. The increase in acridine orange intensity in acid vesicles was inhibited by the perfusion of 10^{-6} M BAFA during the elevation of CO_2 in the perfusion fluid. The peritubular perfusion of 10^{-6} M BAFA suppressed the alkalinaization of pH_i with 20 mM NH_3/NH_4^+ application to peritubular perfusion fluid. These results indicate that intracellular H^+ transport in the acid vesicles as well as Na^+/H^+ exchanger or Na^+-HCO_3^- cotransporter in the cell membrane play an important role of the pH_i regulation in the proximal tubule of bullfrog kidney.

P126 (2P1-009)

Galanin inhibits spontaneous firing of rat subfornical organ neurons

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Activation by angiontensin II (ANGII) of subfornical organ (SFO) neurons induces water intake and vasopressin release. On the other hand, central and peripheral administrations of galanin (GAL) have been reported to inhibit water intake and vasopressin release in recent *in vivo* studies. We anatomically, molecularbiologically and electrophysiologically investigated existences of GAL-receptors, and GAL-induced responses in SFO neurons of rats. Immuno-electron microscopic observation revealed existence of GAL-containing synaptic vesicles in the SFO. Conventional RT-PCR analysis demonstrated that there were present all mRNAs of three known GAL receptor subtypes, GALR1, GALR2 and GALR3, in the SFO tissues. In the extracellular recordings, application of GAL inhibited spontaneous firing rate in more than half of SFO neurons, even after synaptic blockade by a low [Ca^{2+}] and high [Mg^{2+}] solution. The inhibitory responses were dose-dependent. Seventy-two percent of GAL-sensitive neurons were also responsive to ANGII. ANGII increased the firing rate. These results suggest that GAL functions as an inhibitory neurotransmitter or neuromodulater in the SFO. Furthermore, the electrophysiological results offer a plausible explanation for the *in vivo*-observed opposite effects of ANGII and GAL on water intake and vasopressin release.

P127 (2P1-010)

Modulation of two types of apical ion channels in cultured mouse renal collecting tubule cells

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Ion channels in the apical membrane of cultured mouse renal collecting tubule cells were investigated using the patch-clamp technique. At least two types of ion channels were observed with cell-attached patches under control conditions. One was an inwardly rectifying small conductance (20-30 pS) K channel (SK channel) and the other was an intermediate conductance (40-50 pS) non-selective cation channel (NC channel). Activity of the SK channel was relatively high, whereas that of NC channels was usually low in the control condition. Addition of membrane-permeant cAMP analogue, 8Br-cAMP (100 μM), stimulated the activity of SK channels but did not affect that of NC channels in inside-out patches. In the presence of 1mM Ca^{2+}, addition of Ca^{2+}-ionophore, ionomycin (1 μM), resulted in marked enhancement of NC channel activity and reduction of SK channel activity. In inside-out patches, SK channels required cytosolic ATP (1 mM) to maintain their activity, which was not affected by cytosolic Ca^{2+}. On the other hand, NC channel was activated directly by elevation of cytosolic Ca^{2+}, but not by ATP in inside-out patches. These results suggest that regulatory mechanisms of these channels are quite different, which may play a role in the electrolyte transport along the collecting duct under various conditions.

P128 (2P1-011)

Analysis of ANP- and cGMP-activated cation channels in the frog urinary bladder

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It has been known that atrial natriuretic peptide (ANP) inhibits Na^+ reabsorption in renal tubules of the mammalian kidney. We, however, recently reported that ANP and cGMP stimulate amiloride-blockable Na^+ absorption through PKA-dependent pathway in the urinary bladder of the Japanese tree frog, *Hyla japonica*. We also suggested that there is a possibility of expression of cyclic nucleotide-gated channels in the frog urinary bladder using Ussing chamber voltage-clamp and whole cell patch-clamp techniques (Yamada *et al.*, 2005). In the present study, we examined to identify characteristics of the channels activated with ANP and cGMP by using a single channel recording in epithelial cells of urinary bladder. In the recordings of cell-attached patch with NaCl in a patch pipette, a variety of ion channels were revealed the activity with conductance ranging from 5 to 30 pS. Ion channels with conductance for inward current of 5-7 pS were examined because the channels have the same properties, such as conductance and slow opening and closing kinetics with the epithelial Na channels in the toad urinary bladder. When Cl^- in the pipette solution was replaced by gluconate, the conductance of the channel did not change. This shows the channel carries cations but anions. Addition of 10^{-4} M 8-Br-cGMP to the bath solution significantly stimulated the inward current in the cell-attached patches. These results suggest that ANP- and cGMP-dependent increases in Na^+ absorption are mediated by 5-7 pS cation channels in the epitherlial cells of the frog urinary bladder.

POSTERS
Gastrointestinal functions

P129 (3P1-071)

The effects of central neuropeptide Y on gastric relaxation of the proximal stomach.

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Potent appetite-stimulating effects of neuropeptide Y (NPY) are well known. The present study undertook to demonstrate that the role of NPY in gastric relaxation of the proximal stomach was examined in anaesthetized rats. Intragastric pressure (IGP) was measured using a balloon situated in the proximal part of the stomach. The administration of NPY into the fourth ventricle induced relaxation of the proximal stomach in a dose-dependent manner. The administration of an Y1 receptor agonist [Leu^{31}, Pro^{34}] NPY induced a larger relaxation than NPY. The administration of an Y2 receptor agonist (NPY13-36) did not induce significant changes in motility. Microinjection of [Leu^{31}, Pro^{34}] NPY into the caudal part of the dorsal vagal complex (DVC) induced relaxation of the proximal stomach. In contrast, similar injections into the intermediate part of the DVC increased IGP of the proximal stomach. The administration of NPY into the fourth ventricle did not induce relaxation after bilateral injections of the Y1 receptor antagonist (1229U91) into the caudal DVC. Bilateral vagotomy below the diaphragm abolished the relaxation induced by the administration of NPY into the fourth ventricle. The intravenous injection of atropine methyl nitrate reduced the magnitude of relaxation induced by the administration of NPY. These results indicate that NPY induces relaxation in the proximal stomach via Y1 receptors situated in the DVC. This work was supported by a Grant-in-Aid for Scientific Research from the Japanese Society for the Promotion of Science to MK.

P130 (3P1-072)

Effects of four drugs those affect gastrointestinal motility on proximal gastric tone in conscious dogs

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Aim: Mosapride, selective 5-HT$_4$ receptor agonist, and itopride, selective D$_2$ receptor antagonist, were known to enhance motility of the distal stomach and facilitate gastric empting. On the other hand, sumatriptan, selective 5-HT$_1$ receptor agonist, and paroxetine, 5-HT reuptake inhibitor, were reported to induce a relaxation in the proximal stomach. In this study, effects of these four drugs on proximal gastric tone were researched using a barostat in conscious dogs with a gastric fistula. ***Methods:*** The effects of the drugs and those vehicles on the proximal gastric volume at a constant intragastric tone were studied using a barostat. Simultaneously, proximal and distal gastric contractility, and duodenal contractility was measured using three force transducers. Furthermore, stepwise isobaric gastric distensions were performed before and after the administration of the drugs. The same experiments were done in fasting phase and in postprandial phase. ***Results & Conclusion:*** Effects of the drugs on proximal gastric tone were more clearly seen in postprandial phase than fasting phase, and sometimes differed from those on distal gastric and duodenal contractility. Such a measurement of proximal gastric tone using a barostat in animal experiments seems to be useful for estimation of effectiveness of some drug on gastrointestinal disorders.

P131 (3P1-073)

Decrease in gastric acid secretory capacity in aged mice

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The gastrointestinal (GI) tract demonstrates a number of changes that accompany advanced age. In the present study, we investigated gastric acid secretion in young and old mice in response to chemical stimulation and mechanical stimulation. Gastric acid secretion was measured in anesthetized mice. Proton pump (H^+,K^+ ATP ase) is a good maker of the parietal cell function, therefore, protein expression of proton pump was determined by Western blotting. After 60 min basal collection, acid secretion was stimulated by histamine (500 and 1000 mg/kg) or carbachol (10 and 20 mg/kg). To investigate the response to mechanical stimulation, the stomach was distended by an intragastric injection of isotonic saline (0.5, 1.0, 1.5 and 2.0 ml). 1) Administration of 2 doses of histamine produced a dose-dependent increase in acid secretion in young mice, whereas a higher dose of histamine could not produce a significant increase in acid secretion in old mice. 2) Stimulatory effect of carbachol on gastric acid secretion did not differ between young and old mice, although the mean values tended to be lower in old animals than in the young. 3) The response to mechanical stimulation tended to be lower in old animals. 4) Protein expression of H^+, K^+ ATPase was significantly lower in old mice than the young. The present study showed that gastric acid secretion was different between young and old mice concerning to various kinds of stimulations. The decrease in the secretory function of the stomach in the old mice is partly associated with a decrease in parietal cells (H^+,K^+ ATPase).

P132 (3P1-074)

Effects of exercises on cardiac and gastric parasympathetic nerve activities in young adults

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Thirteen young male subjects first lied in the supine position on bed with eyes opened for 10 min, followed by 10 min exercise of a cycle ergometer with a load randomly chosen among three different ones inducible heart rates as high as 15, 30, or 60% of the predicted maximal heart rate, and again took a supine position as during the first rest for 40 min. ECG and EGG were measured by using bio-amplifiers during all the experimental period, AD converted at the rate of 1kHz and stored into PC. RR-intervals obtained from ECG were Fourier transformed by every 64, serially from the top to the end of the file with doubling about its 50%. Cardiac sympathetic (CS) as well as parasympathetic nerve activities (CPS) were assumed from the power in high-frequency band (HF), and the relative magnitude of the power in low-frequency band (LF) to HF, respectively. EGG re-sampled by 10 Hz from the row EGG datum was Fourier transformed by every 512, serially from the top to the end, with doubling about its 50%. The power summed in the range between 2.4 and 3.6 cpm was assumed to be gastric parasympathetic nerve activities (GPS). CPS tended to increase only during 10-20 min district after 30% exercise (ex30). GPS was significantly increased during 10-15 min district ($p<0.05$) in the post-ex30 period. Either CPS or GPS after 60% exercise was significantly depressed. These results suggested that moderate exercise could augment parasympathetic nerve activities, particularly in the gastric region.

P133 (3P1-075)

Effect of intracellular Cl^- concentration on acetylcholine-induced response in endothelial cells of the guinea-pig mesenteric artery

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In vascular endothelial cells acetylcholine (ACh) increases $[Ca^{2+}]_i$ which in turn activates charybdotoxin-sensitive IK_{Ca}, apamin-sensitive SK_{Ca} and Cl_{Ca} channels. As a result, ACh induces a membrane hyperpolarization, but the response is sometimes transient and followed by the membrane depolarization. To investigate the possible mechanism for this variation, a sheet of endothelial cells was isolated from a guinea-pig mesenteric artery and perforated whole-cell clamp experiments using amphotericin B were performed. The intracellular Cl^- concentration was modified by using either low-Cl^- (20 mM) or high-Cl^- (150 mM) pipette solutions. In the current clamp mode, application of ACh (0.5 μM) induced a large and sustained hyperpolarization when $[Cl^-]_i$ was low, while the response was small and transient when $[Cl^-]_i$ was high. In the voltage clamp mode in low $[Cl^-]_i$ condition, the reversal potential of ACh-induced current was -54.1 mV and it was changed to -27.8 mV after the K conductance was blocked by charybdotoxin and apamin. On the other hand, the reversal potential was changed from -57.8 mV to -76.0 mV by blocking Cl conductance with DIDS. When $[Cl^-]_i$ was high, the reversal potential was -22.0 mV and it was changed to -0.6 mV after the K channels were blocked. These results indicate that enhanced Ca^{2+}-activated Cl^- current modifies ACh-induced hyperpolarization to be small and transient when the $[Cl^-]_i$ is increased in some pathological conditions.

POSTERS
Muscle physiology

P134 (3P1-076)

Contribution of each amino acid residue to the dual effects of a synthetic peptide of actin binding region of heat shock protein 20 on the contraction of skinned smooth muscles

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Heat shock protein 20 (HSP20) has actin binding capacity and its amino acid sequence of actin binding region (residues 110-121; GFVAREFHRRYR) is highly homogenous to the inhibitory region of skeletal muscle troponin I (residues 104-115). Our previous study showed that, in Triton-X-100 skinned muscle preparations from guinea pig taenia caeci, a synthetic peptide of the actin binding region of HSP20 ($HSP20_p$) had both a suppressing effect on the maximal Ca^{2+} induced force and an enhancing effect on the Ca^{2+} sensitivity for the force (Yoshino et al.,). In the present study, to evaluate the contribution of each amino acid residue of $HSP20_p$ to the dual effects of $HSP20_p$ on the skinned taenia, we compared the effects of 11 $HSP20_p$ analogues, which consisted of single glycine replacement, on the Ca^{2+}-induced contraction. Every residue of $HSP20_p$ was necessary to achieve maximum inhibition of the Ca^{2+}-induced contraction. On the other hand, replacing F111, V112, A113, E115, F116 or R121 for glycine resulted in losing enhancing effects on the Ca^{2+} sensitivity for the force. These results suggest that, in phasic smooth muscles, the pathways through which $HSP20_p$ modulated the maximal Ca^{2+} induced force and Ca^{2+} sensitivity for the force appears to be different.

P135 (3P1-077)

Interaction between P2 antagonists and Ca^{2+}-dependent K^+ channel antagonists in the contraction of the guinea-pig vas deferens

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Contractions evoked by electrical stimulation of 50 pulses with 40 Hz to the guinea-pig vas deferens consist of L- and T-type Ca^{2+} channel-mediated adrenergic (α_1) component and L-type Ca^{2+} channel-mediated purinergic component. SK and BK channels closely located L- and T-type Ca^{2+} channels greatly affect on the contraction, especially α_1 adrenergic component. The α_1 component consisting of the early and late components was obtained by the treatment of P2 antagonist. P2 antagonist suramin (300 μM) not only antagonizes P2x purinoceptors but also activates SK channels via P2Y2 purinoceptors (Ming et al). PPADS (30 μM)-treated vas deferens revealed large early α_1 component suggesting that PPADS itself might inactivate SK channels. α,β-Methylene ATP (desensitization of P2x purinoceptors, 10 μM) did not affect directly K^+ channels. SK channel antagonist apamin (100 nM) amplified both early- and late-α_1 components. BK channel antagonist iberiotoxin (100 nM) amplified the late-α_1 component without affecting on the early-α_1 component. On the contrary, another BK channel antagonist CTX (100 nM) amplified not only the late-α_1 component but also the early-α_1 component. CTX seemed to deprive the action of PPADS to antagonize P2x purinoceptors. The results suggest that α,β-methylene ATP was suitable for antagonizing P2x purinoceptors, and that apamin and iberiotoxin were more suitable for antagonizing SK and BK channels, respectively.

P136 (3P1-078)

Effects of hypoxia on intracellular mechanisms of smooth muscle cells isolated from the guinea-pig stomach

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Gastric smooth muscle cells generate slow waves spontaneously, and their activities may be originated from interstitial cells of Cajal. Experiments were carried out to investigate the effects of hypoxia on the activities of smooth muscle cells isolated from the guinea-pig stomach antrum by recording intracellular membrane potentials using conventional microelectrode methods, and also by measuring intracellular Ca^{2+}concentration ($[Ca^{2+}]i$) with fura-2 fluorescence. Hypoxic condition was produced by bubbling Krebs solution with N_2-gass containing 5% CO_2. Hypoxia increased the frequency of slow waves with no significant alteration to the membrane potential, and these were associated with the increase in frequency and decreased in amplitude of Ca^{2+}-transients. In the presence of 10 μM cyclopiazonic acid (CPA, an inhibitor of Ca^{2+}-ATPase at internal Ca stores), the increase in frequency of slow waves in response to hypoxia was abolished. In the presence of 1 mM KCN (a blocker of oxidative phosphorylation), the frequency of slow waves was decreased, with associated reduction of the amplitude. In the presence of KCN, hypoxia again increased the frequency of slow waves. These results suggest that hypoxia-induced increase in the frequency of slow waves is associated with the elevated release of Ca^{2+} from internal CPA-sensitive stores, possibly in the pacemaker cells. As the hypoxia-induced increase in the frequency is not altered by KCN, no significant contribution of mitochondrial factor in pacemaker cells is considered.

P137 (3P1-079)

Contribution of each amino acid residue to the force suppressing effect of cardiac troponin I inhibitory peptide on the contraction of skinned smooth muscles

Watanabe, Masaru; Yoshino, Yasumasa; Saifuding, Musha (*Tokyo Med. University, Tokyo, Japan*)

TnIp, a synthetic peptide originating from an actin tropomyosin binding region of rabbit cardiac troponin I [residues 136-147; GKFKRPTLRRVR], had biphasic effects on the relaxation of skinned smooth muscle, as accelerating the initial phase and slowing the following latter phase of the relaxation, resulted from accelerating fast cross-bridge dissociation and also transformation from fast to slow (latch) cross-bridges (Watanabe et al., 2004). To evaluate the contribution of each amino acid residue of TnIp to its biphasic effects on the relaxation, we compared the effects of 11 TnIp analogues, which consisted of single glycine replacement, on the relaxation time course by lowering Ca^{2+} concentration in contracting skinned taenia caeci from guinea pig. Replacing K_{137}, F_{138}, K_{139}, R_{140}, T_{142}, L_{143}, R_{144}, or R_{145} for glycine resulted in loss of the accelerating effect of TnIp on the fast cross-bridge dissociation, seeming that the entire residues of TnIp might be necessary for acceleration of the cross-bridge dissociation by TnIp. On the other hand, the enhancing effect of TnIp on the translation from fast cross-bridges to latch bridges was kept except only when R_{147} was substituted for glycine. R_{147} seems to be a key residue for regulation of latch bridge formation by TnIp.

P138 (3P1-080)

Functional overloading facilitates the regeneration of injured skeletal muscles

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Muscle satellite cells have been considered to play an important role in postnatal growth and the regeneration of skeletal muscle. Recently it has been suggested that the activation of muscle satellite cells is also associated with muscle hypertrophy. Therefore, the regenerative process of injured muscles may be facilitated by the activation of muscle satellite cells induced by various hypertrophic stimuli. The purpose of this study was to investigate the effects of overloading on the regenerative process of injured skeletal muscle in mice. Male mice (C57BL/6J) were divided randomly into four groups: (1) cage control (CC), (2) cardiotoxin-injected (CX), (3) functional-overloaded (FO) and (4) CX+FO groups. In the groups of FO with and without CX, overloading on soleus of both hindlimbs was performed by cutting the distal tendons of both plantaris and gastrocnemius muscles. Two weeks after the surgery, cardiotoxin was injected into soleus muscles of both limbs in CX and CX+FO groups. Soleus muscles were dissected 14, 28 and 35 days after cardiotoxin-injection. Responses of muscular protein contents and Pax7-positive muscle satellite cells during the regeneration were analyzed. Evidences suggest that functional overloading may facilitate the regeneration of injured skeletal muscles.

P139 (3P1-081)

Characteristics of stem cells derived from interstitial spaces of skeletal muscle: Behavior and differentiation potential in culture

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Recently, we have identified multipotent stem cell populations residing in the interstitial spaces of skeletal muscle. We characterized these cells using FACS on the basis of cell surface antigen expression, and sorted them as a CD34+/45- and CD34-/45- cell fractions from enzymatically isolated cells. Cells in the CD34+/45- fraction (designated Sk-34 cells) were >94% positive for Sca-1 and mostly negative (<3% positive) for CD14, 31, 49, 144, c-kit and FLK-1showing that Sk-34 cells are not committed endothelial progenitors. However, Sk-34 cells formed colonies in clonal cell culture, and CFU displayed the potential to differentiate into adipocytes, endothelial, and myogenic cells. We also identified cells in the CD34-/45- (designated Sk-DN cells) fraction as a putative cell population that includes further immature stem cells; that can form clonal sphere-like colonies in a collagen based cell culture with bFGF and EGF; and that exhibits the potential to differentiate into myogenic and endothelial cells. In addition, the Sk-34 and Sk-DN cells included only a few side-population (SP) cells and mostly composed by main-population cells (1:1000). These findings demonstrated that multipotent stem cells (Sk-34 and Sk-DN cells) residing in the interstitial spaces of skeletal muscle, and potentially contribute to postnatal myogenesis and vasculogenesis following muscle growth and/or muscle hypertrophy, and there were not SP cells.

P140 (3P1-082)

Characteristics of stem cells derived from interstitial spaces of skeletal muscle: Effect of transplantation to the sever muscle damage model and differentiation potential in vivo

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We have shown that stem cell populations residing in the interstitial spaces of skeletal muscle can give rise to myogenic-endothelial cell lineages, and were designated Sk-34 (CD34+/45-) and Sk-DN (CD34-/45-) cells. Potential therapeutic use of these cells, such as the functional significance of the transplanted tissue, and vasculogenesis, myogenesis was investigated in detail. For this purpose, we developed a severe-damage model of mouse tibialis anterior muscle with a large deficit of nerve fibers, muscle fibers, and blood vessels. Freshly isolated and cultured Sk-34 and Sk-DN cells were transplanted directly into damaged portion of the muscle. Results showed that, after transplantation, implanted cells give rise to myogenic, vascular (pericytes, vascular smooth muscle and endothelial cells), and neural (Schwann cells) cells, as well as contributed to the synchronized reconstitution of blood vessels, muscle fibers, and peripheral nerves, with significant recovery of both mass and contractile function after transplantation. Intrinsic plasticity of these cells was also revealed by fluorescence in situ hybridization (FISH) analysis for the transplanted muscle detecting the Y chromosome. As well, there were no donor-derived Sk-34 and Sk-DN cells in the muscle of lethally irradiated bone marrow-transplanted animals, indicating that the Sk-34 cells were not derived from bone marrow.

P141 (3P1-083)

Characteristics of stem cells derived from interstitial spaces of skeletal muscle: Differentiation potential after transplantation to non-muscle tissues

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We have shown that multipotent stem cell populations residing in the interstitial spaces of skeletal muscle (Sk-34 and Sk-DN cells) can give rise to myogenic, vascular (pericytes, vascular smooth muscle and endothelial cells), and neural (Schwann cells) cells, as well as contributed to the synchronized reconstitution of blood vessels, muscle fibers, and peripheral nerves, with significant recovery of both mass and contractile function after transplantation. Intrinsic plasticity of these cells when there were transplanted into non-skeletal muscle tissue environment was investigated. The renal capsule, spinal cord and heart were selected as non-skeletal muscle tissues. In the renal capsule, these cells could also give rise to muscle fibers, nerve fibers, and blood vessels as well as in the skeletal muscle tissue. In the spinal cord, however, MAP-2 positive neural cells and blood vessels were observed. Interestingly, in the cardiac muscle tissue milieu, these skeletal muscle derived stem cells could differentiate into mono-nucleated cardiac muscle cells with an apparent desmosome junctions between donor-recipient cells, and/or donor-donor cells. Donor cell derived blood vessels were also observed. These results suggest that Sk-34 and Sk-DN cells can give rise to myogenic, neural and vascular cell lineage intrinsically, and these capacities can be affected by the different tissue milieus.

P142 (3P1-084)

Improvement of therapeutic neovascularization using skeletal muscle-derived stem cells

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We have found multipotent stem cell populations residing in the interstitial spaces of skeletal muscle (designated Sk-34 and Sk-DN cells) can give rise to myogenic, vascular (pericytes, vascular smooth muscle and endothelial cells), and neural (Schwann cells) cells, as well as contributed to the synchronized reconstitution of blood vessels, muscle fibers, and peripheral nerves. To expecting vasculogenic capacity of these cells, we investigated the hypothesis that Sk-34 (CD34+/45-) cells may play an important role for the ischemic tissues as a "tissue specific vasculogenic cells" and may be contribute to vasculogenesis. For this purpose, the Sk-34 cells, obtained from the muscles of 3-6 week-old GFP mice, were administrated intramuscularly into the nude mice with hindlimb ischemic models (n=4 each), and same amount of physiological saline was administrated for control group. Two weeks after transplantation, the Sk-34 cells transplanted group demonstrated significantly less toe necrosis ($p<0.05$), and enhanced recovery of peripheral perfusion measured by Laser Doppler ($p<0.05$) compared to control group. Moreover, increase in donor derived (GFP positive) CD31 positive cells and/or vessels can be seen in treated animals compared to control by immunohistochemical analysis. These findings indicate that this cell population represents an accessible cell source that can be used therapeutically to improve postnatal neovascularization.

P143 (3P1-085)

Roles of slow-fibers in recovery of skeletal muscles from crush injury

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Skeletal muscle crush injuries occur in an incident when the limb is trapped with a compressing force. Little is known about the recovering mechanisms of injured muscles. To study these, we developed a new apparatus. For induction of crush injury, a pressure of 4.25 kg/cm^2 was applied for 2 h on the left lower limb of the rat. We examined changes in their histological and physiological properties. We report that slow fibers play important roles in recovery of skeletal muscles from such injury. A follow-up survey was done for 8 weeks on 12 species of hind limb muscles; their wet-weight, number of fibers, diameter and area of fibers were measured. Slow fibers were identified by immunohistological staining. Muscle function was analyzed from footprint. Soon after the crush, wet-weight of muscles increased by 10-20% in 1-2 days (acute edematous phase), decreased by 30% during 2 days to 2 weeks (atrophic phase) and then increased gradually for 8 weeks (recovery phase). Diameter and area of muscle fibers, which reduced in the first 2 weeks, started to increase gradually in the recovery phase. At 8 weeks, muscle weight exceeded 100% of the control (opposite-side muscles) although their fibers were thinner than the control. Immunohistology revealed that slow fibers showed a large increment in number in the recovery phase; it indicates that they play a major role in recovery from the injury.

P144 (3P1-086)

The Effect of structural change on single soleus fiber after hindlimb immobilization in rats

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We investigated the effects of hindlimb immobilization (HI) on contractile properties of single skinned soleus fibers in rats. HI (6 weeks) resulted in reduced wet weight of soleus muscle (~40%). In immobilized fibers, maximal Ca^{2+}-actiavated force was reduced by ~40% and the force-pCa curve was shifted to the lower pCa side by ~0.15 pCa units (sarcomere length, 2.20 μm). Our EM observation revealed thinner myofbrils in immobilized muscle, with the width of Z-line similar in control and immobilized muscles during relaxation and contraction (pCa 4.5). We reconstituted thin filaments of control and immobilized fibers with the identical troponin complex (from rabbit psoas muscle). It was found that Ca^{2+} sensitivity was still lower in immobilized fibers by ~0.15 pCa units after troponin reconstitution, suggesting that troponin isoform switches, if at all, can not account for functional changes of immobilized fibers. We then induced Ca^{2+}-independent active force by lowering the MgATP concentration (= -log [MgATP], from 5 to 7) to investigate whether or not the decreases in Ca^{2+}-activated force are the result of reduced cross-bridge formation. We found that in immobilized fibers, maximal Ca^{2+}-independent active force was reduced by ~40%, with a leftward shift of the force-pMgATP curve. These results suggest that cross-bridge recruitment is suppressed in immobilized muscle, via e.g., structural changes of the sarcomere, resulting in reduced active force production.

P145 (3P1-087)

Sex difference and influence of menstrual cycle on the muscle pressure pain threshold and delayed onset muscle soreness

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Many reports have shown that there are sex differences in pain sensitivity, and that hormonal condition also modulates pain sensitivity. Because muscle pain sensitivity was not studied yet in Japanese, we examined sex differences and differences in different phases of menstrual cycle in muscle pain sensitivity of young Japanese. Healthy subjects (10 males, 10 females in follicular phase, and 10 females in luteal phase) were recruited from students of Aichi Gakusen University and provided informed consent. The age ranged between 18 and 22 years old. Experimental muscle pain was induced in the biceps brachii muscle by eccentric exercise (ECC). A weight was placed around the wrist of undominant arm, and the subjects were asked to flex and extend the arm. Pressure pain threshold of the biceps brachii muscle (PPT), elbow joint angles that the subjects could flex or extend without pain, blood pressure, hemoglobin and skin fold thickness were measured. The females showed a significantly lower basal PPT, maximal blood pressure, and blood hemoglobin content, and their skin fold was thicker than the males. The PPT and blood hemoglobin in luteal phase were significantly lower than the follicular phase. PPT decreased 1 day after ECC and returned to the pre-exercise value 4 days after ECC. This time course in the development of delayed onset of muscle soreness was not different among groups. Present data showed clear sex and menstrual cycle differences in muscle pain threshold.

P146 (3P1-088)

Passive tension of cardiac and skeletal muscle with a reference to the domain structure of connectin.

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Titin/connectin is a giant spring-like protein that is responsible for passive tension generation of striated muscle. We observed the passive tension of the bundles of myofibrils from rabbit cardiac and psoas muscle, and that from chicken breast muscle at various sarcomere lengths (SL) to compared it with the domain structures of connectin predicted from the cloned cDNAs. The bundles of the myofibrils of rabbit cardiac muscle were prepared mechanically from the homogenized muscle tissue in the relaxing solution. The specimen was held by a pair of sharp glass capillaries, and tension was estimated from their bending. SLs were observed microscopically. The passive tension of rabbit psoas and chicken breast muscle was measured with tension transducers. Moderate extension of the specimens developed a small passive tension that rapidly decayed to a steady level. Significant tension development began with a stretch beyond particular SL characteristic for each muscle type. That is, rabbit cardiac myofibrils began to develop tension at the shortest SL (around 2.0 μm), and chicken breast and rabbit psoas muscle began to develop tension at longer SLs (at around 2.3 and above 2.6 μm, respectively). On the other hand, the predicted length of PEVK region of connectin was human cardiac < chicken breast < human psoas < human soleus muscle. Assuming the connectin of rabbit to be similar to human, these data strongly suggests that the length of PEVK region correlates closely with the passive tension development at short SL.

P147 (3P1-089)

Molecular dynamics study on mutant troponin related to cardiomyopathy

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A mutant troponin T of which glutamate is replaced with aspartate (Glu244Asp) is one of the causes of familial hypertrophic cardiomyopathy (HCM). Incorporation of this mutant to skinned fibers has been reported to increase calcium sensitivity as well as maximal tension (Nakaura et al. 1999). However, mechanism has not been elucidated. Therefore, we constructed a model structure of this mutant troponin by introducing the mutation to the crystal structure of human cardiac troponin (TIC complex) obtained from Protein Data Bank (ID number 1J1E). Molecular dynamics simulation of the wild and the mutant structure was carried out in water at 310 K to estimate a dynamic structure of the mutant troponin and search a possible mechanism for the enhanced tension development. Dynamics was calculated by the use of software Amber ver.7. Iteration was done in TIP3 water with 0.5 or 1 fs time step in periodic condition at constant temperature (310 K). It was found that the electrostatic interaction between T244 and I111 which linked troponin T and I in the wild was lost in the mutant. Furthermore, when a terminal residue of troponin I was pulled toward an actin molecule, troponin T seemed to be easier to follow troponin I in the mutant than that in the wild. This may be involved in the enhanced tension development in the mutant myofilament.

P148 (3P1-090)

Crossbridge dynamics during length clamp after preload shortening in rat papillary muscle

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The intensity ratio of (1,0)/(1,1) equatorial x-ray diffraction indicates the mass transfer from myosin filament to actin filament. This ratio decreases during isometric contraction. During a very low load shortening, the amount of changes in this ratio reduced. To examine this reduced ratio carefully, the length clamp method was introduced in rat papillary muscle. When the shortening was stopped at any moment during a preload shortening, the isometric force was developed in accordance with the residual activity of crossbridges (length clamp). During the length clamp, both the shortening length and the force were located between those of preload and isometric contractions. Similarly the intensity ratio was also located between those of preload and isometric contractions. After the clamp of length, the time course of intensity ratio quickly separated from that of preload contraction. If the number of crossbridges actually reduced during the preload shortening, the time course of intensity ratio during length clamp was expected to follow that of preload shortening because of the latency in crossbridge formation. These results suggest that the number of crossbridges will not decrease during the preload shortening, and that not so small number of cross-bridges that can contribute the force development are left even under the low load shortening.

P149 (3P1-091)

Effect of troponin exchange on length-dependent activation in porcine ventricular muscle

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At the basis of the Frank-Starling mechanism of the heart is the intrinsic ability of the contractile system to produce active force in response to stretch. It has been reported that length-dependent activation is, at least in part, modulated via interfilament lattice spacing reduction due to titin-based passive force (e.g., Fukuda *et al*., *J. Physiol*. **553**, 147-154, 2003). In the current study, we examined whether or not length-dependent activation is modulated at the thin filament level. We used skinned porcine ventricular muscle that had been treated with 1% (w/v) Triton X-100. An increase in sarcomere length (SL) from 1.9 to 2.3 μm exerted a marked increase in Ca^{2+} sensitivity with a concomitant increase in passive force. The SL-dependent increase in Ca^{2+} sensitivity was markedly attenuated by perfusing preparations with rigor solution containing exogenous fast skeletal troponin (T-I-C complex; rabbit psoas muscle), with little or no effects on passive force. The magnitude of SL dependency was similar to what was observed in rabbit psoas muscle. Our SDS-PAGE analyses showed that endogenous cardiac troponin subunits were replaced with skeletal counterparts by ~100% and that titin was not degraded upon troponin exchange. These results suggest that, presumably downstream of titin-based lattice spacing reduction, length-dependent activation is modulated at the thin filament level.

P150 (3P1-092)

Plasmalemmal estrogen receptor (ER) regulates ERα expression via PKC/MAPK pathway

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We investigated cellular signaling cascades linked to estrogen-induced ER synthes is in mouse C2C12 myoblasts. By immunoblotting, ER was detected in the post-nuclear fraction with a corresponding molecular weight of 66 kDa. The amount of ER protein was dose-dependently (10^{-12}-10^{-5} M increased after treatment with 17β-estradiol for 24 hours. 17α-estradiol (10^{-8}M), the stereoisomer of 17β-estradiol, and a BSA-17β-estradiol-conjugate which is incapable of penetrating the plasma membrane, mimicked the increasing effects on ER. The level of ER expression was reduced by an ERK1/2 inhibitor, PD 98059(10μM), or a specific p38 inhibitor, SB 203580 (10μM) regardless of the presence of estradiol. Using ^{35}S-methionine for immunoprecipitation, newly-synthesized ER was increased by 17β-estradiol. Novo-synthesis of ER was further increased by the protein kinase C (PKC) activator, TPA (1μM). These results suggest that ERs located at the plasma membrane of mouse skeletal myoblast cell are a target of estrogen's actions. The estrogen-stimulated ER synthesis involves the PKC/MAPK signaling system, thereby presumably regulating the proliferation process. However, somewhat contra-intuitively, up-regulation of estrogen receptor was also observed in the mouse skeletal muscle treated with antiestrogens, such as tamoxifen and ICI182780.

P151 (3P1-093)

Regulation of gastrointestinal pacemaker activity via type 3 serotonin receptors

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We assessed the involvement of serotonin receptors (5-HT-R) on pacemaker Ca^{2+} activity in gastrointestinal (GI). Small tissues of 100-300 mm diameter (cell cluster preparations) were prepared from the stomach and small intestine muscle (including the enteric neurones) of mice by enzymatic and mechanical treatments. After 2 to 4 days of culture, the fluo-3 AM (acetoxymethly ester of Fluo-3) was loaded to measure the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). In the presence of nifedipine, we measured ($[Ca^{2+}]_i$) oscillations in c-Kit-immunopositive pacemaker cells (= interstitial cells of Cajal: ICCs) and examined the effects of several drugs: LY-278584, 2-Methylserotonin maleatesalt, GR113808, Fluoxeline, SK&F96365, etc. RT-PCR and immunohistochemical examinations were carried out to characterize expression of serotonin receptor subtype in pacemaker cells. We conclude that endogenous 5-HT plays a crucial role in generating and maintaining pacemaker Ca^{2+} activity in ICCs via type 3 serotonin receptors ($5\text{-}HT_3$) under basal conditions, and a potentiates Ca^{2+} responses to electrical stimuli by facilitating cholinergic neurons. Our results may provide a new therapeutic target in diseases associated with abnormal GI motility, and add an important member (i.e. ICCs) to assess the brain-gut (The Second Brain) interaction.

P152 (3P1-094)

Identification and spatio-temporal analysis of the rat pelvic pacemaker region using a macro zoom microscope and voltage-sensitive dye

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As we reported in the last meeting in Sendai, we established *in situ* Ca^{2+} imaging technique of the rat renal pelvis using a macro zoom microscope (Olympus MVX 10). The clear view and smooth zooming operation with this technique enabled us to search upstream of Ca^{2+} transient and to identify the pacemaker region easily. With higher magnification of the region, we could observe that not only one cell but several cells increased their intracellular Ca^{2+} concentration simultaneously. Interestingly their propagating pathway were slightly different every time. Spontaneous Ca^{2+} rises in the other part of renal pelvis rarely occurred; they never propagated to the downstream. Contrary many spontaneous but asynchronous Ca^{2+} rises were observed in the presence of low concentration of heptanol, a gap junction blocker. Using the same technique we could also successfully record image of di-4-ANEPPS, a voltage-sensitive dye. The initial depolarization occurred at the exactly same place as the Ca^{2+} rise. These results might suggest that the smooth muscle cells connected via gap junctions and with synchronous Ca^{2+} rise played an important role in the pacemaker mechanisms.

P153 (3P1-095)

Effects of synthetic peptides orinating from small heat shock proteins on the skinned carotid artery from guinea pig

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Heat shock protein 20 (HSP20) has actin binding capacity and its amino acid sequence of actin binding region (residue 110-121; GFVAREFHRRYR) is highly homogenous to the inhibitory region of skeletal muscle troponin I (residue 104-115; TnIp). Previous study by Rembold et al (2000) showed that, in carotid artery, HSP20 monomer might directly interfere with actin-myosin resulting in the smooth muscle relaxation. In phasic skinned taenia caeci, however, a synthetic peptide of $HSP20_{110\text{-}121}$ enhanced Ca^{2+}-induced force development except under the condition of high concentrations of Ca^{2+}, so increased in the Ca^{2+}-sensitivity for the force (Yoshino et al., 2003). To determine whether HSP20p also enhance Ca^{2+}-induced contraction in tonic skinned carotid artery as well as in taenia, we studied the effects of HSP20p on contractile properties of beta escin skinned muscle preparations from guinea pig carotid artery. In skinned carotid artery, HSP20p suppressed Ca^{2+}-induced contraction with little effects on the Ca^{2+}-sensitivity for the force. Also a synthetic peptide originating from HSP27 actin binding region (residues 131-142; GYISRCFTRKYT; HSP27p) suppressed Ca^{2+}-induced contraction. These results suggest that, different from the case of phasic smooth muscles, HSP20p might mainly affect thin filament and interfere with actin-myosin interaction causing muscle relaxation in tonic carotid artery.

POSTERS
Ionic channels & receptors

P154 (1P1-048)

Effect of 8-bromo-cAMP on the tetrodotoxin-resistant sodium (Nav 1.8) current in rat small-diameter nodose ganglion neurons

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We examined whether 8-bromo-cAMP (8-Br-cAMP)-induced modification of tetrodotoxin-resistant (TTX-R) sodium current (I_{Na}) in neonatal rat nodose ganglion neurons is mediated by the activation of protein kinase A (PKA) and/or protein kinase C (PKC). In 8-Br-cAMP applications ranging from 0.001 to 1.0 mM, 8-Br-cAMP at 0.1 mM showed a maximal increase in the peak TTX-R Na^+ (Nav1.8) current and produced a hyperpolarizing shift in the conductance-voltage (G-V) curve. The PKC inhibitor bisindolylmaleimide Ro-31-8425 (Ro-31-8425, 0.5 μM) decreased the peak Nav 1.8 current. The Ro-31-8425-induced modulation of the $G_{V1/2}$ baseline (a percent change in G at baseline $V_{1/2}$) was not affected by additional 8-Br-cAMP application (0.1 mM). The application of a PKC activator, phorbol 12-myristate 13-acetate (PMA, 0.1 μM), increased the Nav 1.8 current, and this increase was not significantly affected by additional 0.1 mM 8-Br-cAMP application. Intracellular application of a PKA inhibitor, protein kinase inhibitor (PKI, 0.1 mM), greatly attenuated the 8-Br-cAMP-induced increase in the peak Nav 1.8 current but caused a significant increase in the slope factor of both activation and inactivation curves. The PKI application lowered the G max to below the control level. These results suggest that the 8-Br-cAMP-induced increase in Nav 1.8 currents may be mediated by activation of PKC.

P155 (1P1-049)

Differences in the sensitivity of the TTX-resistant Na^+ channel to the PKCβ inhibitor LY333531 in small dorsal root ganglion neurons of control and diabetic rats

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Experimental evidence has been presented to suggest that the protein kinase Cβ inhibitor LY333531 is effective at alleviating diabetic hyperalgesia to some extent. The present study was designed to examine the acute action of LY333531 (0.01-1μM) on the tetrodotoxin (TTX)-resistant Na^+ current (I_{Na}) in small (<25 mm in soma diameter) dorsal root ganglion (DRG) neurons in control and streptozocin (STZ)-induced diabetic rats, using the whole-cell patch-clamp method. The cell membrane was initially hyperpolarized from a holding potential of -70 mV to -120 mV for 20 ms and then depolarized to various test potentials ranging from -50 to +50 mV, in the presence of TTX (0.1 mM) and the appropriate blockers for Ca^{2+} and K^+ currents. I_{Na} was measured as a transient inward current during depolarizing steps. The maximal density of I_{Na} was significantly increased in diabetic rats compared with control (50.5 $pApF^{-1}$ vs 32.3 $^{-1}$). I_{Na} recorded from diabetic rats was found to exhibit a significantly higher sensitivity to inhibition by LY333531 compared with control (IC_{50}, 6 nM vs 30 nM). Thus, our results provide experimental evidence to show that the sensitivity of I_{Na} to LY333531 is substantially enhanced by diabetic state, which suggests that I_{Na} is considerably inhibited by LY333531 at clinically relevant concentrations of <100 nM in diabetic state.

P156 (1P1-050)

Prostaglandins have no detectable effect on the tetrodotoxin-resistant sodium currents mediated by Nav1.8 and Nav1.9 in small neurons from mouse dorsal root ganglia

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One possible mechanism that may contribute to the inflammatory hyperalgesia, an inflammation-induced sensitization of primary afferent neurons, is the modulation of tetrodotoxin-resistant (TTX-R) voltage-gated Na currents by inflammatory mediators such as prostaglandins (PGs). Evidence for this compelling idea is mainly based on the electrophysiological finding that the TTX-R Na current, which is expressed preferentially in small primary afferent neurons and likely to be involved in nociception, is augmented by inflammatory mediators. However, the detailed mechanism underlying this observation has not been fully investigated. We re-investigated the effect of PGE2 (and also PGI2) on the heterogeneous TTX-R Na currents mediated by Nav1.8 and Nav1.9 in mouse dorsal root ganglion neurons, using conventional and nystatin-perforated whole-cell patch-clamp recordings on wild-type and Nav1.8-null mutant DRG neurons. Unexpectedly, PGE2 (and also PGI2) had no detectable effect on these TTX-R Na currents, raising a question regarding the well-known modulatory role of PGs on TTX-R Na currents in inflammatory hyperalgesia.

P157 (1P1-051)

CaMKII phosphorylates the C-terminal tail of Cav1.2 Ca^{2+} channel and modulates interaction of the channel with calmodulin

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Cav1.2 Ca^{2+} channel is suggested to be modulated by calmodulin (CaM) and Ca^{2+}/CaM-dependent protein kinase II (CaMKII), that are proposed to underlie Ca^{2+}-dependent inactivation and facilitation of channel activity. To explore phosphorylation sites for CaMKII, three GST-fusion fragment peptides derived from the C-terminal tail of guinea-pig Cav1.2, CT-1 (amino acids number 1509-1791), CT-2 (1777-2003) and CT-3 (1944-2169) were examined in vitro. Only CT-1 was consistently phosphorylated by CaMKII. By using mutated CT-1, the phosphorylation site was suggested to be threonine residue at position 1603. In pull-down assay, CT-1 but not CT-2 nor CT-3 was found to interact with CaM at both low and high [Ca^{2+}] conditions. CT-1 treated with CaMKII showed a higher affinity for CaM than that treated with alkaline phosphatase. These results suggest that interaction between CT-1 and CaM is modulated by phosphorylation mediated by CaMKII, and that they are consistent with the hypothesis that both CaM and CaMKII are involved in maintaining basal activity of the channel and in Ca^{2+}-dependent inactivation and facilitation of the channel.

P158 (1P1-052)

Calcium- and dose-dependent effects of calmodulin on activity of Cav1.2 Ca^{2+} channels in guinea-pig ventricular myocytes

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We have previously reported that calmodulin (CaM) + ATP can reverse rundown of Cav1.2 L-type Ca^{2+} channels observed in the inside-out patch mode. In this study we have examined Ca^{2+}- and dose-dependent effects of CaM on activity of the Ca^{2+} channels in guinea-pig ventricular myocytes. Application of CaM (0.1-14 μM) + ATP (3 mM) to the intracellular side of the channels within 1 min after patch excision resulted in a bell-shaped dose-dependent effect with a maximum effect at 2-4 μM CaM at low [Ca^{2+}] condition (Ca^{2+}-free to 80 nM). This relation between CaM and channel activity may be related to Ca^{2+}-dependent facilitation and inactivation of the channel. This bell-shaped curve for the dose-dependent effect of CaM on channel activity was significantly shifted toward left (lower concentration) by increasing [Ca^{2+}]. These results suggest that CaM plays a crucial role in regulation of the Cav1.2 Ca^{2+} channels, and that not only [Ca^{2+}] but also CaM concentration are important factors for functional modulation of the Ca^{2+} channels.

P159 (1P1-053)

Simulation study of the current through the L-type Ca^{2+} channels

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L-type Ca^{2+} channel currents have the voltage-dependent inactivation (VDI) and Ca^{2+} dependent inactivation (CDI) processes. These inactivation processes regulate the the amount of Ca^{2+} influx into cardiac myocytes. Under the β-adrenergic stimulation, the CDI dominate the decay of Ca^{2+} current since the VDI is slow (I. Findlay, J. Physiol., 2002). In this study, we have modeled the VDI and CDI under the control and the β-adrenergic stimulation (100nM isoproterenol). We have developed the VDI model by using the experimental time course of VDI estimated from the outward-going K^+ current through the Ca^{2+} channel in the absence of extracellular Ca^{2+}. The current dacay calculated by using the pure VDI was close to the experimental Ba^{2+} current decay which possessed minimal CDI. After that, we modeled the CDI satisfying the experimental current. The inactivation rate was expressed as a function of the Ca^{2+} current. In this formula, two components CDI was adopted to reproduce the strong bi-phasic decay for the β-adrenergic stimulation. The Ca^{2+} current dependent K^+ current through the Ca^{2+} channel are introduced into this model to reproduce the experimental reversal potential. The calculated time course of the inward Ca^{2+} current was in excellent agreement with the experimental results. Therefore we have successfully established the inactivation model of the L-type Ca^{2+} channel in consistent with the experimental results.

P160 (1P1-054)

Store-operated Ca^{2+} entry activated by hyperpolarization in rat submandibular acinar cells

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Store-operated Ca^{2+} entry activated by hyperpolarization in rat submandibular acinar cellsYoshida, H. and Nakahari, T.Department of Physiology, Osaka Medical College, 2-7 Daigaku-cho, Takatsuki 569-8686, JapanIn rat submandibular cells, ACh evoked a biphasic increase in $[Ca^{2+}]_i$, that is, an initial transient phase followed by a sustained phase. The initial transient phase is induced by a Ca^{2+} release from the intracellular stores and the sustained phase is maintained by a Ca^{2+} influx from the extracellular fluid, which is the so-called "store-operated Ca^{2+} entry". Store-operated Ca^{2+} entry were stimulated using 4 μM thapsigargin, sarco(end)plasmic reticulum Ca^{2+} ATPase inhibitor, in Ca^{2+}-free solution followed by superfusion with control (Ca^{2+}-containing) solution. This protocol resulted in a $[Ca^{2+}]_i$ increase, which was inhibited in the presence of 1 μM Gd^{3+}. A restoration from 150 mM K^+ solution(Ca^{2+}-containing) to control solution also evoked an $[Ca^{2+}]_i$ increase during thapsigargin stimulation, and an $[Ca^{2+}]_i$ increase following reintroduction of Ca^{2+} during thapsigargin stimulation was completely inhibited by addition of 1 μM Gd^{3+}. Although a restoration from 7.5 mM K^+ solution to control solution did not evoke an $[Ca^{2+}]_i$ increase during thapsigargin stimulation, addition of tetraethyl ammonium into 7.5 mM K^+ solution evoked an $[Ca^{2+}]_i$ increase. Consequently, Ca^{2+} entry pathways is activated by the store-depletion and hyperpolarization.

P161 (1P1-055)

BK channels in Ca^{2+} store control Ca^{2+} release

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Ca^{2+} release from intracellular Ca^{2+} stores terminates after a rapid release of a fraction of releasable Ca^{2+} (called "quantal" release). To explain the quantal nature, it has been hypothesized that a decrease in luminal Ca^{2+} attenuates Ca^{2+} efflux. However, the mechanism remains an enigma. We show that voltage- and Ca^{2+}-activated potassium channels in Ca^{2+} store control Ca^{2+} release. The potassium channel was identified as BK-type by patch-clamp recordings from an enlarged nuclear envelope in the nucleus-attached mode and by immunolabeling. The store BK channel was activated by positive shifts in luminal potential and luminal Ca^{2+} increases. The closing or blockage of store BK channels developed lumen-negative potentials and suppressed Ca^{2+} release. Ca^{2+} uptake by store Ca^{2+} pumps would reactivate the store BK channels regeneratively with K^+ entry to allow repetitive Ca^{2+} release. Indeed, the luminal potential oscillated bistably ~45 mV in amplitude as revealed with an organelle-specific voltage-sensitive dye [$DiOC_5(3)$]. Our study suggests that Ca^{2+} release-induced closings of store BK channels cause a lumen-negative potential towards the equilibrium potential for Ca^{2+} to attenuate Ca^{2+} efflux.

P162 (1P1-056)

Facilitation of HERG channel can be induced by various HERG blockers

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Blockers of cardiac I_{kr} channels encoded by HERG gene is reported as the drug clinically effective for lethal ventricular arrhythmias. However the kinetic property of a HERG channel by these blockers is not yet studied in detail. We attempted to find the characteristics.

By using the standard two-microelectrode voltage clamp technique on HERG channels expressed in Xenopus oocytes, HERG blocker (nifekalant, quinidine, carvedilol, E-4031, and dofetilide) blocked HERG channels in a use-dependent manner . However, some characters were found under the block effect. Nifekalant, quinidine or carvedilol increased HERG channel current at low voltages only in the presence of a previous strong depolarizing pulse, and so this pulse could separate the facilitation effect from the block effect. On the other hand, with the facilitation effect, they caused a significant negative shift in the voltage-dependence of activation. In the case of E-4031 etc, the facilitation effect was not found. Moreover, some mutant HERG channel blocked by quinidine induced stabilization of the closed state of channel. These results reveal that each HERG blocker has a different mechanism of block effect, and has not only block effect but also other effects.

P163 (1P1-057)

Analyses of heteromultimeric assembly of Kir2.1 and Kir3.4 inward rectifier K^+ channel subunits

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Kir2 subfamily members of inward rectifier K^+ channel are known to co-assemble to form heteromultimers, and it is also known for Kir3. Here we examined whether Kir2.1 (IRK1) and Kir3.4 (GIRK4) belonging to different subfamilies can assemble each other or not. First, we examined the association by co-immunoprecipitation experiments using FLAG or myc tagged constructs co-transfected in HEK 293 cells. We observed "GIRK4-FLAG and IRK1-myc" as well as "IRK1-FLAG and GIRK4-myc" co-immunoprecipitated at a comparable level with a positive control pair, IRK1-FLAG/IRK1-myc. This clear co-immunoprecipitation was not observed in a negative control pair, $P2X_2$ receptor-FLAG/GIRK4-myc. As a next step, we anlayzed electrophysiologically using Xenopus oocyte expression system the effect of co-injection of GIRK4 or GIRK4/GIRK1 cRNA on IRK1 current. We could not record a clear emergence of current with unique features which reflects formation of functional heteromultimers, but observed a decrease in the amplitude of IRK1 current, suggesting a suppression effect possibly by hetero-multimerization. Finally, we carried out FRET analysis of IRK1-CFP/GIRK4-YFP pair and GIRK1-CFP/IRK1-YFP pair expressed in CHO cells, and obtained preliminary data supporting association of these two subunits. Taken together, these results suggest that IRK1 and GIRK4 subunits have a capability to form heteromultimers in heterologous expression systems.

P164 (1P1-058)

Analyses of the voltage and ATP-dependent "gating" of ATP receptor channel

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P2X receptors are ligand-gated cation channels activated by extracellular ATP. The $P2X_2$ channel current at the steady-state after ATP application is known to have voltage-dependence, i.e. - it shows inward rectification, and a gradual increase in the inward current is observed upon hyperpolarization. We analyzed this "activation" phase quantitatively under two-electrode voltage clamp using *Xenopus* oocytes expression system, and also approached its structural background by mutating a glycine residue (G344) in the 2nd-transmembrane helix (2nd-TM), a putative kink for the "gating". We observed that the inward current of G344A mutant increased instantaneously upon hyperpolarization without a gradual increase. On the contrary, G344P mutant showed a slower "activation" than that of WT. We also analyzed the conductance-voltage relationship by measuring the tail current, and observed that the half-maximal voltage of "activation" of G344P was shifted to the hyperpolarized potential in comparison with that of WT. The mutation did not affect the ATP dose-response relationships significantly. By the glycine scanning mutageneses on the G344A mutant background, we observed a recovery of the "activation" phase by introducing a glycine residue to the middle region of 2nd-TM. Taken together, we speculate that the flexibility of G344 in the 2nd-TM contributes to the voltage dependent "gating" of $P2X_2$ channel, which could be caused by an intrinsic or extrinsic mechanism to the channel molecule.

P165 (1P1-059)

Two opposing roles of 4AP-sensitive K^+ current in spike-initiation and invasion in mesencephalic trigeminal neurons

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The axon initial segment plays important roles in spike-initiation and invasion of axonal spikes into the soma. Among PSNs, those in the MTN can exceptionally initiate spikes in response to synaptic inputs, consequently displaying two kinds of spikes, one caused by invasion of an axonal spike arising from the sensory receptor and the other initiated by somatic inputs. We addressed where spikes are initiated in MTN neurons and whether there are any differences between initiated and invaded soma spikes (S-spikes). Simultaneous patch-clamp recordings from the soma and axon hillock revealed a spike-backpropagation from the initiation site in the stem axon to the soma in response to somatic current pulse, which brought about the delayed emergence of S-spikes after the offset of the current pulse. These initiated S-spikes were smaller in amplitude than invaded ones generated by stimulation of the stem axon; however, 4AP (<=0.5mM) eliminated the amplitude difference. Furthermore, 4AP markedly shortened the delay in spike-initiation without affecting the latency to spike-invasion, whereas it prolonged the refractory period of invaded S-spikes without affecting that of presumed axonal spikes markedly. These observations suggest that 4AP-sensitive K^+ currents exert two opposing effects on S-spikes depending on their origins; suppression of spike-initiation and facilitation of spike-invasion at higher frequencies.

P166 (1P1-060)

Regulation of the Kir2.1 potassium channel current by intracellular pH

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The Kir2.1 channel currents show a strong inward rectification under the whole-cell recordings because of a voltage-dependent block of the channel by intracellular polyamines and Mg^{2+}. Here we examined the effects of the intracellular pH on the Kir2.1 channel current using a 293T cell expression system. When the inside-out patch membrane was exposed to the intracellular solution of pH 7.2 containing no polyamines and Mg^{2+}, the currents still showed a relatively strong inward rectification, but the closing of the channel in response to depolarizing voltage steps usually required several tens of seconds. Acidification of the intracellular solution from pH 7.2 to 6.8 and 6.4 induced a voltage-dependent decay of the outward currents during depolarizing voltage steps and increased the slow time-dependent component of the inward tail currents on hyperpolarization. Lowering of the intracellular pH from 7.2 to 6.0 and 5.6 also decreased the amplitude of the inward currents. In the presence of 0.1-5 μM spermine or 1-10 μM spermidine, a decrease in pH changed the amplitude of the outward currents with a complex voltage-dependence. Analyses of the inward tail currents suggested that the contribution of the pH-induced gating to the decrease of the outward currents increased as the intracellular pH was decreased.

P167 (1P1-061)

Determination of a critical amino acid residue associated with calmodulin-dependent kinase II (CAMKII)-mediated activation of vascular receptor-operated Ca^{2+} entry channel TRPC6

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The molecular background for CaMKII-mediated regulation of a murine transient receptor potential protein homologue TRPC6 was explored by mutation analysis of CAMKII phosphorylation motifs (RXX(S/T)), which were found on the N-terminal (NT) and transmembrane (TM) (but not C-terminal) regions of wild-type TRPC6 and its chimera T776 with the NT and TM domains of TRPC7. Substitution of the last serine or threonine in these motifs with alanine revealed that, out of eight and five candidate sequences, only the mutations T487A in wild type TRPC6 and T433A in T776 respectively can strongly attenuate Ba^{2+} influx or inward cationic current evoked by muscarinic receptor stimulation with 100μM carbachol. Assuming a smilar membrane topology suggested by a recent structural analysis of TRPC1, T487 in TRPC6 and T433 in T776 may be located on a long intracellular stretch between the second and third TM domains (II-III loop) of each channel. Thus, considering the absolute requirement of a putative calmodulin/IP3 receptor binding site (CIRB) for TRPC6 channel activation, it is conceivable that close spatial arrangements of CIRB and the II-III loop might allow effective phosphorylation of T487 via the actions of CAMKII, thereby increasing the availability of TRPC6 channel for opening in response to subsequent receptor stimulation.

P168 (1P1-062)

Role of TRPV4 in control of body temperature under heat radiation

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TRPV family, identified as thermosensitive, Ca^{2+}-permeable channels, consists of six subtypes. TRPV3 and 4 were described as heat transducers operative at moderately warm temperatures (>34 °C), whereas TRPV1 is activated by temperature above 42 °C. In natural environment, infrared light is detected as thermal radiation through skin. TRPV3 and 4 were reported to express in keratinocytes. They might, therefore, respond to infrared radiation and transfer the thermal signal to CNS. In this study, we examined the role of TRPV4 in regulation of body temperature by using transgenic mice defecting in TRPV4 protein. As thermal stimulus, infrared laser irradiation (λ=830 nm, 150 or 300 mW, 15 min) was applied to the back skin of the mouse, and temperatures of both skin surface and rectum were monitored. In wild type mouse, laser radiation which caused the increase in skin temperature up to 55 °C did not induce the change in body temperature. In TRPV4-knockout mice, however, moderate thermal stimulus, which increased the skin temperature less than 43 °C, resulted in the increase in the body temperature during the laser irradiation suggesting the loss of autonomic temperature regulation. The processing of moderate thermal radiation may partly depend on the TRPV4 expressed in skin cells.

P169 (1P1-063)

Mechanical stress activates TRPM7 channels expressed in HEK293T cells

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Mechanical stress activates TRPM7 channels expressed in HEK293T cells Numata, Tomohiro; Shimizu, Takahiro; Okada, Yasunobu (Dept. Cell Physiol., Natl. Inst. Physiol. Sci., Okazaki, Japan)Stretch-activated cation channels play an essential role in sensing and transducing external mechanical stresses in living cells. In the previous meeting we reported that TRPM7 channels endogenously expressed in human epithelial HeLa cells are activated by membrane stretch or osmotic cell swelling. However, it has not been known whether TRPM7 shows mechanosensitivity when heterologously expressed. HEK293T cells overexpressed with TRPM7 exhibited whole-cell currents typical of TRPM7, such as outward rectification, conductivity to Ca^{2+}, and sensitivity to Mg^{2+} and ruthenium red. In addition, TRPM7 currents were augmented by following three kinds of mechanical stimuli: shear stress imposed by perfusion of extracellular solution, membrane stretch produced by patch membrane suction, cell swelling due to hypotonic stimulation. We thus conclude that the TRPM7 channel can be activated by mechanical stress in the heterologous expression system.

P170 (1P1-064)

Regulation of TRPV4 activity by the PDZ-LIM protein

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TRPV4, a member of TRPV subfamily, is a nonselective cation channel that is activated by hypotonic stimulus, warm temperatures (about 25-34°C) or chemical compounds such as 4α-PDD, and is expressed in various tissues. To investigate TRPV4 function in the epithelial tissue, we screened a cDNA library from epithelial cells to identify TRPV4 interacting protein using a yeast two-hybrid system. Sequence analysis revealed that one of the positive clones encodes a protein containing PDZ and LIM domains. We found that the N terminal region of TRPV4 bound to LIM domains of the PDZ-LIM protein. When both TRPV4 and the PDZ-LIM protein were co-expressed in HEK293 cells, patch-clamp analysis showed that 4α-PDD-evoked currents were larger than those observed in cells expressing TRPV4 alone. Increase in the 4α-PDD-evoked currents was not observed in the cells expressing TRPV4 and the PDZ-LIM protein lacking LIM domains. These results suggest that the PDZ-LIM protein regulates TRPV4 activity by physical binding through LIM domains.

P171 (1P1-065)

Identification of TRPM8 Ion Permeation Region

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TRPM8 was cloned as the family of thermally activated TRP channels. TRPM8 is cooling activated channel involved in cold sensation. However, it is unclear which amino acid residues are critical for ion permeation as the pore.

We hypothesized that the pore is made by three consecutive hydrophobic amino acid residues between TM5 and TM6, highly conserved among different species or related TRP channels. WIF (898-900) were residues consistent with above hypothesis. We examined the involvement of this region on ion permeation by constructing TRPM8 mutant WIF898AAA, in which corresponding amino acid residues were replaced by alanine. Ion permeation was investigated with Fura-2 calcium imaging method. Menthol or cooling (from 30 °C to 15 °C) elevated intracellular calcium ion concentration in wild type TRPM8 -expressing cells but not in WIF898AAA -expressing cells.

We conclude that the conserved three consecutive hydrophobic amino acid residues between TM5 and TM6 (WIF) are critical for ion permeation of TRPM8.

P172 (1P1-066)

Transient receptor potential A1 is non-selective cation channel, activated by cooling.

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In TRP (Transient Receptor Potential) cation channels family, cooling activated channels, TRPM8 and TRPA1, were identified in peripheral sensory neurons. However, it is still controversial whether TRPA1 is activated by cooling. Story et al [Cell,2003] reported that TRPA1 is activated by cooling below 17ºC. In contrast, Jordt et al [Nature,2004] reported that cooling did not activate TRPA1. Here, we investigated thermal sensitivity of TRPA1 by Fura-2 microfulorimetry and patch-clamp recordings in TRPA1-expressing HEK293 cells.

1: Cooling increased intracellular calcium levels.

2: In whole-cell voltage-clamp recording (-60mV), cooling below threshold evoked inward current. Threshold temperature was 17.5 ± 2.7 ºC.

3: In inside-out single-channel recording, cooling induced activities of ion channels with non-selective cation channel properties. This indicated that channel activities occurred without intracellular soluble component. Single channel currents showed inward rectification. Single channel conductance was 74.1 ±18.8pS

We conclude that TRPA1 is non-selective cation channel, activated by cooling.

P173 (1P1-067)

Roles of two putative coiled-coil domains in the cytoplasmic C-terminal region of KCNQ channels

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KCNQ channels have two putative coiled-coil domains in the cytoplasmic C-terminal region. These two domains, also known as A-domain and B-domain, are well conserved among KCNQ channel family and are recognized as subunit interaction domains. We have previously shown that serine residues in A-domain may be phosphorylated when PKC shifts the voltage-dependence of KCNQ channels. Therefore, we hypothesized that the stability of coiled-coil domain might determine the voltage-dependence of KCNQ channels. The amino acid sequence of the coiled-coil domain is characterized by heptad repeats (*a-b-c-d-e-f-g*) in which positions *a* and *d* are occupied by hydrophobic amino acid residues. To decrease the stability of the coiled-coil domain, we introduced double glutamine (Q) mutations at positions a and d of each heptad repeat. We analyzed electrophysiological properties of four double glutamine mutants for A-domain (I551Q/Y554Q, H558Q/M561Q, L565Q/I568Q, V572Q/I575Q) and three for B-domain (L606Q/V609Q, V613Q/M616Q, L620Q/L623Q). Three of them (I551Q/Y554Q, L565Q/I568Q, L620Q/L623Q) did not express detectable current. Among mutants showing functional expression, V572Q/I575Q showed drastic changes in its voltage-dependence: $V_{1/2}$ of the *G-V* curve was negatively shifted (-65.1 ± 1.1 mV vs. -39.0 ± 0.7 mV) and the apparent valence of charge movement (z) became smaller (1.54 ± 0.04 vs. 4.08 ± 0.07). The results of V572Q/I575Q suggest A-domain may also be responsible for the voltage-dependence other than the subunit interaction.

P174 (1P1-068)

Voltage dependence of the adaptation in MscS occurs independent of the charged residues in the transmembrane domain

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MscS (mechanosensitive channel of small conductance) is ubiquitously found among bacteria and has been proposed to play an important role in osmoregulation. Although the MscS gating is regulated by membrane-stretch and voltage, little is known how MscS senses membrane potential. Three arginine residues (Arg-46, Arg-74, and Arg-88) in the transmembrane domain have been proposed to serve as voltage sensors. To examine whether some of these three residues constitute voltage sensors, we neutralized the charge of each residue by substitution with asparagine (R46N and R74N) or glutamine (R88Q). Mechanical threshold for the opening of the expressed wild-type MscS did not change with voltage in the range from -40 to +100 mV. Replacement of the arginine residues with asparagine or glutamine did not alter the threshold. By contrast, inactivation process of wild-type MscS was strongly affected by voltage. At the pipette potential of -40 to -80 mV the current of the wild-type MscS rapidly declined whereas the current at -20 to +60 mV sustained for a longer time, as reported previously (Akitake et al., 2005). The voltage dependence of the inactivation rate of all mutants tested, was almost indistinguishable from that of the wild-type MscS. These findings indicate that the voltage dependence of the inactivation occurs independently of the positive charges of Arg-46, Arg-74, and Arg-88.

P175 (1P1-069)

Role Of Cytoplasmic Domain In Voltage-Dependency Of Zebrafish VSP

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A protein called Ci-VSP (Voltage Sensor containing Phosphatase) has recently been reported (Murata et al, Nature 2005). VSPs have ion channel like transmembrane segments from S1 to S4 as the voltage sensor domain and the C-terminal cytoplasmic domain. It exhibits phosphatase activity in a voltage dependent manner. We have previously compared zebrafish ortholog of VSP (Z-VSP) with Ci-VSP and found that Z-VSP shows more robust charge movements in mammalian heterologous expression than Ci-VSP. To understand voltage-sensing mechanisms of VSP, we focused on the double mutant of Z-VSP in which two arginine residues were inserted into the S4 (DM). This shows negative shift of the Q-V curve (threshold was around -40 mV). Furthermore, DM showed biphasic profile of the Q-V curve that could not be fitted by a single Boltzmann equation: the movement of the voltage senor saturates around at 100 mV but then it increases as the membrane potential is more depolarized. Such biphasic profile of the Q-V curve did not depend on the phosphatase activity as examined from C302S mutant. This exhibited the threshold of charge movement even more negative (around -60 mV) and the simple Q-V curve that can be fitted with the single Boltzmann equation. These results suggest that the C-terminal domain but not its enzyme activity affects charge movement probably through exerting some constraint on the movement of the VS domain.

P176 (1P1-070)

Maxi-anion channels in rat cardiomyocyte sarcolemma are heterogeneously distributed

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ATP conductive maxi-anion channels are functionally expressed in rat cardiomyocytes and activated in hypotonic, hypoxic or ischemic conditions. In the present study, using a newly developed Scanning Ion Conductance Microscopy (SICM) technique combined with patch-clamp, we studied the spatial distribution of maxi-anion channels over the surface of sarcolemma of primary cultured neonatal rat cardiomyocytes and isolated adult cardiomyocytes. In primary cultured rat cardiomyocytes, maxi-anion channels were found to be predominantly expressed around the center of the cell body compared to the cell extensions. Using a P2X-receptor-based biosensor technique, it was found that the local concentration of ATP released in response to hypotonic stress was higher around the cell center compared to that at cell extensions. In isolated adult rat cardiomyocytes, maxi-anion channel activity was observed when a fine-tipped patch pipettes were giga-sealed at the grooved areas of sarcolemma using a conventional patch-clamp method. After taking a 3-D image of isolated cell by SICM technique, we specifically patched T-tubules, Z-grooves and scallop crest areas. We found that maxi-anion channels are predominantly expressed in T-tubules and Z-groove areas, but not in scallop crests, in adult cardiomyocytes. The results obtained indicate that maxi-anion channels are heterogeneously expressed over the surface of the rat cardiac sarcolemma.

P177 (1P1-071)

Effects of opioid- and cannabinoid-receptor agonists on calcium channels in rat nucleus tractus solitarius

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The profile of opioid- and cannabinoid receptors in neurons of the nucleus tractus solitarius (NTS) have been studied using the whole cell configuration of the patch clamp technique. Experiments with selective agonists and antagonists of opioid, opioid-receptor-like-1 (ORL-1) receptor and cannabinoid receptors indicated that μ-opioid, κ-opioid, ORL-1 and CB1, but not δ-opioid, receptors inhibit VDCCs currents in NTS. Application of DAMGO (μ-opioid receptor agonist), Orphanin FQ (ORL-1 receptor agonist) and WIN55,122 (CB1 receptor agonist) caused inhibition of VDCCs currents in a concentration-dependent manner with an IC50 of 390 nM, 220 nM and 2.2 μM, respectively. Intracellular dialysis of the $G\alpha_i$-protein antibody attenuated DAMGO-, Orphanin FQ- and WIN55,122-induced inhibition of I_{Ba}. Both pretreatment with adenylate cyclase inhibitor and intracellular dialysis of the protein kinase A (PKA) inhibitor attenuated WIN55,122-induced inhibition of I_{Ba}, but not DAMGO- and Orphanin FQ-induced inhibition. Mainly N- and P/Q-type VDCCs were inhibited by both DAMGO and Orphanin FQ, while L-type VDCCs were inhibited by WIN55,122. These results suggest that μ- and κ-opioid receptors and ORL-1 receptor inhibit N- and P/Q-type VDCCs via $G\alpha_i$-proteins β γsubunits, whereas CB1 receptors inhibit L-type VDCCs via $G\alpha_i$-proteins involving PKA in NTS.

P178 (1P1-072)

Functional interactions between P2X receptors and GABA- or Glycine-receptor in rat area postrema neurons

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We previously reported the negative interaction between excitatory P2X receptor (P2XR)- and nicotinic ACh receptor-channels in rat area postrema (AP) neurons. We now investigated whether there was also the interaction between excitatory P2XR and inhibitory $GABA_A$- or glycine-receptors (GlyR). At a holding potential of -70mV, the amplitude of the GABA- or Gly-induced current was significantly reduced in the presence of ATP, and there was an inverse correlation between the amplitudes of these responses. On the other hand, the ATP-induced current in the presence of GABA or Gly was only slightly reduced. As the GABA- or Gly-induced current desensitizes faster than the ATP-induced current, the weaker inhibition of the ATP-induced current by GABA or Gly could be due to the reduced amplitude of the GABA- or Gly-induced current at the time of ATP application. In fact, the current caused by the concomitant applications of ATP and GABA or Gly was smaller than the predicted sum of the individual currents.These results suggest that the negative interactions between the different receptors modify the strength of excitatory or inhibitory neurotransmission when plural transmitters are simultaneously released from presynaptic nerve terminals.

P179 (1P1-073)

Specific molecular actions of sarcolemmal phosopholipid metabolites on cardiac ryanodine receptors

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We examined with a lipid bilayer method how the single channel currents of cardiac RyR channels are modified by pathophysiological metabolites from the sarcolemmmal membrane phospholipids. During the apoptosis and the hyperlipidemia, sphingosylphosphatidylcholine (SPC) is metabolized from sphingomyelin (SM) of a minor sarcolemmal phospholipid. (1-1) The cytoplasmic-side addition of SPC blocked the RyR channels at the μM level, while the SR luminal-side addition of SPC did not affect. (1-2) SPC unaltered the membrane capacitance. Thus, SPC could exert a specific effect via an intermolecular binding to the cytoplasmic domain of the RyR molecule, although SPC belongs to the lipid. Kinetics of a long-lived blocking state of the SPC-modified channels is characterized by an extremely low dissociation rate constant. During the cardiac ischemia, lysophosphatidylcholine (LPC) is produced from phosphatidylcholine (PC) of a major sarcolemmal phospholipid. (2-1) Both cytoplasmic-side and SR luminal-side additions of LPC activated the RyR channels at the μM level. (2-2) LPC increased the membrane capacitance. In contrast to SPC effects, LPC could thus exert an indirect effect via a fusion of LPC into the membrane lipids on the RyR channel. Here we propose that a second messenger metabolited from SM of sarcolemmmal membrane phospholipids specifically regulated *in vivo* cardiac RyR channel activities.

P180 (1P1-074)

The mechanism of intracellular Ca^{2+} oscillation during P2Y2 receptor activation in rat bone marrow stromal cells

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Rat bone marrow stromal cells express G protein-coupled purinergic receptor ($P2Y_2$ receptor). We have investigated intracellular Ca^{2+} signals in these cells using fura-2 AM and found that UTP, an agonist of $P2Y_2$ receptor, induced not only Ca^{2+} rise but also Ca^{2+} oscillation. Removal of extracellular Ca^{2+} diminished UTP-induced Ca^{2+} oscillation. This fact indicates that the Ca^{2+} oscillation involves Ca^{2+} entry from extracellular space. SKF96365, a blocker for store-operated Ca^{2+} entry channel suppressed UTP-induced Ca^{2+} oscillation. Carbenoxolone (CBX), a gap junction blocker, also suppressed Ca^{2+} oscillation at higher concentration than 50μM, but enhanced at 10μM in some cells. At 10μM, CBX enhanced the peak amplitude of UTP-induced Ca^{2+} rise and kept high Ca^{2+} concentration until CBX was washed out. L-type voltage-dependent Ca^{2+} channel blockers, nifedipine or verapamil did not affect UTP-induced Ca^{2+} oscillation. These results suggest that UTP-induced Ca^{2+} oscillation may be regulated by a complex mechanism including Ca^{2+} entry through store-operated Ca^{2+} channel at plasma membrane and Ca^{2+}-transport system via gap junction.

P181 (1P1-075)

Vasopressin activates orexin neurons through a V1a receptor

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Orexin A and B are a pair of neuropeptides which are implicated in the regulation of sleep-wakefulness and energy homeostasis. The regulatory mechanism of orexin neurons is poorly understood so far. In this study, we studied the effects of various neuropeptides on the activity of orexin neurons by calcium imaging using transgenic mice in which orexin neurons specifically express calcium sensing protein (Yellow Cameleon 2.1). We screened 21 neuropeptides and found that arginine-vasopressin (AVP), cholecystokinin-8s and oxytocin triggered a robust, concentration-dependent calcium increase in orexin neurons.

We revealed the intracellular mechanisms and the subtype of AVP receptors involved in the AVP-induced activation of orexin neurons. The V1a AVP receptor antagonist, SR49059, inhibited AVP-induced activation of orexin neurons in a concentration-dependent manner, whereas the V1b and V2 receptor antagonists (SSR149415 and SR121463) had little effect. Removing extracellular calcium eliminated the AVP-induced increase in intracellular calcium concentration.

These results suggested that the V1a receptor is involved in the AVP-induced activation of orexin neurons. This AVPergic excitatory input to orexin neurons might have an important role in the physiological regulation of sleep-wakefulness.

P182 (1P1-076)

Interdomain interaction within type 1 ryanodine receptor is involved in dysfunction of Ca^{2+} release channel in malignant hyperthermia

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Malignant hyperthermia (MH) is an inherited disorder caused by a missense mutation of type 1 ryanodine receptor (RyR1) of skeletal muscle. We have recently showed that Ca^{2+}-induced Ca^{2+} release (CICR) activity of RyR1 in the SR is selectively stabilized by a probable interdomain interaction between the N-terminal and the central regions of RyR1 where many of mutated sites for MH are clustered (Murayama et al., Am. J. Physiol. 288; C1222-C1230, 2005). According to our hypothesis, a mutation within these regions will weaken the interdomain interaction, resulting in an increased CICR activity. We are presenting here further evidence to support this hypothesis using SR vesicles from skeletal muscles of wild type and MH pigs carrying the N-terminal Arg615Cys mutation in RyR1. Furthermore, we will show results using RyR1 channels carrying several human MH mutations including those in the C-terminal region that are stably expressed in HEK293 cells.

P183 (1P1-077)

Regulation of I_{Kr} potassium current by α_1 receptor in HL-1 cells

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Long QT syndrome (LQTS) results from mutations of several genes encoding cardiac ion channels. It was reported that in LQTS2, form associated with dysfunction of the rapid component of I_K (I_{Kr}), acute auditory stimuli could trigger the symptoms thus suggesting fast neural control of I_{Kr}. The aim of this study was by means of whole-cell patch-clamp method to investigate acute regulation of native I_{Kr} by α_1-adrenergic receptor (AR) in HL-1 cardiomyocytes.In the cells transiently transfected with AR, bath-application of 30 µmol phenylephrine (PHE) reversibly decreased I_{Kr} density by 29.4%, shifted activation curve (V_h from -17.6 to -9.2 mV) and accelerated deactivation. These effects remained in the presence of protein kinase C (PKC) inhibitor bisindolylmaleimide (200 nmol). In non-transfected cells 30 µmol PHE did not affect I_{Kr}. In HL-1 cells expressing muscarinic M1-receptor (known to be coupled to Gq - PLC pathway as AR), 10 µmol acetylcholine (Ach) suppressed I_{Kr} even more (37.2%).To confirm involvement of membrane PIP_2 breakdown in I_{Kr} modulation, HL-1 cells cotransfected with PH (PLCδ pleckstrin homology domain) -GFP and AR or M1-receptor were used for confocal microscopy. 30 µmol PHE or 10 µmol Ach induced translocation of PH-GFP fluorescence from the cell membrane to citosol, which was not observed in the cells transfected with PH-GFP alone.AR stimulation in HL-1 cells acutely suppressed I_{Kr} by depletion of membrane PIP_2 and was not dependent on PKC. This effect could explain onset of symptoms in the LQTS2 patients.

P184 (1P1-078)

Muscarinic receptor-operated cation channels as calcium entry pathways in bovine ciliary muscle

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In the ciliary muscle, tonic contraction requires a sustained influx of Ca^{2+} through the cell membrane. However, little has been known about the routes for the Ca^{2+} entry in this tissue that lacks voltage-gated Ca^{2+} channels. Recently we have shown by whole-cell voltage clamp experiments that in bovine ciliary muscle cells (BMCs) there are two types of non-selective cation channels (NSCCs) with widely different unitary conductances (35 pS and 100 fS), which are opened by muscarinic stimulation with carbachol (CCh). Here we examined effects of inhibitors of the NSCCs, La^{3+} and Gd^{3+}, on CCh-induced changes of the intracellular Ca^{2+} concentration $[Ca^{2+}]_i$. BMCs cultured for 18~48 hours in a serum-free media were used. The $[Ca^{2+}]_i$ was monitored by a Fluo-4 fluorescence method. Application of CCh to the BMCs in normal Krebs solution caused an initial phasic increase in the $[Ca^{2+}]_i$ followed by a plateau which was abolished by La^{3+} or Gd^{3+} (10-100 µM) as well as by removal of external Ca^{2+}. The CCh-induced elevation of $[Ca^{2+}]_i$ was also completely inhibited by 100 nM of atropine or 4-DAMP. These results support the idea that the muscarinic receptor-operated NSCCs serve as entry pathway of Ca^{2+} during the sustained phase of contraction. We also conducted immunofluorescence microscopy of the plasma membrane of BCMs and thereby detected transient receptor potential (TRP) channel homologues (TRPC1, TRPC3, TRPC4 and TRPC6), which are now regarded as possible molecular candidates for receptor-operated NSCCs.

P185 (1P1-079)

Functional involvement of tempromandibular joint P2X receptor in the jaw reflex activities in rats

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Purinergic receptor mechanisms have recently been implicated in peripheral (Rong et al 2000) and central (Hu et al 2002, Chiang et al 2005) nociceptive processing. Of the 7 iontotropic purinergic receptor subtypes (P2X receptor family), the expression of the P2X3 receptor is reported to be much higher than that of the other P2x receptor subtypes in trigeminal ganglia (Xiang et al 1998) and it has recently been found in TMJ tissues (Ichikawa et al 2004, Shinoda et al 2005). To clarify further the role of P2X receptors in TMJ-related functions, the application of P2X receptor agonist to the TMJ elicits nociceptive behaviors (Oliveira et al 2005, Shinoda et al 2005). The first aim of the present study was to test if the application of a P2X receptor agonist to the rat TMJ induces reflex activity in the jaw muscles and if this excitatory effect can be blocked by peripheral application of P2X receptor antagonist. The second aim was to test if blockade of peripheral NMDA receptors can influence the reflex jaw muscle activity evoked by ATP agonist.

P186 (1P1-080)

Conformational changes in the cytoplasmic domain of KcsA potassium channel upon gating

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For the cytoplasmic domain (CD) of potassium channels, crucial roles for mediating intracellular stimuli and assembling subunits have been investigated. KcsA channel, with only 160 amino acid residues, possesses intracellular stretches in the C-terminus, which forms CD in the tetrameric channel. KcsA channels are activated by intracellular acidic pH, the mechanism of pH-sensing remains unsolved. Also only predicted structure is available for the CD. Present study investigated surface structure of the CD in KcsA channel by developing a novel approach. Single cystein was introduced into various parts of the channel and specific reaction between introduced cystein and a flat gold surface was evaluated by surface plasmon resonance signals. All mutations did not alter single-channel properties, such as single-channel current-voltage curves and the gating characteristics. In contrast to the closed channel at pH 7.5, various sites in the CD became exposed to the surface when channels were activated (pHi = 4.0). These observations indicate that the cytoplasmic domain takes several conformational states when the channel is actively gating. We have also investigated the effect of open channel blocker, tetrabutylammonium, on the conformational changes in the CD.

P187 (1P1-081)

Role of negatively charged residues of the transmembrane segments of the voltage-sensitive phosphatase, Ci-VSP

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We previously reported an ascidian protein Ci-VSP which has a transmembrane voltage sensor motif with significant homology to voltage-gated channels and a phosphatase domain just downstream of the transmembrane region. We showed that the voltage sensor functionally couples with the phosphatase domain (Nature, 2005). However, it remains unknown whether similar mechanisms for voltage sensing of voltage-gated channels operate in Ci-VSP.There are conserved negative-charged residues in S2 and S3 regions of both Ci-VSP and voltage-gated ion channels. These residues are known to contribute to the gating charge of the channels. We have previously shown that a mutation which neutralized the negative-charged residue in S2 altered voltage dependency of its gating current. In this mutant, voltage-dependence of the coupling was shifted in the same direction as the change of the Q-V curve. In this work, we systematically mutated negatively charged residues in S1-S3 regions of Ci-VSP. cRNAs encoding these mutants were expressed in *Xenopus* oocytes and the two-electrode voltage clamp recording was performed to record the gating current of these mutants. In addition, these mutants were co-expressed with GIRK2 channels and changes of the phosphatase activity with membrane potentials were detected by monitoring changes of ion currents through GIRK2 channels. We also analyzed the voltage dependence of phosphatase activity of the mutant constructs of which the properties of voltage sensor movement are altered.

P188 (1P1-082)

Myofibrillogenesis regulator 1 (MR-1) as a causative gene for a hereditary channelopathy; A study on a large Japanese family of paroxysmal dystonic choreoathetosis (PDC)

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Paroxysmal dystonic choreoathetosis (PDC) is thought to be a hereditary channelopathy mapped to chromosome 2q32-36. By means of linkage analysis on a large Japanese family, we have narrowed the PDC locus that contains 32 candidate genes. Here, we report that a heterozygous mutation (A7V) in one of such genes, myofibrillogenesis regulator 1 (MR-1), is responsible for PDC in the Japanese family. This is consistent with the finding in American PDC families. We further report that there are several other polymorphisms in MR-1 in the Japanese PDC family. To characterize MR-1, we generated specific antibodies against MR-1 and performed the immunohistochemical analysis in rat brain. The results of the MR-1 localization will be discussed. Similar to other channelopathies such as epilepsy and migraine, PDC is characterized by involuntary movement attacks, and is presumed to be induced by abnormalities of ion channels. Although MR-1 may be associated with some ion channels, its physiological functions remain unclear. Further characterization of MR-1 including its molecular function and relationship to ion channels, may facilitate not only to understand pathophysiology of PDC, but also to develop effective therapies for paroxysmal neurological disorders.

P189 (1P1-083)

Dopamine induces slow afterdepolarization in lateral amygdala neurons in vitro.

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The amygdala plays significant roles in regulating emotional states and behaviors. Certain aspects of emotion are well known to be affected by the domaminergic projection system, of which targets includes the amygdala. Indeed, a large number of in vivo studies have shown that activation of dopamine (DA) receptors in lateral amygdala (LA) neurons alter emotional expression. For understanding DA-based modulation of emotion in the LA, it would therefore be beneficial to study effects of dopamine on intrinsic properties of the LA neurons, which remain largely unknown. In the present experiments, whole cell patch clamp recordings were carried out in rat brain slices to investigate DA effects on LA neurons. Application of DA depolarized resting membrane potential markedly, and induced slow afterdepolarization (sADP) in LA neurons. This sADP is induced in a voltage-dependent manner, and lasts for more than 5 seconds. D1, but not D2, receptor agonists induced the same type of sADP. Previous reports have repeatedly suggested that sADP in general is triggered by the calcium influx. Consistently, calcium channel blockers inhibited the present DA-induced sADP, but sodium channel blockers did not. Also, application of flufenamic acid (FFA), a calcium activated non-selective cation channel (CAN) blocker, inhibited the DA-induced sADP and canceled out the DA-induced depolarization as well. These results suggest that DA induces sADP in LA neurons by activating D1 receptors, and this sADP is attributable to activation of CANs.

P190 (1P1-084)

Bidirectional Ca^{2+} coupling between endoplasmic reticulum and mitochondria and multimodal regulation of plasmalemmal Ca^{2+} entry in rat brown adipocytes

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We have studied how endoplasmic reticulum and mitochondria communicate each other via Ca^{2+} and whether mitochondrial activity affects plasma Ca^{2+} entry. Intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) and mitochondrial membrane potential were measured by fluorometry in brown adipocytes in culture. FCCP, a protonophore, caused bi- or triphasic rises in $[Ca^{2+}]i$. The first phase was accompanied by mitochondrial membrane depolarization. The second phase, whose rising phase paralleled mitochondrial membrane repolarization, was blocked by a Ca^{2+} free solution, indicating the activation of plasmalemmal Ca^{2+} entry. The third phase was blocked by a Ca^{2+} free, EGTA solution, but not by thapsigargin, and enhanced at pH 9, but not in a Na^+-free solution, indicating the activation of store-operated Ca^{2+} entry (STOC). A blocker of phospholipase C, U73122, accelerated the decay of the first phase and enhanced the second phase. At a high $[Ca^{2+}]i$ under the effect of thapsigargin, FCCP produced a large rise in $[Ca^{2+}]i$ and subsequent reduction, or directly reduced $[Ca^{2+}]i$ for ten to tens of minutes. These results suggest that mitochondrial Ca^{2+} release and/or depolarization activates plasmalemmal Ca^{2+} entry different from STOC and Ca^{2+} release from ER, which leads to STOC activation, while Ca^{2+} release from ER activates Ca^{2+} accumulation in, or release from, mitochondria.

P191 (1P1-085)

Multimodal regulation of mitochondrial activity and plasmalemmal Ca^{2+} entry by noradrenaline and glucagon in rat brown adipocytes

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In brown adipocytes, mitochondria and endoplasmic reticulum (ER) are found to couple each other via Ca^{2+}, regulating plasmalemmal Ca^{2+} entry (Kuba et al., this meeting). To study how noradrenaline and glucagons that cause thermogenesis regulate this coupling and how it regulates plasmalemmal Ca^{2+} entry, intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) and mitochondrial membrane potential were measured by fluorometry in cultured rat brown adipocytes. Isoprotelenol and glucagon caused bi- or triphasic rises in $[Ca^{2+}]i$. The first phase was accompanied by mitochondrial membrane depolarization. The second phase was paralleled by mitochondrial membrane repolarization and blocked by Ca^{2+} free solution, indicating plasmalemmal Ca^{2+} entry. The third phase was blocked by Ca^{2+} free, EGTA solution, but not by thapsigargin, a blocker of Ca^{2+} pump at ER, and enhanced at pH 9, but not in a Na^+-free solution, indicating activation of STOC. A blocker of phospholipase C, U73122, enhanced the second and third phases of β_3-adrenergic and glucagon responses, while it blocked Ca^{2+} release by α_1-adrenoreceptor activation from ER and subsequent activation of store-operated Ca^{2+} entry. Thus, the activation of β_3-receptor and glucagons receptor causes multimodal plasmalemmal Ca^{2+} entry via changes in mitochondrial membrane potential and depletion of Ca^{2+} in ER via mitochondrial ER coupling.

P192 (1P1-086)

Kinetics of the divalent cation gate of gap-junction channel in guinea-pig ventricular myocytes

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Myocardial gap junction channels (Gap) are indispensable to action potential propagation. The channel gate (chemical gating) is regulated by intracellular cations such as Ca^{2+}, Mg^{2+} and H^+, but its dynamic gating properties have been scarcely examined. In this study, we investigated effects of Mg^{2+} on the Gap conductance in paired cells dissociated from guinea-pig ventriculues.The two-electrode whole-cell patch-clamp technique was applied to one of the paired ventricular myocytes (cell1). The current response to±5 -mV voltage pulses was recorded every 400 ms. In order to apply Mg^{2+}instantaneously, we perforated the membrane of the other pair of myocytes (cell 2), using a sealed pulsed nitrogen laser in the presence of a given concentration of Mg^{2+} in the bath solution. Under these conditions, the recorded current flowed from the cell 1 mostly through the Gap into the cell 2 whose interior was short circuited to the ground.The Gap conductance decreased in response to various Mg^{2+} concentrations ($[Mg^{2+}]_i$) in a dose-dependent manner (Hill coefficient: 3.84 EC50: 0.603mM). The conductance decay was well fit by a single exponential function. The $1/\tau$ - $[Mg^{2+}]_i$ relationship was almost linear over the range of $[Mg^{2+}]_i$ from 1 to 10 mM. Our results suggest that the Gap gating is regulated by multiple bindings of divalent cations, including one rate-limiting step.

P193 (1P1-087)

Molecular mechanisms of regulation of receptor type-specific Gq signaling by RGS8

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The regulator of G-protein signaling type 8 (RGS8) has a high affinity for Gαi and only a low affinity for Gαq. We previously reported that RGS8 decreased the amplitude of Gq-mediated response in a receptor type-specific manner and that RGS8S, a splice variant of N-terminus region, induced less inhibition. Although molecular mechanisms underlying receptor type-specific attenuation by RGS8 still remains unclear, recent evidences have raised a possibility that RGS may interact with certain GPCRs. Here we show by co-immunoprecipitation experiments that RGS8 directly binds to the third intracellular (i3) loop of M1- and M3-muscarinic AChR but not of M2, and that binding of RGS8S is weaker. We observed that a deletion of N-terminal 9 aa of RGS8 or substitutions of Arg-8 and Arg-9 of RGS8 for Ala reduced binding with M1i3, suggesting the importance of N-terminal region. To examine whether or not the interaction between RGS8 and M1 may occur in living cells, we performed BRET analysis. The results showed that RGS8 actually interacts with M1 and that the interaction of RGS8S is less clear. We next analyzed electrophysiologically the inhibitory effects of RGS8 w.t. and R8A/R9A mutant on Gq-mediated responses using *Xenopus* oocytes, and observed that the inhibitory effect of RGS8 was decreased by the mutations. These biochemical and electrophysiological results show that RGS8 inhibits M1-muscarinic AChR-mediated responses by a mechanism which involves direct interaction between N-terminus of RGS8 and i3 loop of M1.

P194 (1P1-088)

Swelling-activated chloride currents in rabbit articular chondrocytes: inhibition by arachidonic acid

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Articular chondrocytes play an important role in the formation of the cartilage in synovial joints, which is closely influenced by mechanical or osmotic stress. In the present study, whole-cell membrane currents were recorded from isolated rabbit articular chondrocytes during exposure to hyposmotic external solution, under conditions where Na^+, Ca^{2+} and K^+ channels and electrogenic transporters were minimized. Articular chondrocytes responded to a hyposmotic external solution (Na^+ reduction to about 70% of control) with an osmotic cell swelling, which was consistently accompanied by the activation of an outwardly-rectifying Cl^- current (swelling-activated Cl^- current, $I_{Cl,swell}$). $I_{Cl,swell}$ was practically time-independent at potentials negative to +30 mV but exhibited a gradual inactivation at more positive potentials. Bath application of arachidonic acid (AA) reversibly and concentration-dependently blocked $I_{Cl,swell}$ with an IC_{50} of 0.58 μM and Hill coefficient of 1.9. The maximal effect (100% block) was obtained with 10 μM AA. Neither cyclooxygenase inhibitor indomethacin (10 μM) nor lipoxygenase inhibitor nordihydroguaretic acid (NDGA, 3 μM) significantly affected the inhibitory action of AA. In addition, PGE_2, LTB_4 and LTD_4 did not have any appreciable effect on $I_{Cl,swell}$, suggesting that AA directly affected $I_{Cl,swell}$. The present study thus confirms the presence of $I_{Cl,swell}$ which exhibits a high sensitivity to inhibition by AA in rabbit articular chondrocytes.

P195 (1P1-089)

TRPM2 activation in rat pancreatic islets is involved in insulin secretion

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There are six thermosensitive TRP channels in mammals, and there might be other TRP channels sensitive to temperature stimuli. Recently, we have demonstrated that TRPM2 can be activated by exposure to warm temperatures (>35°C) apparently via direct heat-evoked channel gating. β-NAD^+- or ADP-ribose-evoked TRPM2 activity is robustly potentiated at elevated temperatures. We have also reported that, even though cyclic ADP-ribose (cADP-ribose) does not activate TRPM2 at 25°C, co-application of heat and intracellular cADP-ribose dramatically potentiates TRPM2 activity. Here we show that in rodent pancreatic islets, TRPM2 is co-expressed with insulin, and mild heating (around body temperature) of these cells evokes increases in both cytosolic Ca^{2+} and insulin release which is K_{ATP} channel-independent and cAMP-mediated. Heat-evoked response in pancreatic islets was significantly diminished by applying the known TRPM2 inhibitors; nonsteroidal anti-inflammatory drug flufenamic acid (FFA), anti-fungal reagent econazole or 2-aminoethoxydiphenyl borate (2-APB), and by treatment with TRPM2-specific siRNA. These results suggest that TRPM2 regulates Ca^{2+} entry into pancreatic β-cells at body temperature depending on production of cADPR-related molecules, thereby regulating insulin secretion.

P196 (1P1-090)

Expression of Na^+/Ca^{2+} exchangers in mouse osteoclasts and their functional role during bone resorption

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The plasma membrane Na^+/Ca^{2+} exchangers (NCXs) are bi-directional transporter that catalyzes the exchange of Na^+ for Ca^{2+} depending on the electrochemical gradients. Mammalian NCX forms a multigene family comprising NCX 1, NCX 2 and NCX 3. However, the expression and functional role of NCXs in mammalian osteoclasts are still unknown. The aim of present study is to clarify the expression of NCX and their functional role during bone resorption in mouse osteoclasts. We examined the expression of NCX using RT-PCR, immunocytochemical and Western blotting methods. The activation of NCX during bone resorption were assessed by measurement of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) using fura-2 and the effect of NCX inhibitors on pit formation assay. Mouse osteoclasts were expressed NCX 1 and 3, not NCX 2 using RT-PCR, Western blotting and immunocytochemical methods. There are some isoforms in mouse osteoclasts; NCX 1.3 and 1.4 in NCX 1 and NCX 3.5 in NCX 3. Under the measurement of $[Ca^{2+}]_i$, low or free extracellular sodium increased $[Ca^{2+}]_i$ in osteoclasts. The $[Na^+]_o$ free-induced $[Ca^{2+}]_i$ i increase was inhibited by NCX inhibitors. The NCX inhibitors also decreased in pit area resorbed by osteoclasts in dose dependent manner. These results suggest that NCXs are expressed in mouse osteoclasts and act as Ca^{2+} regulation during bone resorption.

P197 (1P1-091)

Effects of a Gq inhibitor, YM-254890, on carbachol-induced contraction of bovine ciliary muscle

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In the ciliary muscle, a smooth muscle under parasympathetic control, contraction is initiated by stimulation of muscarinic receptors of M_3subtype. It is established that the initial phasic component of the contraction is triggered by Ca^{2+} release from intracellular stores mediated by G_q-linked signalling pathway. The tonic component is also known to be highly dependent on Ca^{2+}, but Ca^{2+} is now provided by influx through receptor-operated cation channels rather than by release from stores [Takai et al.(2005) *J Physol* 559, 899-922]. However, little is known about the signalling mechanism involved in this Ca^{2+} influx. Here we have examined effects of YM-254890, a putative specific G_q inhibitor on contraction and $[Ca^{2+}]_i$ elevation induced by carbachol (CCh). For mechanical experiments ciliary muscle bundles dissected from bovine eyes were vertically mounted in an organ bath continuously perfused with normal saline, and isometric tension was recorded using a U-gauge transducer. Bath application of 2 μM-CCh caused a contraction. Both phasic and tonic components of this response were abolished by YM-254890 (3-10 μM). Using a Fluo-4 fluorescence method, we observed that CCh (10 μM) induced an elevation of the $[Ca^{2+}]_i$ in dispersed bovine ciliary muscle cells. Both initial phase and sustained phase of this response were also abrogated by YM-254890 (3-10 μM). G_q appears to be critically involved in Ca^{2+} mobilization in tonic as well as phasic component of the contraction of bovine ciliary muscle.

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P198 (1P1-092)

Abnormal regulation of ENaC and SGK1 by aldosterone in Dahl salt-sensitive rat

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Disturbance of renal Na^+ reabsorption develops hypertension in Dahl salt-sensitive (DS) rat. Aldosterone plays a critical role in controlling renal Na^+ reabsorption by stimulating expression of epithelial Na^+ channel (ENaC) and also activate an ENaC-regulating protein kinase, serum and glucocorticoid-regulated kinase 1 (SGK1). Therefore, we studied how aldosterone regulates ENaC expression and SGK1 in DS rat. Aldosterone (1.5 mg/kg B.W.) was subcutaneously injected into adrenalectomized DS and Dahl salt-resistant (DR) rats kept with normal (0.3% NaCl) diet and saline for 2 weeks after adrenalectomy. RNA and protein were extracted from the kidney 6 hr after the aldosterone application. Aldosterone decreased mRNA expression of β- and γ-ENaC in DS rat unlike DR rat, while aldosterone increased α-ENaC mRNA expression in DS rat similar to DR rat. Further, we found that aldosterone did not affect SGK1 expression in DS rat but elevated it in DR rat. These observations indicate that ENaC and SGK1 are abnormally regulated by aldosterone in DS rats, suggesting that these abnormal responses to aldosterone would be one of factors causing salt-sensitive hypertension.
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P199 (3P1-096)

The activation of phosphatidylinositol-linked D1-like dopamine receptor profoundly suppresses the exaitatory transmission in the developing hippocampus

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The disorder of dopamine (DA) system may be related to neurodevelopmental dysfunction. However, the action of DA on synaptic transmission during development is largely unknown. We studied the effect of DA on GABAergic and glutamatergic transmission in neonatal rat hippocampus from the early period of synapse formation by whole-cell patch-clamp recordings from CA1 pyramidal cells. DA (100 μM) profoundly decreased the amplitude of $GABA_A$ receptor-mediated postsynaptic currents ($GABA_A$-PSCs) to 32% in the first postnatal week, when GABA provides excitatory drive. DA also decreased the amplitude of AMPA receptor-mediated excitatory postsynaptic currents (EPSCs) to 29% in the second postnatal week, when glutamate responses first appear. The DA-induced inhibition declined after these periods and became only partial after postnatal day 30. Further we identified the receptor subtype involved in the DA-induced inhibition as phosphatidylinositol (PI)-linked D1-like receptor, since SKF 83959, a selective agonist for PI-linked D1-like receptor, clearly mimicked the action of DA, and U-73122, an inhibitor of phospholipase C, significantly reduced the DA-induced inhibition. DA did not change the response to puff-applied GABA or kainic acid, nor miniature $GABA_A$-PSC or EPSC amplitudes. These results suggest that the activation of PI-linked D1-like receptor profoundly suppresses the excitatory

P200 (3P1-097)

Chronic nicotine treatments increases cholinergic modulation of GABAergic synaptic transmission in the mouse striatum.

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The striatum, an input stage of the basal ganglia, contributes to habit formation as well as motor functions. Recent studies suggest the involvement of the dorsal striatum in the advanced stages of drug addiction. Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the striatum and can control the excitability of medium-sized spiny (MS) neurons, which provide the striatal output. We investigated the effect of chronic nicotine treatment on GABAergic synaptic transmission in mouse MS neurons. Twice-daily subcutaneous injections of nicotine (1 mg/kg) for 10-15 days did not change the electrophysiological properties of MS neurons or of three types of interneurons. However, intrastriatal stimulation evoked multiphasic IPSCs more frequently in MS neurons of nicotine-treated mice than in those of PBS-treated mice. Multiphasic IPSCs consisted of early and late components, both of which were mediated by $GABA_A$ receptors. However, the $GABA_B$ receptor agonist SKF97541 suppressed the early but not the late IPSC, suggesting heterogeneity of GABAergic inputs to MS neurons. Furthermore, generation of the late IPSC required the activation of nicotinic acetylcholine receptors (nAChR) because dihydro-β-erythroidine, an antagonist of nAChR, suppressed only the late IPSC. These results suggest that chronic nicotine treatment enhances the cholinergic modulation of GABAergic synaptic transmission in the striatum and that the enhanced inhibitory inputs.

P201 (3P1-098)

Muscarinic suppression of Golgi cells excitability in the mammalian cochlear nucleus

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Dorsal cochlear nucleus (DCN) is known to process complex sounds. The principal cells are known to integrate inputs from auditory nerve fibers (ANFs) and parallel fibers. Axons of granule cells form parallel fibers and convey multimodal information. Granule cells cluster around ventral cochlear nucleus and DCN, and have mutual synapses between inhibitory interneurons; Golgi cells. Thus, Golgi cells may have some modulatory effects on parallel fiber activities; however, little is known.

We studied the excitability of Golgi cells and interpreted the roles played by Golgi cells in the neuronal activity of DCN. By depolarizing current injection, Golgi cells fired repetitively and the firing frequency increased with current injection. At higher current intensity (300 pA-400 pA), steep firing adaptation was observed. By hyperpolarizing current injection, a depolarizing voltage sag emerged due to h-current activation. EPSCs evoked by ANFs stimulation were of multiple-peaks suggesting inputs through polysynaptic pathway. Because some cholinergic projections were expected, we tested cholinergic agonists: Carbachol induced a membrane hyperpolarization, accompanied with a decrease in the input resistance; muscarine evoked similar responses. These indicate the activation of muscarinic receptors, which hyperpolarized Golgi cells through the activation of GIRK.

Therefore, cholinergic innervation may contribute in modulation of parallel fiber activity through inhibitory Golgi cells.

P202 (3P1-099)

α-Adrenoceptive dual modulation of inhibitory GABAergic inputs to Purkinje cells in the mouse cerebellum

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In the cerebellar cortex, previous reports indicated that noradrenaline (NA) enhances inhibitory synaptic transmission via β-adrenoceptor-pathways. However, the effects of α-adrenoceptor activation on cerebellar inhibitory postsynaptic currents (IPSCs) have not yet been fully understood. Therefore, we investigated the effects of the α_1- or α_2-adrenoceptor agonist on IPSCs recorded from mouse Purkinje cells (PCs). The selective α_1-adrenoceptor agonist phenylephrine (PE) increased both the frequency and amplitude of spontaneous IPSCs (sIPSCs). PE also enhanced the amplitude of evoked IPSCs (eIPSCs) and increased the frequency but not the amplitude of miniature IPSCs (mIPSCs). Moreover, PE decreased the paired-pulse ratio of eIPSCs and did not change GABA receptor sensitivity in PCs. Conversely, the selective α_2-adrenoceptor agonist clonidine significantly reduced both the frequency and the amplitude of sIPSCs. Neither eIPSCs nor mIPSCs were affected by clonidine. Furthermore, presynaptic cell-attached recordings showed that spontaneous activity of GABAergic interneurons was enhanced by PE, while reduced by clonidine. These results suggest that NA enhances inhibitory neurotransmitter release via α_1-adrenoceptors, which are expressed in presynaptic terminals and somatodendritic domains, whereas suppresses the excitability of interneurons via α_2-adrenoceptors, which are expressed in presynaptic somatodendritic domains. Thus, cerebellar α-adrenoceptors play roles in a presynaptic dual modulation of GABAergic inputs from interneurons to PCs.

P203 (3P1-100)

Disruption of Clathrin-mediated Endocytosis of Synaptic Vesicles by Calpain-dependent Cleavages of Amphiphysin I

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Amphiphysin I, a member of the BAR (Bin-Amphiphysin-Rvsp) protein super family, plays a key role in clathrin-mediated endocytosis of synaptic vesicles. Amphiphysin I mediates invagination and fission of synaptic vesicles in cooperation with dynamin, which senses and facilitates membrane curvature by its BAR domain. In vitro, when present at higher concentrations, amphiphysin I can stabilize membrane curvature, generating lipid tubules, forming ring structure with dynamin, and increasing dynamin GTPase activity. In the present study, we found that amphiphysin I was cleaved to three fragments by treatment with high KCl (80 mM) in the mouse hippocampus slices. The cleavages were inhibited by pretreatment with calpain inhibitors. Calpain also cleaved amphihysin I to three fragments in vitro. We identified the three cleavage sites by mass spectrometry. Amphiphysin I was cleaved at the sites of 322, 349 and 386. Calpain-dependent cleavages of amphiphysin I can induce the liposome tubulation as the same as wild- type amphiphysin I, but it cannot form the ring structure with dynamin I under electron microscope. Moreover, transferrin uptake was inhibited by overexpression of the truncated form of amphiphysin I compared with that of wild-type amphiphysin I in COS-7 cell. These results suggest that amphiphysin I may be a substrate of calpain in presynaptic terminus and the cleavages are important for the regulation of clathrin-mediated endocytosis of synaptic vesicles.

P204 (3P1-101)

Protein kinase inhibitor staurosporine reduces the number of readily releasable synaptic vesicles at the calyx of Held

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The efficacy of synaptic transmission is determined by the quantal parameters such as the number of readily releasable pool vesicles (*N*), release probability (*p*) and postsynaptic response to a single quantum of transmitter (*q*). After massive vesicle exocytosis, *N* is replenished by vesicle trafficking for maintaining synaptic efficacy. However the mechanism underlying vesicle dynamics remains unknown. As a first step, we investigated whether staurosporine, a general kinase inhibitor, affects quantal parameters at the calyx of Held synapse in the brainstem slices of 12- to 15-day-old rats. Pretreatment of slices with 2 μM staurosporine for 1h decreased the amplitude of evoked EPSCs and slowed their rise time, but had no effect on their decay time. Staurosporine also reduced the frequency of spontaneous miniature EPSCs without affecting their amplitude (*q*) or kinetics. Estimation of quantal parameters, using the tetanic stimulation protocol, revealed that staurosporine reduced *Nq*, but not *p*. Staurosporine increased the magnitude of synaptic depression during repetitive stimulation (1-100 Hz) supporting the depletion model of synaptic depression. Staurosporin slowed recovery from depression caused by 10-100 Hz-stimulation without affecting the fast phase of recovery time course after 100 Hz-stimulation. We conclude that staurosporine-sensitive protein kinases affect the size of readily releasable pool and specifically accelerate a slow component of vesicle mobilization thereby contributing to the replenishment of readily releasable vesicles.

P205 (3P1-102)

Melittin Enhances Inhibitory Synaptic Transmission in Adult Rat Spinal Dorsal Horn Neurons

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We have recently reported that a phospholipase A_2 (PLA_2) activator melittin reversibly enhances glutamatergic excitatory synaptic transmission in substantia gelatinosa (SG; lamina II of Rexed) neurons of the rat spinal cord. The SG neurons receive not only excitatory but also GABA- and glycine-mediated inhibitory transmission. In order to know the effect of melittin on the spontaneous inhibitory transmission, we applied the blind whole-cell patch-clamp technique to SG neurons in adult rat spinal cord slices. Melittin (1 μM) superfused for 3 min gradually increased the frequency and amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs) at a holding potential of 0 mV, which were visible about 2 min after the beginning of its superfusion and subsided within 8 min after washout. These facilitatory actions of melittin were observed for GABAergic and glycinergic sIPSCs, which were recorded in the presence of a glycine-receptor antagonist strychnine (1 μM) and a $GABA_A$ receptor antagonist bicuculline (10 μM), respectively, and were reduced in extent by a PLA_2 inhibitor 4-bromophenacryl bromide (10 μM). A voltage-gated Na^+ channel blocker tetrodotoxin (TTX, 0.5 μM) had a tendency to inhibit the effect of melittin on the GABAergic but not glycinergic sIPSC. It is concluded that melittin enhances GABAergic and glycinergic inbibitory transmission in a pre- and postsynaptic manner through the activation of PLA_2 in the SG; a part of the effect of melittin on GABAergic transmission is due to its action on excitatory transmission.

P206 (3P1-103)

Positive modulation by proteinase-activated receptor agonist peptides of nociceptive transmission in the rat spinal dorsal horn

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G-protein coupled proteinase-activated receptors (PARs) have a unique activation mechanism in that a proteolytically exposed N-terminal region acts as a tethered ligand. Four members of PARs such as PAR-1 and PAR-2, which were identified by molecular cloning, can be activated by synthetic peptides whose amino acid sequences correspond to the tethered ligands. Although PARs appear to be involved in nociceptive transmission in peripheral terminals of primary afferents, there is no report about involvement of PARs in the transmission in the spinal dorsal horn. In order to clarify a role of PARs in the nociceptive transmission, we examined how PAR-1 and PAR-2 agonist peptides affect glutamatergic spontaneous excitatory postsynaptic currents (sEPSCs) in substantia gelatinosa (SG; lamina II of Rexed) neurons which are thought to play a pivotal role in regulating nociceptive transmission to the CNS. We applied the blind whole-cell voltage-clamp technique to the SG neurons in adult rat spinal cord slices. PAR-1 agonist peptide (SFLLRN-NH_2; 1 μM) reversibly increased the frequency of sEPSC without a change in its amplitude, while PAR-2 agonist peptide (SLIGKV-NH_2; 1 μM) had no effects on sEPSCs. Both peptides did not alter holding currents at -70 mV. These results indicate that PAR-1 but not PAR-2 agonist peptide enhances the spontaneous release of L-glutamate from nerve terminals in the SG. It is suggested that PAR-1s expressed in nerve terminals in the SG may play an important role in producing nociception.

P207 (3P1-104)

Muscarinic responses in rat PAG neurons

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The periaqueductal grey (PAG) of the brainstem is a central site for the various physiological functions including cardiovascular control, defensive behavior and pain. Since the cholinergic modulation on PAG activity is still unknown, we used infrared-videomicroscopy in conjunction with whole-cell recordings to elucidate the effects of acetylcholine on PAG neurons in midbrain slices of juvenile rats. In current clamp mode, 40% of all PAG neurons examined showed depolarization of their membrane potential during the application of carbachol (CCh). On the other hand, 20% of all PAG neurons examined were hyperpolarized by CCh. The remaining PAG neurons (40%) were insensitive to CCh. Both the depolarizing and the hyperpolarizing action of CCh were atropine-sensitive, indicating that they were mediated by muscarinic receptors. The depolarizing response to CCh was abrogated by M1 antagonist, pirenzepine, while the hyperpolarizing response was abolished by M2 antagonist, gallamine. These results suggest that rat PAG neurons have functional muscarinic receptors.

P208 (3P1-105)

Facilitated NMDA-receptor activities in the hippocampal CA1 area of mutant mice lacking D-amino-acid oxidase

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D-serine which is thought to facilitate the NMDA receptor, present in the forebrain and co-localize with N-methyl-D-aspartate (NMDA) receptor. We reported that the spatial learning in the water maze and tetanus induced hippocampal LTP in the slice preparations were facilitated in the mutant mice lacking D-amino-acid oxidase, an enzyme which metabolizes D-serine. In the present report, we compared NMDA dependent synaptic current and the effect of D-serine on NMDA dependent synaptic current in the hippocampal CA1 area between the wild-type mice and mutant mice lacking D-amino-acid oxidase, to clarify whether NMDA dependent synaptic current was enhanced in the mutant mice and whether the enhancement was D-serine dependent. Excitatory postsynaptic currents (EPSCs) were recorded in CA1 pyramidal cells using whole cell patch-clamp techniques by stimulating Schaffer collateral-commissural fibers of the hippocampal slices. The ratio of NMDA receptor-mediated EPSC amplitudes to non-NMDA receptor-mediated EPSC amplitudes was significantly larger in the mutant mice than in wild-type mice. The ratio of NMDA component to non-NMDA component of the wild-type mice was significantly increased to the levels observed in the mutant mice, when D-serine was added to perfusion medium. We suggest that D-serine increased in the mutant mice brain facilitates NMDA-mediated synaptic current.

P209 (3P1-106)

Lidocaine transiently inhibits evoked EPSPs in the rat hippocampal CA1 region

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Extracellular recordings were made from CA1 regions in the rat hippocampal slice tissues. Superfusion of slice preparations with lidocaine at low concentrations (1 - 100 μM) induced a transient inhibition and subsequent augmentation of the maximal slope of the field excitatory postsynaptic potentials (fEPSPs). On the other hand, high concentrations (300 - 1000 μM) of lidocaine only suppressed the maximal slope of the fEPSPs. The amplitude of presynaptic volleys was simply suppressed by lidocaine (1 - 1000 μM) in a dose-dependent manner. Pretreatment of adenosine 1 (A_1) receptor antagonist, DPCPX (1 μM) diminished the transient inhibition of the fEPSPs. Intracellular recordings from CA1 neurons showed that lidocaine (3 - 100 μM) transiently suppressed the amplitudes of the evoked fast EPSPs and, of the fast and late IPSPs. Lidocaine at low concentrations (3 - 30 μM) also induced a DPCPX-sensitive transient hyperpolarization in the CA1 and CA3 neurons. In the presence of TEA (20 mM) and TTX (0.3 μM), lidocaine at the low concentrations reduced the amplitude and duration of Ca^{2+} spikes recorded from CA3 neurons. These results suggest that lidocaine at the low concentrations (3 - 100 μM) transiently inhibites the evoked fEPSPs via activation of A_1 receptors at both pre- and post-synaptic sites in the rat CA1 hippocampal neurons.

P210 (3P1-107)

Apparent lack of desensitization in the AMPA-component of EPSC evoked in the CA1 pyramidal neuron of hippocampal slice of adult rat

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To study differences in nature between synaptic and extrasynaptic AMPA-receptor in the adult brain, we recorded stimulus-evoked EPSC and AMPA-induced current response from hippocampal pyramidal neuron of rat brain slice under whole-cell patch clamp. Application of either NBQX or GYKI52466, AMPA antagonists, suppressed the both EPSC and AMPA-induced current response. Current(I)-voltage(V) relationship of the EPSC and AMPA-response intersected the voltage axis at 0 mV showing linear I-V curve. The AMPA-induced response was markedly augmented as much as twice of the control in the presence of cyclothiazide (CTZ), an inhibitor of desensitization of AMPA-receptor, whereas either EPSC or spontaneously evoked miniature EPSC was not affected at all by the CTZ. Furthermore, neither the amplitude nor the time course of the consecutive EPSCs evoked by repetitive stimulation of Schafer collateral at 100 Hz was not affected significantly by the presence of CTZ, although cumulative current responses to repetitive application of AMPA, which exhibited occlusion, were greatly augmented by CTZ in similar fashion to the single application of AMPA. The amplitude of the EPSC decreased in the presence of dihydrokainic acid (DHK), an inhibitor of GLT-1 glutamate transporter, but addition of CTZ to the DHK did not augment the EPSC amplitude. All these results suggest that synaptic AMPA receptor may lack the nature of desensitization unlike the extrasynaptic AMPA-receptor.

P211 (3P1-108)

Facilitation of glutamatergic excitatory synaptic transmission in hippocampal CA1 regions of traumatic brain injury model rats

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We investigated the effects of fluid percussion injury (FPI) on the glutamatergic excitatory synaptic transmission of CA1 pyramidal neurons using conventional intracellular recording techniques. A moderate impact (3.8-4.8 atm) was applied to the left hemisphere of the parietal cerebral cortex by using a FPI device (HPD-1700, Dragonfly, Inc.). After a survival period of 7 days, horizontal brain slices containing hippocampus were cut. The slice preparations were devided into three groups (control, FPI-ipsilateral, and FPI-contralateral). The control group includes the data from normal and sham-operated rats. There were no significant differences in resting and acting membrane properties among three groups. However, the EPSPs evoked by stimulations of Schaffer collaterals in the presence of bicuculline showed steeper input-output relationship (I-O) in the FPI-ipsilateral group. The number of spikes evoked by EPSP in the presence of bicuculline was larger in FPI-ipsilateral group although there were no significant differences among three groups in the absence of bicuculline. The paired-pulse facilitation ratio in FPI groups was smaller than that of control group. Frequency of mEPSPs recorded in the presence of tetrodotoxin was higher than that of control while the amplitude of mEPSPs was not different. These results suggest that the post-traumatic facilitation of gultamatergic transmission in pyramidal neurons of rat hippocampal CA1 is mediated by presynaptic origin.

P212 (3P1-109)

Effects of propofol on tonic GABAergic inhibition in rat neocortex layer V pyramidal neuron

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$GABA_A$receptor mediated two inhibition models, phasic and tonic. Midazolam, benzodiazepine, and a widely used anesthetic, propofol potentiate $GABA_A$ receptor function. Bai et al. reported propofol, compared with midazolam, had a lower potency but higher efficacy for increasing the amplitude of the tonic current in the hippocampal CA1 pyramidal neurons. We compared the effect of midazolam and propofol on the tonic current in neocortex layer V pyramidal neuronsusing whole-cell patch clam techniques from 2- to 3- week old rats. Propofol and midazolam cause a concentration-dependent increase in the amplitude of the tonic current. In the neocortex, midazolam increased tonic current more than propofol. Picrotoxin blocked both midazolam and propofol effect on the tonic current. In contrast, bicuculline blocked tonic current induced by midazolam but not proporol. These suggest that these two drugs mediate tonic current via different subtypes of $GABA_A$ receptors Referance (1)Molecular Pharmacology 2001;59:814-824

P213 (3P1-110)

Descending inhibitory synaptic inputs to rat superior salivatory nucleus

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We showed the excitatory (glutamate) and inhibitory (GABA and glycine) synaptic inputs to the superior salivatory (SS) neurons innervating the submandibular salivary glands and tongue. However, the relationships between the higher and lower centers in the synaptic inputs have not yet been examined. In the present study, we studied electrophysiologically the inhibitory synaptic inputs in brainstem slices obtained from normal and decerebrate rats. The SS neurons were labeled by retrograde axonal transport of a fluorescent dye. Whole-cell patch-clamp recordings were made from the labeled neurons. The currents were evoked by agonists (GABA and Glycine) perfusion, and electrical stimulation near the recording cell. After decerebration, agonists perfusion induced larger currents, but their decay time constant were not altered as compared with those of normals. This increase may result from receptor up-regulation at postsynaptic membrane. By electrical stimulation, in 83% (n=34/41) neurons, enhanced IPSCs were evoked, suggesting that the neurons have the inhibitory inputs from both the higher and lower centers. In 17% neurons (n=7/41), no IPSCs were evoked, suggesting that the neurons have only the descending inhibitory inputs from the higher centers. It is suggested that all SS neurons have the descending inhibitory synaptic inputs from the higher centers.

P214 (3P1-111)

Neuronal glutamate transporters regulate the activation of metabotropic glutamate receptors in cerebellar Purkinje cells

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Glutamate transporters are essential to remove synaptically released glutamate in excitatory synapses. Glial glutamate transporters expressed in Bergmann glia remove the majority of glutamate at excitatory synapses in cerebellar Purkinje cells (PCs) at early times after transmitter release. The neuronal glutamate transporter, the excitatory amino acid transporter 4 (EAAT4), is concentrated at perisynaptic sites of PCs, where metabotropic glutamate receptors (mGluRs) are located. To clarify the contribution of EAAT4 to the regulation of mGluR activation, we recorded mGluR-mediated excitatory postsynaptic currents (mGluR-EPSCs) in cerebellar slices of mice deficient in EAAT4 and compared them with those in wild-type (WT) mice. The amplitude of mGluR-EPSCs evoked by the stimulation of parallel fibers (PFs) was larger in EAAT4-deficient mice than that in WT mice. However, the amplitudes of PF-evoked mGluR-EPSCs in EAAT4-deficient and wild-type mice were similar in the presence of the glutamate transporter antagonist DL-threo-β-benzyloxyaspartic acid (TBOA). mGluR-EPSCs evoked by the stimulation of climbing fibers (CFs) were observed in EAAT4-deficient mice but not in WT mice in the normal saline. When the function of EAAT4 was inhibited by a pharmacological treatment, mGluR-EPSCs were elicited by the stimulation of CFs even in WT mice. These results indicate that EAAT4 plays a critical role in the regulation of the activation of perisynaptic mGluRs at both PF and CF synapses in PCs.

P215 (3P1-112)

Removal of extracellular divalent cations induced a slowly synchronized rhythmic activity in immature rat dorsal spinal cord

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Immature rat spinal motoneurons showed a slowly synchronized rhythmic activity (sSRA) in divalent cation- free solutions. This sSRA was attributed to rhythmic oscillations of extracellular potassium ions in spinal cord. So, possible occurrence of the same phenomena in the dorsal horn at this same special situation, was investigated in this experiment. In the isolated spinal cord preparation from newborn rats, extracellular neuronal activity was recorded from dorsal and ventral roots using suction electrodes. After removal of extracellular divalent cations, sSRA could be recorded from both the dorsal root (dorsal sSRA) and the ventral root (ventral sSRA). The dorsal and ventral sSRA had a mirror image, but ventral sSRA occurred slightly earlier. Both were recorded in rats soon after birth, but not in older rats. Sectioning the border between the dorsal and ventral horn eliminated ventral sSRA, but not dorsal sSRA. It is suggested that both dorsal and ventral sSRA depends on the activity of a rhythm generator located in the dorsal horn. Several characteristic features of these two sSRA will be discussed.

P216 (3P1-113)

Relationship between 5'-nucleotidase activity and nicotine effects in rat hippocampus

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It has been demonstrated that AMP is hydrolyzed to adenosine(ADO) in the synaptic cleft by 5'-nucleotidase. This enzyme in the central nervous system is known to participate in neuroprotective properties. However, little is known about the relationship between 5'-nucleotidase activity and nicotine effects. Nicotine significantly increased 5'-nucleotidase in synaptosomes from hippocampus of adult rats. This activity was significantly inhibited by AMPCP which was a 5'-nucleotidase inhibitor. Nicotine significantly increased the hydrolysis of ATP, ADP, AMP and ADO by analysis with HPLC. These hydrolysis of adenine nucleotides were completely blocked in EGTA Ca^{2+}-free buffer solution. We also examined the effect of ovariectomy (OVX) and estrogen replacement therapy (ER) on the activity of the enzyme that degrade adenine nucleotides in female rats. The release of ATP, ADP, AMP and ADO from hippocampus slice preparations by nicotine application in the buffer solution tended to decrease in the OVX group when compared to a control group. ER reversed the inhibition of the release of these nucleotides observed in OVX rats. The regulation of enzymes that hydrolyze these nucleotides in the hippocampus is essential in the modulation of the processes of neuronprotective properties. Results suggest the presence of a strong relationship between 5'-nucleotidase activity and nicotine effects to the ADO formation, and also to estrogen binding-sites.

P217 (3P2-114)

Differential effects of propofol on inhibitory postsynaptic currents in CA1 pyramidal cells and dentate gyrus granule cells of rat hippocampal slices

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We have recently reported that there exist regional differences of benzodiazepine effects in GABA-mediated inhibitory synaptic transmission in vitro studies. In this study, we examined the effects of propofol, one of the most popular intravenous anesthetic agents, on the inhibitory postsynaptic currents ($IPSC_s$) in CA1 pyramidal cells ($CA1\text{-}PC_s$) and dentate gyrus granule cells ($DG\text{-}GC_s$) in rat hippocampal slices. The monosynaptic $IPSC_s$ were evoked by electrical stimulation of GABAergic interneurons and recorded from $CA1\text{-}PC_s$ and $DG\text{-}GC_s$ by whole cell patch-clamp technique. The effects of specific concentrations of propofol (0.1, 1, 10 and 100μM) on the $IPSC_s$ in $CA1\text{-}PC_s$ and $DG\text{-}GC_s$ were examined at varying membrane potentials (20 mV steps, from -120 to +40 mV). In the absence of propofol, at the clamped membrane potential of-120mV and +40mV, IPSC amplitudes and decay time constants in both $CA1\text{-}PC_s$ and $DG\text{-}GC_s$ were kept stable for at least 20 minutes. When tested within this stable period, propofol was observed to increase the amplitudes and prolonged the decay time constant of $IPSC_s$ in $CA1\text{-}PC_s$. However, propofol changed neither the amplitude nor decay time constant of the $IPSC_s$ in $DG\text{-}GC_s$. These results suggest that propofol possesses differential effects on $IPSC_s$ in $CA1\text{-}PC_s$ and $DG\text{-}GC_s$ similar to benzodiazepines. The mechanism for these differential effects could be due to the different sensitivity to propofol of the $GABA_A$receptor subtypes in the $CA1\text{-}PC_s$ and $DG\text{-}GC_s$.

P218 (3P2-115)

NMDA receptor activity-dependent oscillatory signal outputs from the retrosplenial cortex triggered by a signal input from the visual cortex

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The retrosplenial cortex is located at a critical juncture between the visual cortex and hippocampal formation. Herein we show how signals traveling from the visual cortex behave in local circuits of the retrosplenial cortex, using optical recording methods and application of caffeine to rat brain slices. Electrical signals evoked in the primary visual cortex penetrated into the deep layer of the retrosplenial granular a cortex (RSGa), and propagated further toward postsubiculum and upper layer. Non-NMDA receptor-dependent initial traveling signal from the visual cortex triggered NMDA receptor-dependent neural oscillation in the RSGa. Oscillatory signals originated from the local area in the deep layer of the RSGa, and the signal spread back and forth toward the visual cortex and postsubiculum, in addition to spreading toward the upper layer. From the perspective of the RSGa, extrinsic signal inputs from the visual cortex switched on neural oscillators in the RSGa that deliver NMDA receptor-dependent intrinsic signal outputs. Opening and strengthening of non-NMDA receptor-dependent input pathways from the visual cortex required NMDA receptor-dependent oscillatory neural activities. These input and output relationships indicate that the retrosplenial cortex may represent an important relay station between the visual cortex and hippocampal formation.

P219 (3P2-116)

Muscarinic inhibition on IPSC at SNr GABA neurons is induced through the $G_{\beta\gamma}$ activation from $G_{q/11}$ linked with M_3-muscarinic ACh receptor.

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Although I have analyzed the mechanism of muscarinic inhibition on the IPSC at substantia nigra pars reticulata GABA neurons of rat, the intracellular transduction mechanism is not clear yet. N-ethylmaleimide (NEM), a membrane permeable inhibitor of PTX-sensitive G-proteins, did not have any significant effect on the muscarinic inhibition at the concentration of 100 μM. NEM itself significantly increased in the amplitude of IPSC to 1.627 ± 0.166 of the control amplitude (n=5, mean ± S.E.M., p=0.019 by Student's *t* test paired). Muscarine (10 μM) reduced the IPSC amplitude to 0.420 ± 0.104 under the control condition and to 0.370 ± 0.050 of the increased IPSC in the solution with NEM, respectively (p=0.738). ω-Agatoxin TK (ω-Aga TK), a selective P-type Ca^{2+} channel blocker, exerted no inhibitory influence on the muscarinic inhibition. The amplitude of IPSC was significantly attenuated to 0.443 ± 0.139 (n=4, p=0.031) in the solution with ω-Aga TK (100 nM). The muscarinic inhibition ratios were 0.430 ± 0.089 in control and 0.326 ± 0.077 of the decreased IPSC in the solution with ω-Aga TK, respectively (p=0.386). These results mentioned above and those previously reported suggest that $G_{\beta\gamma}$ subunits dissociated from $G_{q/11}$ linked with M_3-receptors reduce the GABA release through the direct effect on the release machinery, when M_3-muscarinic ACh receptors at the presynaptic terminal of a striato-nigral projection fiber is activated. Hopefully, these observations contribute to the new approach of drug therapy for the basal ganglia disturbance.

P220 (3P2-117)

Visualization of synaptically released glutamate by a novel opticalglutamate sensor

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Glutamate is an essential excitatory neurotransmitter in the central nervous systems. For the understanding of mechanisms underlying synaptic transmission, we developed a novel optical glutamate probe called S403C-OG which consists of a recombinant glutamate binding domain derived from GluR2 subunit of AMPA receptor and a fluorescent dye. To visualize synaptically released glutamate, we immobilized S403C-OG on the cell surface of the cultured hippocampal neurons and captured fluorescence images with CCD camera. With this maneuver, we successfully detected the glutamate release along active synapses in response to electrical stimuli. The amount of glutamate release considerably varied among locations within the same neuron, suggesting the spatial heterogeneity among the release sites. We also observed spontaneous and transient glutamate release events without electrical stimuli, which was stochastic, variable in amplitude and spatially confined in small regions. Neither application of tetrodotoxin nor removal of extracellular calcium blocked the spontaneous glutamate release. The frequency of the release increased upon application of high concentrations of sucrose which is known to increase the frequency of miniature EPSC. Furthermore, we succeeded in continuous monitoring of the changes in presynaptic activity induced by phorbol ester, indicating that our probe enable to directly visualize the presynaptic activity. In summary, S403C-OG is useful to address many fundamental issues related to glutamatergic synaptic transmission in the central nervous system.

P221 (3P2-118)

Effect of MEK inhibitor U-0126 on wind-up action of dorsal horn nociceptive neurons in rats

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It is well known that wind-up is the phenomenon that the second order neurons increase in firing frequency following repetitive stimulation of C-fibers. Recently, several lines of evidences suggest that the phosphorylation of the extracellular signal-regulated protein kinase (ERK) is involved in the hyperexcitability of the nociceptive neurons. However, it is not known how the ERK is involved in the windup phenomena. The present study was designed to evaluate the change in wind-up of DH nociceptive neurons i.t. administration of MEK inhibitor, U0126. Rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). When single neural activity was isolated, the left sciatic nerve was stimulated (0.5 Hz, 3 times higher than C-fiber threshold). After windup was observed, U0126 (2.5 mM, 25 mM and 250 mM) was applied to the DH. The increased firing was significantly depressed following application of U0126. DH nociceptive neurons were classified as WDR neurons. All WDR neurons were located in laminae I-II in the DH. We could not observe any clear dose dependent effect of U0126 on DH nociceptive neurons. The mechanical and thermal responses were not affected by U0126. The present findings suggest that the intracellular MAP kinase cascade is involved in the central sensitization of the DH nociceptive neurons without any effects on naturally evoked responses.

P222 (3P2-119)

Rapid elevation of the synaptophysin mRNA expression level in rat somatosensory cortex induced by tactile stimulation

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Synaptophysin is an integral membrane protein abundant in the synaptic vesicle and is found in nerve terminals throughout the brain. Its function has been implicated in various aspects of synaptic vesicle cycling such as biogenesis of vesicles, the regulation of the SNARE complex formation, synaptic vesicle fusion with plasma membrane, endocytosis and recycling of synaptic vesicles. It was recently suggested that synaptophysin is also involved in the modulation of activity-dependent synapse formation under a competitive condition. In this study, we examined at the individual level whether tactile stimulation selectively influenced the synaptophysin mRNA expression level in the somatosensory cortex of rats. Anesthetized rats were caressed on the back by an experimenter' s palms for twenty minutes and the mRNA expression levels in the somatosensory cortex responsible for the back and in the visual cortices five minutes afterwards were determined using quantitative PCR methodology. The synaptophysin mRNA expression level was selectively higher in the experimental group than in the control group in the somatosensory cortex but not in the visual cortex. This result suggests that the mRNA expression level of synaptophysin induced by neuronal activity is related to the regulation of synapse formation or remodeling or both.

P223 (3P2-120)

Neurosteroid pregnenolone sulfate enhances glutamatergic synaptic transmission by facilitating presynaptic calcium channels

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Pregnenolone sulfate (PREGS) is an abundant neurosteroid in the brain. PREGS presynaptically facilitates glutamatergic synaptic transmission, but underlying mechanism is not known. At the giant synapse, the calyx of Held in the rat brainstem slices, PREGS potentiated nerve-evoked excitatory postsynaptic currents (EPSCs), and increased the frequency, but not the amplitude, of spontaneous miniature EPSCs (mEPSCs). The EPSCs potentiations by PREGS and those by forskolin or phorbol ester did not occlude with each other. In direct whole-cell recordings from presynaptic terminals PREGS accelerated activation kinetics of voltage-dependent Ca^{2+} channel currents. BAPTA (10 mM) loaded into the terminal only partially attenuated this PREGS effect, suggesting that the main effect is independent of intracellular Ca^{2+}. PREGS had no effect on presynaptic voltage-dependent K^+ currents or resting conductance. Ca^{2+} imaging at the nerve terminal showed that PREGS increased Ca^{2+} influx into the terminal at the resting membrane potential. Consistently the PREGS-dependent increment of the mEPSC frequency was attenuated by 300 μM Cd^{2+}. The PREGS-induced Ca^{2+} current facilitation was reversed by the PREGS scavenger cyclodextrin applied from outside, but not from inside, of the nerve terminal. We conclude that PREGS, by acting from outside of the nerve terminal, activates Ca^{2+} channels, thereby increasing both evoked and spontaneous transmitter release at the calyx of Held.

P224 (3P2-121)

In vivo transduction of murine cerebellar Purkinje cells by HIV-derived lentiviral vectors

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Cerebellar Purkinje cells are key elements regulating motor learning and motor coordination. Gene transfer into neurons, followed by the assessment of the effects on neural function, is an effective approach for examining gene function. However, this method has not been used fully in the study of the cerebellum, because of the obstacle of delivering genes into Purkinje cells. To overcome this, we used a human immunodeficiency virus (HIV)-derived lentiviral vector and examined the transduction profile of the vector in the cerebellum. A lentiviral vector expressing GFP was injected into the cerebellar cortex. Seven days after the injection, GFP was predominantly expressed in Purkinje cells. GFP was also expressed, though less efficiently, in other cortical interneurons and Bergmann glias. In contrast to reported findings with other viral vectors, no transduced cells were observed outside of the cerebellar cortex, even in the deep cerebellar nuclei, pontine nuclei and inferior olivary complex, which are synaptically linked with Purkinje cells or granule cells. Thus, when HIV-derived lentiviral vectors were injected into the cerebellar cortex, transduction was limited to the cells in the cerebellar cortex, with the highest tropism for Purkinje cells. These results suggest that HIV-derived lentiviral vectors are useful for the study of gene function in Purkinje cells as well as for application as a gene therapy tool for the treatment of diseases that affect Purkinje cells.

P225 (3P2-122)

Transneuronal regulation of synapse formation and plasticity by Cbln1

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Cbln1 is the prototype for a family of four brain-specific proteins (Cbln1-Cbln4) of unknown function that was first identified by virtue of its harboring a naturally occurring 16 amino acid peptide, cerebellin. Cbln1 is a cerebellum-specific protein and structurally related to the C1q and Tumor Necrosis Factor families of proteins. We show here that Cbln1 is secreted from cerebellar granule cells as a glycoprotein and is essential for three processes in cerebellar Purkinje cells: the matching and maintenance of pre- and post-synaptic elements at parallel fiber-Purkinje cell synapses, the establishment of the proper pattern of climbing fiber-Purkinje cell innervation, and the induction of long-term depression at parallel fiber-Purkinje cell synapses. Interestingly, the behavioral, physiological and anatomical phenotype of cbln1-null mice precisely mimics loss-of-function mutations in the orphan glutamate receptor, GluRΔ2, a gene selectively expressed in Purkinje neurons. Therefore, Cbln1 secreted from presynaptic granule cells may be a component of a previously undocumented trans-neuronal signaling pathway that controls synaptic structure and plasticity.

P226 (3P2-123)

Depolarizing GABAergic mechanisms of hippocampal seizure-like activity in post-tetanic and low-Mg2+ conditions

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GABA is known to be a major inhibitory neurotransmitter in mature mammalian brains. However, the effect of GABA can be converted into depolarizing or even excitatory when the postsynaptic Cl^- concentration becomes relatively high. We have recently shown that seizure-like afterdischarge induced by tetanic stimulation in normal ACSF (post-tetanic afterdischarge) is mediated by GABAergic excitation in mature hippocampal CA1 pyramidal cells. Here we investigated the possible contribution of similar depolarizing/excitatory GABAergic input to seizure-like afterdischarge induced in a low extracellular Mg^{2+} condition, as another experimental seizure model (low-Mg^{2+} afterdischarge). Perfusion of $GABA_A$ antagonists abolished low-Mg^{2+} afterdischarge in most cases. Each oscillatory response during low-Mg^{2+} afterdischarge was dependent on Cl^- conductance and contained an F^--insensitive depolarizing component in the pyramidal cells. Perforated patch-clamp recordings revealed that GABA responses were indeed depolarizing during low-Mg^{2+} afterdischarge. Moreover, interneurons in the strata pyramidale and oriens discharged in oscillatory cycles more actively than those in other layers. These results suggest that the depolarizing GABAergic input may facilitate oscillatory synchronization among hippocampal CA1 pyramidal cells during low-Mg^{2+} afterdischarge in a fashion similar to the expression of post-tetanic afterdischarge.

P227 (3P2-124)

Possible roles of synaptic vesicles in plasticity of enhanced neurotransmission via Ca^{2+}-induced Ca^{2+} release at frog motor nerve terminal.

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Ca^{2+}-induced Ca^{2+} release (CICR) takes place in response to Ca^{2+} entry via the activation of type-3 ryanodine receptors (RyRs) after its use- and Ca^{2+}-dependent priming, and amplifies impulse-evoked transmitter release in frog motor nerve terminals. Since the activation of CICR occurs in less than 1 msec after a nerve impulse, the site of Ca^{2+} release is close to the high $[Ca^{2+}]_i$ microdomain, where the machinery of the exocytosis is activated. Then, the most possible Ca^{2+} stores, on which RyRs reside, would be synaptic vesicles. We studied here the effects of loading Ca^{2+} chelator into synaptic vesicles on the priming and induction of CICR. EGTA was loaded into synaptic vesicles by incubating preparations in a Ca^{2+}-free, EGTA (1mM) and Mg^{2+} (1 or 10mM) solution for 20-30 min, in which endocytosis still took place following high frequency stimulation of the nerve in low Ca^{2+} (0.15-0.5mM), high Mg^{2+} (6-10mM) solutions. After loading EGTA, tetanus-induced rises in end-plate potential (EPP) amplitude and miniature EPP (MEPP) frequency, reflecting the priming and activattion of CICR, became slower in onset and smaller in amplitude and rate of rise. Results are in favor of the idea that synaptic vesicles are involved in the priming and activation of CICR and so synaptic plasticity.

P228 (3P2-125)

Blockade of L-type, but not T-type, calcium channels enhanced LTD magnitude induced with low frequency stimulation at hippocampal CA 1 synapses

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To induce long-term depression (LTD) at hippocampal CA1 synapses, $[Ca^{2+}]i$ must sufficiently rise in CA1 pyramidal cells. Each type of calcium entry is believed to have a distinctive role in LTD induction. The present study focuses on roles of voltage gated calcium channels (VGCCs) in LTD induction at Schaffer collateral-to-CA1 synapses in the hippocampal slices. VGCCs are known to be involved in many important cellular processes including synaptic plasticity. In control experiments, the magnitude of LTD varied depending on stimulus frequencies. Also, LTD evoked by any of the tested frequencies (0.5-2Hz) required NMDA receptor activation. T-type VGCCs turned out to have no significant role in LTD induction, since T-VGCC blockade did not change normal LTD induction. When L-type VGCCs were blocked with nimodipine, LTD magnitude was enhanced with low frequency (0.5-Hz) stimulation and was reduced with high frequency (1- or 2-Hz) stimulation. We were particularly interested in the enhancement of LTD by L-VGCC blockade in response to low frequency (0.5 Hz) stimulation, since we had previously observed ryanodine receptor activation could also enhance LTD in response to the same low frequency stimulation. Application of calcium store depleter, thapsigargin, in addition to nimodipine canceled out the LTD enhancement. Hence, intracellular calcium release seemed to play a part in nimodipine-induced LTD enhancement that we observed in a low stimulus frequency range.

P229 (3P2-126)

Auditory fear conditioning increases cell proliferation in the basomedial nucleus of rat amygdala

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It has been known that proliferating cells exist in the amygdala, but their functions remain to be clarified. On the other hand, the amygdala is known to play essential roles in the emotional learning. Therefore, we examined whether the auditory fear conditioning affects the cell proliferation in the rat amygdala as well as other brain areas. Male SD rats were used. On day 1, the rats of conditioned group received five pairings of tone (20 sec) as the conditioned stimulus (CS) and foot shock (1 sec) as the unconditioned stimulus (US) (CS/US group). The control group rats received only CS (CS group). After the training, rats were injected with BrdU. On day 2, rats were placed in a cage and exposed to the tone and their freezing responses were evaluated. Thereafter, rats were perfused and their brains were removed. Serial 40 μm-thick coronal sections of the brains were cut with a cryostat and BrdU immunohistochemistry was performed. BrdU-labeled cells were quantified in each brain area. There was no statistical difference in the number of BrdU-labeled cells in subventricular zone, septal nucleus, subgranular zone of dentate gyrus, or entire amygdala between two groups. In the basomedial amygdala (BMA), BrdU-labeled cells were more abounding in CS/US group. These results suggest that the cell proliferation in the BMA may be involved in the formation of the auditory fear conditioning.

P230 (3P2-127)

Layer-dependent effects of Homer1a/Vesl-1S on long-term depression at corticocortical synapses in the rat visual cortex

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In the central nervous system, synaptic efficiency is modifiable in activity-dependent manners. Homer1a/Vesl-1S, an activity-dependently inducible member of the scaffold protein family Homer/Vesl, has been implicated in long-term up-regulation of synaptic efficiency (various forms of LTP), as well as in short-term modification of AMPA receptors (arguably, both up- and down-regulation). It is not clear, however, whether Homer 1a takes part in induction of LTD (long-term depression). The present experiments examined roles of Homer 1a in inducing LTD at a variety of corticocortical synapses in rat visual cortex slices by using whole-cell patch clamp. Homer 1a was injected by diffusion from patch pipettes. With or Without Homer 1a injected, LTD induction was attempted by pairing 1 Hz stimulation with post-synaptic depolarization for 10 min. Without Homer 1a, LTD was induced at synapses between layer IV axons onto layer II/III pyramidal cells, those between layer II/III axons onto layer V pyramidal cells and those between layer II/III axons onto layer VI pyramidal cells. However, at synapses between layer IV axons onto layer VI pyramidal cells, LTD induction was failed. With Homer 1a protein injected, on the other hand, LTD induction was reduced in magnitude at layer II/III-to-layer VI pyramidal cell synapses, but not at the other synapses examined, suggesting a synapse-specific effect of Homer 1a.

P231 (3P2-128)

Interaction of GluRδ2 and PICK1 implicated in the induction of cerebelallar LTD

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The glutamate receptor δ2 subunit (GluRδ2) is selectively expressed in cerebellar Purkinje neurons (PNs) and is involved in the long-term depression (LTD). However, little is known about the mechanism of its action. Acute expression of the wild-type GluRδ2 in the GluRδ2-deficient PN rescued the LTD induction, suggesting the direct role of GluRδ2 in LTD. To identify the critical region of GluRδ2 necessary for the LTD induction, we constructed and expressed various mutant GluRδ2 proteins in the GluRδ2-deficient PNs. The mutant GluRδ2 possessing the membrane-proximal 21 amino acid residues (AAs) in C-terminal cytoplasmic region rescued the LTD induction, whereas the mutant with membrane-proximal 13 AAs failed. In addition, overexpression of the membrane-proximal 14-20 AAs fused to EGFP suppressed the LTD induction in a wild type PN. These results suggest that the membrane-proximal 14-20 AAs of GluRδ2 plays an essential role in LTD. Then, we identified protein interacting with C kinase 1 (PICK1) as a molecule interacting with the membrane-proximal C-terminal region of GluRδ2 by yeast two-hybrid screening. PICK1 co-localized with GluRδ2 at spines of PNs, and immunoprecipitation assays showed that GluRδ2 bound to PICK1 mainly through the membrane-proximal 14-20 AAs. These results indicate that the membrane-proximal 14-20 AAs of GluRδ2 are essential for both LTD and interaction with PICK1, and suggest that interaction between GluRδ2 and PICK1 might be critical for the LTD induction.

P232 (3P2-129)

Activity pattern-dependency of BDNF release from mossy fiber terminals of hippocampus

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Brain-derived neurotrophic factor (BDNF), which was first identified as a molecule regulating neuronal survival and differentiation, has a pivotal role in the regulation of synaptic plasticity, especially long-term potentiation (LTP). Recent studies have shown that BDNF is transported anterogradely along axon and stored in the presynaptic terminals. The mossy fiber (MF) terminals, which are axon terminals of the granule cells in the dentate gyrus (DG) of the hippocampus, contain the highest concentration of BDNF in the CNS. These observations led us to hypothesize that BDNF is released from MF terminals and that its release is dependent on the activity pattern. We made a Sindbis virus vector containing a mRNA coding the fusion protein constract of BDNF and Venus, one of green fluorescent protein derivatives, and inoculated it stereotaxically to the DG cells of mouse hippocampus (P14-21). After 2-3 days, MF boutons accumulating BDNF-Venus were identified in the acute slice under confocal microscopy. The activity-dependent BDNF release was measured as a reduction of the fluorescence intensity of the individual presynaptic terminal. We found that the activity patterns as high frequency stimulation and theta-burst stimulation are more effective on the BNDF release than low frequency stimulation. It is suggested that BDNF is released from the MF terminals during induction of LTP.

P233 (3P2-130)

Repeated LTD induction causes long-lasting synaptic elimination, requiringprotein synthesis

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Synaptic plasticity is assumed as the cellular basis of memory. Long-termplasticity that lasts for days/weeks has not been fully analyzed, mainlybecause of the lack of model system. We found previously that the repeatedinduction of LTP in cultured hippocampal slice caused a long-lastingsynaptic enhancement accompanied by the formation of new synapses, which wasseparate from LTP itself. We found recently that the repeated induction ofLTD by mGluR activation in the same specimen caused a long-lasting decreasein synaptic strength accompanied by the elimination of synapses. Thus wepropose that these repetition-dependent synaptic changes can serve as themodel system for the analysis of long-term plasticity. Here we addfollowing findings that support this proposal. 1) The synapse eliminationis independent of the means of LTD induction, since LTD induced not only byDHPG (dihydroxyphenylglycine, a class I mGluR agonist) but also by a lowdose of NMDA (N-methyl-D-aspartate, an NMDAR agonist) or by DHO(dihydroouabain, a Na/K-ATPase inhibitor) led to the equivalent synapticelimination, when repeated three times (as monitored by electrophysiologicaland morphological indices). 2) The elimination required protein synthesis,since the application of anisomycin (an inhibitor of mRNAs translation toproteins) suppressed the development of synapse elimination.

P234 (3P2-131)

Functional development of neural circuit formation in the embryonic chick olfactory pathway: Optical imaging of voltage-sensitive dyes

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To understand the functional organization of the central nervous system (CNS), it is essential to know how sensory information is processed within the CNS. We have been approaching this topic by following the ontogenetic patterning of neural circuit formation related to the cranial and spinal sensory inputs using multiple-site optical recording techniques with voltage-sensitive dyes. In this study, we surveyed developmental organization of neural networks related the olfactory nerve (N. I) in the embryonic chick forebrain. Stimulation applied to the olfactory nerve elicited excitatory postsynaptic potential (EPSP)-related optical signals in the olfactory bulb from the 7-day old embryonic stage (E7). The EPSP was mediated by glutamate, and NMDA- and non-NMDA-receptor components were identified. In more developed stages, in addition to the responses in the olfactory bulb, another response area was discriminated within the cerebrum, which seemed to correspond to the higher-ordered nucleus of the olfactory pathway. The results suggest that the olfactory pathway is functionally generated at early stages of development when neural networks related to other visceral and general somatic sensory inputs are also in the process of developing.

P235 (3P2-132)

Voltage-sensitive dye imaging of oscillatory activity in the embryonic chick olfactory bulb

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In an accompanying study, using a multiple-site optical recording technique with a voltage-sensitive dye, we examined the developmental organization of the olfactory pathway in the embryonic chick forebrain, and showed that functional synaptic transmission in the olfactory bulb was expressed at around E7. It is known that odor stimuli elicit oscillatory events in the olfactory bulb in various species. We found that oscillatory activity was also generated in the chick olfactory bulb during embryogenesis. At early stages of development (E7-E8), postsynaptic response-related optical signals evoked by olfactory nerve stimulation exhibited a simple monophasic waveform that lasted a few seconds. As development proceeded, the pattern of the optical signal became complicated, and oscillatory activity was observed in a later phase of the postsynaptic response. The oscillation was restricted to the olfactory bulb, and this spatial pattern was different from that of the propagating wave activity termed the depolarization wave. We examined spatio-temporal patterns of the oscillatory activity in different stages, and studied its developmental dynamics.

P236 (3P2-133)

Activity-dependent synapse elimination of corticispinal tract in mouse slice co-culture

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We have succeeded in reconstructing the corticospinal (CS) synapses in vitro by co-culturing the rat sensorimotor cortex and cervical spinal cord. In this in vitro model of CS projection, CS synapses are formed diffusely throughout the spinal gray matter but later the synapses in the ventral side are eliminated, which is NMDA-dependent (Ohno et al, J Neurosci 2004) with a critical period of 6-11DIV (Ohno & Sakurai, Neuroscience 2005). In order to use the genetically-modified mice to study the underlying molecular mechanisms of this developmental plasticity, we tried to reconstruct the same system using mice. Entire blocks of the brain and spinal cord were taken from P0 C57 BL/6 mice. Coronal slices of the sensorimotor cortex and axial slices of the cervical cord were sectioned (350 μm), and forelimb areas in the cortex was dissected from each section. By recording field EPSP (fEPSP) along 80 μm-interval lattice in the spinal gray matter in response to the stimulation of cortical deep layer, we evaluated spatial distribution of synapse formation quantitatively. Field EPSPs were recorded diffusely throughout the spinal gray matter at 6-9 DIV, then the amplitudes of fEPSPs in the ventral side began to decrease at 10 DIV, and dominated in the dorsal area at 14-15 DIV. CS axon terminals labeled with biocytin anterogradely distributed diffusely throughout the spinal gray matter at 7-9 DIV but the axons terminals in the ventral area were eliminated until 14 DIV. This synapse elimination from the ventral side was blocked by APV, indicating that this process is also NMDA-dependent.

P237 (3P2-134)

Expression of the ABC transporter ABCA2 in rat myelinating and non-myelinating Schwann cells

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We have previously shown in rat brain that ABCA2, which belongs to the A subclass of ATP-binding cassette (ABC) transporter superfamily, is predominantly expressed in the cytoplasm of oligodendrocytes but not in $GFAP^+$ astrocytes, $CD11b^+$ microglia, or $NG2^+$ progenitors. In addition, onset of ABCA2 expression in oligodendrocytes coincides with the appearance of myelin segments immunolabeled with myelin basic protein, implying a role of ABCA2 in transport of substances related to myelination processes. Consistently, expression of ABCA2 was detected in $S100\beta^+$ Schwann cells in human and rat peripheral nerve. Unexpectedly, however, ABCA2 also was detected in S100β-weakly positive cells containing number of densely packed, thin axons in peripheral nerve, implying expression of ABCA2 in non-myelinating Schwann cells. Indeed, multiple immunolabeling with ABCA2, S100β, GFAP, and a zinc finger transcription factor Krox20, one of the most reliable makers for myelinating Schwann cells, revealed that ABCA2 is expressed not only in myelinating Schwann cells but also in non-myelinating Schwann cells. As number of non-myelinating axons are thinly wrapped by single non-myelinating Schwann cells, ABCA2 might contribute to transport of lipid components commonly required for surrounding myelinating and non-myelinating axons.

P238 (3P2-135)

Neural differentiation of neural stem cells from adult human brain

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Recent reports suggest that the adult human brain contains undifferentiated, multipotent precursor cells or neural stem cells (NSCs). In this study, we tried to isolate the NSCs from the surgically dissected hippocampus of the adult human using "Neurosphere" methods and characterize their ability of the proliferation and the differentiation. When the dispersed cells from the adult human hippocampus were cultured in defined medium containing LIF (10 ng/ml), EGF (20 ng/ml), FGF2 (20 ng/ml) and B-27 (2%), the sphere-forming cells which diameter is approximately 200 μm were observed. These cells can be maintained and expanded in this condition for at least 6 months. The population doubling time was approximately 16 days. Immunocytochemical analysis showed that most of cells under this growth condition expressed neural stem cell marker protein, nestin. To induce differentiation, growth factors were removed from the medium. Immunocytochemical analysis showed that many cells expressed neuron marker protein, Tuj1 and astroglial marker protein, GFAP in this differentiation condition. These results suggest that these cells are multipotent NSCs. To improve the efficiency of neuronal differentiation, we are now examining the effects of various soluble factors in the differentiation medium on the neural and astroglial differntiation of the isolated NSCs.

P239 (3P2-136)

Developmental Changes of GABAergic Inhibitory Synapses in the Deep Cerebellar Nuclei

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Activity of the deep cerebellar nuclei (DCN) takes an important role in outputting processed information from the cerebellum. In this study, we first investigated modulatory effects of serotonin (5-HT) receptor on GABAergic synapses in the rat DCN using whole-cell recordings in the cerebellar slices. Both of an endogenous agonist 5-HT and a 5-HT1 agonist 5-CT decreased the amplitude of stimulation-evoked IPSCs (eIPSC) in DCN neurons, and their effect was reversibly abolished by a 5-HT1A and 1B antagonist, cyanopindolol. Further, a selective 5-HT1A agonist 8-OH-DPAT had no effects of the amplitude of eIPSCs. Based on these results, the activation of 5-HT1B receptor is suggested to be responsible for decreasing the amplitude of eIPSCs. In the developing DCN neurons, we next examined developmental changes in both the kinetics of GABAergic postsynaptic currents and the modulatory effects of 5-HT on GABAergic synapses in the rat DCN neurons. At younger stage (around P14), eIPSCs showed slower kinetics and were more susceptible to the 5-HT-induced modulation than those at older stage (around P21). These pre- and postsynaptic parameters showed time-matched changing during development. These results suggest that the information flow from the cerebellar cortex is finely controlled at younger developmental stages, which is important to form the normal cerebellar function in the adult.

P240 (3P2-137)

Reduction in metabotropic glutamate receptor-mediated presynaptic inhibition of GABA/glycine synapses on developing rat LSO neurons

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The lateral superior olive (LSO) is the first auditory center that processes differences in the sound level between the two ears. Here we report the developmental changes in metabotropic glutamate receptor (mGluR)-mediated presynaptic inhibition of GABAergic/glycinergic synaptic transmission onto developing rat LSO neurons using conventional whole-cell patch clamp technique. In addition to a developmental switch of MNTB-LSO afferents from GABAergic to glycinergic IPSCs during development, immature MNTB-LSO synapses could release glutamate with GABA/glycine. Bath application of DCG IV, a selective mGluR 2/3 (group II) agonist, greatly reduced IPSC amplitude in neonatal (< P5) with a significant change in the paired-pulse ratio, which was eliminated in the presence of group II antagonist, suggesting that DCG IV acts presynaptic mGluR 2/3 leading to reduce the release probability of GABA and/or glycine release from presynaptic nerve terminals. However, the mGluR-mediated presynaptic inhibition was gradually reduced with postnatal development, in which DCG IV had little effect on MNTB-evoked IPSCs recorded from P16-18 LSO neurons. At P5 LSO neurons, presynaptic mGluR could be activated by endogenous glutamate released from the ipsilateral anteroventral cochlear nucleus (AVCN) afferent, but not from MNTB terminals. Based on these results, the functional roles of presynaptic mGluR in the development of LSO neurons will be further investigated and discussed.

P241 (3P2-138)

Single-cell-based analysis of neural differentiation of the NSCs derived from embryonic mouse striatum

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Neural stem cells (NSCs) are defined as self-renewing, multipotent progenitor cells that give rise to neurons, astrocytes and oligodendrocytes.Using single-cell-based on-chip cell-cultivation system, we analyzed the process of neural differentiation from the NSC and examined the effects of BDNF, which is known to enhance neuronal differentiation.The NSCs were obtained from the striatum of E12.5 nestin-promoter GFP transgenic mice and an individual cell was placed into 32 pairs of agar microchambers connected by microchannels.The NSCs were differentiated by media containing FBS, retinoic acid and forskolin in the presence or absence of BDNF and cell adhesion, neurite outgrowth were examined by recording a series of phase-contrast images and immunocytochemistry for neural marker, TuJ1.We observed that the NSCs attached the microchamber and a part of cells exhibited neuron-like morphology and extended some neurites along microchannels.BDNF increased the rate of neurite outgrowth.It, however, failed to affect cell adhesion.Thus, using this system, we could address the process of neural differentiation from the NSCs in a single-cell-based level and could demonstrate that BDNF had the ability to promote the neural differentiation.

P242 (3P2-139)

EGF receptor (ErbB1) stimulation down-regulates synaptic inputs of neocortical GABAergic neurons

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Neurotrophins and cytokines are involved in neuronal differentiation, synaptic development and plasticity. In neocortical culture, we reported epidermal growth factor (EGF) family (ErbB1 ligands; EGF, TGF-alpha, HB-EGF) down-regulates an AMPA receptor molecule, GluR1. Using neocortical cultures and EGF-administered animals, we electrophysiologically evaluated the effects of the EGF family on synaptic development and plasticity in the GABAergic neurons. In neocortical culture, subchronic treatment with TGF-alpha reduced the expression of GluR1-immunoreactivity in glutamic acid decarboxylase (GAD) 67 immunopositive GABAergic neurons. Whole-cell patch-clamp recording from morphologically identified putative GABAergic neurons revealed decreases both in AMPA currents and amplitudes of mEPSCs by TGF-alpha. Subcutaneous administration of EGF for 14 days in neonatal mice also decreased protein levels of AMPA and NMDA receptors in the frontal cortex. Immunohistochemical study revealed that the decrease in GluR1 levels was relatively specific for the parvalbumin-positive GABAergic neurons. Miniature analyses in cortical slices show that the amplitudes of mEPSCs in the GABAergic neurons decreased significantly, whereas no alteration was observed in the pyramidal neurons. Thus, activation of ErbB1 receptors during cortical development negatively regulates synaptic inputs and plasticity in the GABAergic neurons.

P243 (3P2-140)

Influences of neural activity on motility and dendritic development of cortical GABAergic neurons

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During development of cerebral cortex the two major types of neurons, pyramidal and GABAergic neurons, migrate to their destination through different routes and extend their dendrites at different timing. Questions arise whether neuronal activity gives any influence on these processes of neuronal development, and if so, whether its influence differs between the two types of neurons. To address these questions, we used neuron culture preparations of the visual cortex of GAD67-GFP knock-in mice, in which GABAergic neurons can be identified by GFP. With time-lapse imaging analysis we observed effects of drugs, which block or enhance neural activity, on the motility and dendritic development of GABAergic and pyramidal neurons. Until 7 days in vitro (DIV) GABAergic neurons moved very quickly, although the movement was not smooth, while pyramidal neurons did not show such high motility. After their motility diminished, GABAergic neurons started to develop their dendrites. An application of tetrodotoxin (TTX) increased the motility of GABAergic neurons and extended the period when such high motility is maintained. The application of TTX and antagonists for ionotropic glutamate receptors until 7 DIV retarded the dendritic development of GABAergic neurons, while did not significantly affect that of pyramidal neurons. These results suggest that GABAergic neurons are more susceptible to neuronal activity than pyramidal neurons and activity may be a factor to stop migration of GABAergic neurons and to start development of their dendrites.

P244 (3P2-141)

Promoting action of endogenous and exogenous epidermal growth factor-ErbB1 signals on the development of midbrain dopaminergic neurons

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Epidermal growth factor (EGF) binds to ErbB1 receptor and exerts a neurotrophic activity on midbrain dopaminergic neurons. Here, using EGF administrated animals and midbrain cultures, we investigated endogenous and exogenous ErbB1 activity on the development of dopaminergic neurons. Immunostaining of tyrosine hydroxylase (TH) revealed that the chronic administration of ErbB1 inhibitors (PD153035, ZD1839) to rat neonates prevented dopaminergic neurons from axonal fiber outgrowth and striatal innervation. Further, protein levels of TH and dopamine transporter decreased in the striatum but did not change in the frontal cortex. In midbrain culture, EGF had promoting activities in cell-survival and dopamine uptake. To monitor the development of intrinsic excitability of the dopaminergic neurons we prepared midbrain cultures from TH-EGFP transgenic mice. Whole-cell current-clamp recording from EGFP positive dopaminergic cells revealed that chronic treatment with EGF increased the number of action potentials induced by current injections. Further, daily administrations of EGF in vivo increased AMPA-mediated synaptic responses in the dopaminergic neurons. These findings suggest that ErbB1 ligands such as EGF have a neurotrophic activity for the development of midbrain dopaminergic neurons.

P245 (3P2-142)

Corticospinal re-innervation following elimination of early formed synapses during development

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In the previous study, we showed that the corticospinal (CS) synapses and terminals are distributed in the whole spinal cord at P7 and then eliminated from the ventrolateral side until P10. In this study, we further investigated the development of CS synapses subsequent to the elimination electrophysiologically and morphologically. Field EPSPs of CS synapses were evoked by stimulation of the medullary pyramid and recorded from the lower cervical cord (C7). For anterograde labeling of the CS terminals at C7, biotin dextran amine (BDA) was injected to the sensorimotor cortex. The spinal cord were fixed and sectioned several days after the injection. At the beginning of morphological study we determined the area of sensorimotor cortex projecting to C7. The amplitude of the largest negative peak of the field EPSPs continuously increased until 4 or 5 postnatal week. On the other hand, the spatial pattern of the distribution of the potentials was nearly constant after P10. The terminal distribution was analyzed by counting the number of axons within ventrolateral and dorsomedial area. After a relatively stable period of P10 and P11 following the elimination, the number of the terminals increased again in the ventrolateral area, which was the case with the dorsomedial area. The continuous amplitude increase of largest negative field EPSPs may be an electrophysiological counterpart of CS terminals re-innervation. Thus development of the CS innervation seems to consist of at least two steps.

P246 (3P2-143)

Presynaptic TRPV1 and nACh receptors mediate robust synaptic facilitation in area postrema neurons of the rat

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Inhibitory synaptic transmission and its modulation in neurons of the area postrema (AP), one of autonomic nuclei in the medulla, were studied using whole-cell patch-electrodes applied to slices from newborn rats. When external saline containing 20 mM KCl was applied from a "Y tube" to AP neurons, which were whole-cell clamped at -10 mV, massive inhibitory postsynaptic currents (IPSCs) were induced. Most of the evoked IPSCs were blocked by bicuculline, indicating GABAergic identity, while the remaining minority of synaptic currents was sensitive to strychnine. When nicotine (5-100 μM) or capsaicin (0.1-1 μM) was applied to AP neurons, robust appearance of IPSCs with GABAergic identity was induced. After blocking action potential generation in the slice with tetrodotoxin (1 μM), nicotine or capsaicin could still induce GABAergic IPSCs. The nicotine-induced presynaptic facilitation was significantly inhibited by mecamylamine, and it was slightly inhibited by dihydro-β-erythroidine and negligibly inhibited by α-bungarotoxin. Interestingly, responses to capsaicin of the synaptic facilitation showed marked desensitization; even after five minutes of rigorous washout, the magnitude of synaptic facilitation by capsaicin was 10-30% of that evoked by the first application. It is concluded that nicotinic receptors, as well as capsaicin receptors (presumably TRPV1), are expressed at GABAergic presynaptic terminals in area postrema neurons and play a distinctive role in controlling excitability of these neurons for their proper function in the autonomic system.

P247 (3P2-144)

Hydrophilic changed [Ala4Lys5]-des-acyl ghrelin$^{(1\text{-}10)}$ fails to elicit cardiovascular responses in the rat nucleus tractus solitarius

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The neuronal mechanisms underlying the cardiovascular activities of des-acylated ghrelin (DAG) and ghrelin remain unclear. Not only ghrelin but also DAG that is endocrinically inactive form of ghrelin without binding efficacy to growth-hormone secretagogue receptor type 1a (GHSR-1a) on the pituitary grand exerts cardiovascular actions when microinjected into the rat nucleus tractus solitarius (NTS). These responses may be attributable to a receptor(s) other than GHSR-1a. DAG lacks hydrophobic octanoylation at Ser3 from ghrelin but retains hydrophobicity at Phe4Leu5. We studied the cardiovascular effects in the rat NTS of a synthetic peptide with a hydrophobic-to-hydrophilic substitution at Phe4Leu5 to Ala4Lys5 of DAG$^{(1\text{-}10)}$. The intra-NTS microinjection of 80 or 200 pmol/100 nl of the synthetic peptide produced no changes in the rat mean arterial pressure and heart rate. In addition, pretreatment with 200 pmol of the synthetic peptide had no antagonistic effect on the cardiovascular response induced by 80 pmol of DAG or native ghrelin. The synthetic peptide was incapable of evoking hypotensive and bradycardic responses in the NTS. Our results suggest that hydrophobicity at amino acid position 4 to 5 of DAG may be essential to bind a new receptor and to evoke the cardiovascular responses in the NTS.

P248 (3P2-145)

Spontaneous activity of locus coeruleus neurons is reduced in spontaneously hypertensive rat (SHR) , AD/HD model rat.

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The main problem of attention deficit/hyperactivity disorder (AD/HD), one of developmental disorders of children, is difficulty in control or restrain of behavior. An imbalance of dopaminergic and noradrenergic transmission is proposed for neural mechanisms of AD/HD. On adult rats, we have already reported that methylphenidate, the most common therapeutic agent for AD/HD produced a hyperpolarizing response associated with decrease in a membrane resistance by activation of the inward rectifier K^+ channels via α_2-adrenoceptor of locus coeruleus (LC) neurons where the most major source of noradrenergic tones in CNS. The spontaneously hypertensive rat (SHR) are often used as a model of AD/HD. Behavioral studies established that SHR displayed hyperactivity, impulsivity, poor stability of performance and poorly sustained attention, when compared with their normotensive Wistar-Kyoto (WKY) control rats. In the present study, the tonic activity of LC neurons of juvenile SHR was examined by using a whole-cell patch clamp technique and compared with WKY. As a result, the resting membrane potential of LC neurons in SHR (-47.7±0.39 mV) was significantly decreased when compared with WKY (-51.4±0.63 mV). Nevertheless, the frequency of spontaneous action potentials of SHR (0.64±0.24 Hz) was significantly lower than that of WKY (2.14±0.53 Hz). These results suggest that noradrenergic spontaneous activity on LC neurons in SHR is reduced compare with WKY.

P249 (3P2-146)

Norepinephrine content was increased in locus coeruleus and medial prefrontal cortex of spontaneously hypertensive rat (SHR), AD/HD model rat

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It has been reported that the role of norepinephrine (NE) and dopamine (DA) in the brain is closely related to the etiology of attention-deficit/hyperactivity disorder (AD/HD). In this report, by using the HPLC method, the amounts of NE and DA in the locus ceruleus (LC), the medial prefrontal cortex (mPFC), and the striatum (Str) were measured and compared spontaneously hypertensive rats (SHR), an AD/HD model rat, and Wister-Kyoto rats (WKY) as the contrast rat. The results showed that the amount of NE were significantly larger than those in the mPFC and Str of WKY. On the other hand, the amount of DA has proven to be large in Str, and very small in the LC and the mPFC. This is also the case with SHR; the amount of NE and DA in LC, mPFC, and Str shows the same pattern. However, in the LC, the amount of NE in SHR was increased significantly more than that in WKY. The same tendency can be seen in mPFC, however, in Str, the amount of NE did not show any increase, compared with that in WKY. Furthermore, the amount of DA in the LC was increased significantly in SHR, compared with that in WKY, whereas any significant difference could not be recognized in mPFC and Str. These results suggest that the increase of NE and DA in the LC and mPFC of SHR is related to the characteristic behavior in AD/HD.

P250 (3P2-147)

Distinct presynaptic mechanisms underlie firing frequency-dependent modulation of synaptic transmission in the solitary complex

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The afferent fibers in the vagus nerve transmit visceral information encoded as varying firing frequency to the second-order neurons in the nucleus of the solitary tract (NTS) and dorsal motor nucleus of the vagus (DMX). The purpose of this study was to examine how the firing frequency affects transmission efficiency at these synapses. EPSCs evoked by the solitary tract (TS) stimulation were recorded from DMX and NTS neurons in the thick brainstem slices of young rats. TS stimulation at various frequencies (0.1 - 20 Hz) revealed distinct frequency-dependent responses in the EPSC amplitude among different types of neurons recorded. When stimulated at 20 Hz, NTS neurons and low-pass type DMX neurons exhibited marked amplitude reduction (<30% of the first EPSC) within 10 pulses, whereas high-fidelity (hi-fi) type DMX neurons presented only modest attenuation (>40%). These neurons exhibited distinct short-term plasticity as revealed by paired-pulse ratio (PPR) evaluation. Surprisingly, unlike the NTS neurons, PPRs in the low-pass DMX neurons were not significantly affected by changes in $[Ca^{2+}]_o$, suggesting distinct mechanisms for their short-term depression. These results indicate that the transmission efficiency between the visceral afferents and second-order neurons depends largely on the firing frequency mostly through distinct target cell-dependent presynaptic mechanisms, which might result in differential activation of distinct components in the solitary complex network.

P251 (3P2-148)

Expression of exogenous protein in the primary afferent neurons for the visceral sensation by in-vivo gene transfer

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In order to understand the mechanism how the visceral information is transmitted to the brain, it is indispensable to identify the roles played by already-identified molecules in the synaptic transmission between the vagal afferent fibers and the second-order neurons in the nucleus tractus solitarii (NTS). The molecules underlying the regulation of transmitter release at this synapse are synthesized in the cell bodies located in the nodose ganglion (NG). Here we challenged to establish an optimized method for efficient in-vivo gene transfer into the NG neurons and evaluated expression of the gene product in the NG and its centrally projecting axons. In young Wistar rats, electrical pulses optimized for electroporation were delivered to the NG immediately following injection of pCAGGS-EGFP plasmid vector (5 μg/μl; through courtesy of Drs. J. Miyazaki and K. Nakajima). Two days after delivery, the NG was dissected out and fixed in 4% paraforaldehyde. In some cases, acute brainstem slices including the NTS were prepared and examined with a confocal microscopy a few weeks later. A large portion of somata in the NG and a large number of fibers projecting from NG expressed EGFP. EGFP fluorescence was also detected in the solitary tract and presynaptic termini in the NTS in the brainstem slices. This technique might be applicable to analyzing specific molecule function in the transmitter release in the central termini of the visceral afferent nerves.

P252 (3P2-149)

Ca^{2+} uptake to mitochondria accompanied by depolarization of mitochondrial membrane potential occured when $[Ca^{2+}]_i$ increased to a critical level at frog motor nerve terminal

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To investigate the Ca^{2+} clearance mechanisms in presynaptic terminals, we measured Ca^{2+} dynamics during nerve stimulation in frog neuromuscular junctions with a low affinity Ca^{2+} dye, Oregon Green 488 BAPTA 6F (K_d=13μM, pH=7.2). During 100Hz tetanus for 4 sec in a normal Ringer's solution (1.8mM Ca^{2+}, 0mM Mg^{2+}), $[Ca^{2+}]_i$ increased in two phases, firstly steep rising (4 μM/sec for 0.2 sec) and secondly slow increasing (0.8 μM/sec). Whenmitochondria were inhibited by rotenone and oligomycin, slow phase disappeared and $[Ca^{2+}]_i$ increased monotony with rapid rate. Membrane voltage imaging of mitochondria with TMRE suggested that Ca^{2+} uptake to mitochondria accompanied by depolarization of mitochondrial membrane potential. When tetanus frequency was reduced to 20Hz, $[Ca^{2+}]_i$ dynamics did not affected by rotenone and oligomycin, however, CCCP increased $[Ca^{2+}]_i$ during tetanus. The effect of rotenone and oligomycin appeared when $[Ca^{2+}]_i$ increased more than 0.6 μM during 40Hz tetanus. These results suggest that Ca^{2+} uptake to mitochondria started when $[Ca^{2+}]_i$ increased more than 0.6 μM. CCCP had larger effect on $[Ca^{2+}]_i$ increase during 40 Hz tetanus than rotenone and oligomycin. These results suggest that CCCP sensitive some Ca^{2+} clearance mechanisms other than mitochondrial Ca^{2+} uptake contributed largely when $[Ca^{2+}]_i$ was lower than some μM.

P253 (2P1-012)

Effect of noise on contrast detection sensitivity in human visual perception

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Recently it has been reported that background noise can improve detection sensitivity of sensory stimuli not only in animals, but also in humans. The present study was designed to examine how human visual perception may be modified by superposition of noise. Twenty-two undergraduate students with normal or corrected to normal vision participated in this study. In a dim chamber, participants observed a signals of a small light spot (white LED) with its intensity modulated by sine waves, usually at a frequency of 1Hz. Random flickering light modulated by white noise was superposed on the signal. Contrast detection threshold was measured with or without noise, using a psychophysical method (the adjusting method). The threshold first decreased then increased as increase in the noise intensity, with the minimum value at around just above the noise-threshold. Thus the increase in the contrast detection sensitivity was found not only at subthreshold, but also suprathreshold intensity of noise. These findings were independent on the signal frequency (1-15Hz). A further experiment replicated these findings using the up-and-down methods to measure the threshold in five participants (3 male and 2 female, aged 20-25 yr). We concluded from these results that a certain amount of noise, even supra-threshold, can improve contrast detection sensitivity in human visual perception.

POSTERS
Sensory functions

P254 (2P1-013)

Simulation analysis of the high-pass filtering of the rod network in the retina

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It is known that the rod network in the retina behaves as a high-pass filter to electrical signals. In the turtle and toad retinae, it was found that the time to peak of the response was shorter in rods further away from a slit of light. In the tiger salamander retina, it was shown that the voltage responses to square current injection became more transient as they travel through the rod network. In previous studies, the high-pass filtering behavior has been attributed to an inductance element, a hyperpolarization-activated current, or a K conductance activated by Ca. However, biophysical mechanism underlying the high-pass filter is not fully understood. The objective of this study is to analyze the functional roles of individual ionic currents in the temporal filtering properties of rods through computer simulations. A model of the rod photoreceptor network was developed. The model incorporates much of the known parameters in rod photoreceptors, i.e., the phototransduction cascade in the outer segment, membrane ionic currents (I_{Ca}, I_{Kv}, $I_{K(Ca)}$, I_h, $I_{Cl(Ca)}$), intracellular calcium system and electrical junctions between rods.In simulation, the temporal filtering properties of the rod was analyzed. The simulated result shows that single rod itself behaves as a high-pass filter. The mechanism underlying the high-pass filter was examined by changing model parameters. The result suggests that $I_{K(Ca)}$, $I_{Cl(Ca)}$ and I_h are responsible for the high-pass filtering. The model also well reproduced the experimental observation that the shortening of the time to peak as the signal propagates laterally.

P255 (2P1-014)

Efficiency of suprachoroidal-transretinal stimulation on retinal neurons nearby the electrode

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As a method of retinal prosthesis we newly developed suprachoroidal-transretinal stimulation (STS), in which electrical current passed between scleral electrode and vitreous electrode (Kanda et al. '04). Previously we reported the spatial properties of STS (Kanda et al., PSJ meeting '05), however the recorded neurons with the receptive fields close to the scleral electrode were few. Here, we enlarged the sampling especially within 2° from the scleral electrode and investigated whether STS can activate neurons in the localized retinal area.

105 unit activities of relay cells in the lateral geniculate nucleus were recorded from 12 adult cats. The response probability to STS of various current intensities between 50-500 μA was examined for each unit, and its relationship with the distance from the electrode was analyzed. It was confirmed that the response probability depended on the distance from the electrode. As the intensity decreased, the units with high response probability limited near the electrode. For example, at 100 μA of STS, the units with the probability over 80% were within 2.5° from the electrode. The median of the probability of the units within 1° was the highest among those with further distance when STS of 150 μA or 200 μA was applied. These results suggested that the retinal activation by STS can be localized near the electrode with adequate current intensity.

P256 (2P1-015)

Expression pattern of $Na_v1.1$ in the rat retina

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Retinal ganglion cells and subsets of retinal amacrine cells generate TTX-sensitive action potentials evoked by light stimulus. It has been reported that voltage-dependent sodium channel (Na_v) α subunits, $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, and $Na_v1.6$, are expressed in the retinal ganglion cells (Fjell J et al., Mol Brain Res 50:197-204, 1997; Boiko T et al., J Neurosci 23:2306-2313, 2003). However, subtypes of Na_v expressed in retinal amacrine cells have not been identified. To examine the specific Na_v subtypes expressed in the retinal amacrine cells, we applied *in situ* hybridization on the rat retina with the RNA probes that recognize $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, and $Na_v1.6$. We found that $Na_v1.2$, $Na_v1.3$, and $Na_v1.6$ were localized in the ganglion cell layer (GCL). Interestingly, $Na_v1.1$ was expressed not only in GCL, but also in the inner nuclear layer (INL). Cell bodies of $Na_v1.1$ positive cells in INL were located on the border between INL and the inner plexiform layer. It is probable that these cells are subsets of amacrine cells. Difference in expression pattern of Na_v might reflex functional difference of action potentials between amacrine and ganglion cells.

P257 (2P1-016)

Neuroprotective effect of transcorneal electrical stimulation on optic nerve injury

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Traumatic injury to the optic nerve often causes a rapid loss of vision within several hours and leads retinal ganglion cells to cell death with slower time course. The previous experiments demonstrated the neuroprotective effect of electrical optic nerve stimulation on the retinal cell death over several days or weeks. However, it is not clear whether the electrical stimulation effectively prevents the acute impairment of visual function as observed in the traumatic optic neuropathy.

We examined whether the transcorneal electrical stimulation could improve visual function after the optic nerve crush. A screw electrode was secured on the skull over the visual cortex to record visually evoked potential (VEP) in adult rats. VEP elicited by flash stimuli was recorded before and after the optic nerve crush, and after the transcorneal stimulation to estimate the degree of damage and the effect of stimulation in individual animal. Calibrated optic nerve crush reduced the amplitude of VEP significantly in all animals (30% of pre-crush value on average). Transcorneal stimulation given through a bipolar electrode fitted to a contact lens (intensity 500μA, duration 50μsec, 20Hz, 6hours) significantly enhances VEP amplitude (270% of the post-crush value on average) and the enhancement was preserved for one week in most cases. These results suggest that the transcorneal stimulation has a protective effect against acute impairment of visual function by optic nerve crush.

P258 (2P1-017)

Electrophysiological Study of Rat Collicular Responses to Suprachoroidal-Transretinal Electrical Stimulation: Evaluation of Pulse Parameters for Artificial Retina

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We recently developed an electrical stimulation method for retinal prosthesis named Suprachoroidal-Transretinal Stimulation (STS, Kanda et al., IOVS 2004). It was shown that a monophasic pulse of STS focally applied between an anode on the sclera and an intravitreal cathode (inward STS) evokes well-localized field potentials in the superior colliculus (SC) of both normal and retinal dystrophic rats. When the outward STS was delivered by reversing the stimulus polarity, the threshold was much higher than that of the inward STS.

To avoid the electrochemical retinal damage due to charge imbalances, a biphasic pulse must be used in the STS-based artificial retina. Thus, we examined the SC response to biphasic STS comparing with that to the monophasic STS. In normal rats, Electrically-Evoked Potentials (EEP) to the single biphasic or monophasic STS were recorded from the SC contralateral to the stimulated eye. In response to the biphasic STS the late oscillatory components of EEP were greatly suppressed, although the early component of EEP was smaller in amplitude than that to the monophasic inward STS. Thus, biphasic STS may be beneficial for generating artificial perception with high temporal resolution, as well as being harmless.

P259 (2P1-018)

Dynamic change in orientation tuning of neurons in the cat lateral geniculate nucleus

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It is commonly believed that orientation selectivity first emerges in the primary visual cortex (V1). In the present study, first, we examined the orientation selectivity of LGN neurons using optimal and non-optimal (in terms of stimulus size and spatial frequency (SF)) grating stimuli in anesthetized cats. We found that although only about 10% LGN neurons showed significantly orientation-biased response to the grating with optimal size and SF, about 90% of LGN neurons exhibited significant orientation selectivity to grating with diameters larger than its classical receptive field (CRF) and SFs higher than the optimal for CRF response. Then, stimulus-size tuning curves were made for responses to stimulation with preferred orientation tested with large stimulus and with orthogonal orientation. These two stimulus-size tuning curves exhibited profile similar to each other under the optimal SF condition. However, high SF grating caused stronger surround suppression for response to the orthogonally oriented stimulus than that to the optimally orientated stimulus. These results suggest that there is orientation tuned surround suppression that is effective around the CRF boundary and its optimal SF is tuned to higher than that of the CRF responses. We should further address how the orientation selectivity of LGN contributes to that in V1.

P260 (2P1-019)

Temporal-frequency dependent surround suppression in early visual pathway

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Neuronal responses of the primary visual cortex (V1) exhibit stimulus-size tuning property, and they are suppressed by stimulation with grating patches larger than their classical receptive field (CRF). Recently, it has been revealed that size-tuning property of V1 neurons is dependent on stimulus parameters, such as luminance contrast and spatial frequency. In this study, we examined the effect of temporal frequency (TF) of sinusoidal grating stimulus on size-tuning curves of cat V1 neurons. Our results showed that when the TF was higher than optimal, the strength of surround suppression became weak and CRF-size became larger, suggesting that V1 neurons summate visual information from wider region under high TF condition. We also tested the effect of changing stimulus-size on TF tuning curve. Corresponding to above results, large grating made peak and high cut-off of TF-tuning curve higher than those for small grating. Then we examined neuronal responses of the lateral geniculate nucleus (LGN), and obtained basically similar results to those of V1 neurons. These results suggest that neurons in early visual pathway change their spatial integration property according to TF of stimulus, in such a way that neurons integrate wide visual field for fast moving stimulus, whereas localized field for slow stimulus.

P261 (2P1-020)

Spatial-frequency dependent surround suppression in early visual pathway

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Neurons in the primary visual cortex (V1) change their responses depending on stimulus parameters such as orientation, spatial frequency (SF), size and so on. In this study, we investigated how stimulus size effects on SF tuning property of neurons in V1 and lateral geniculate nucleus (LGN) of cats. First, we found that V1 neurons increased sensitivity to high SF stimuli when small gratings were used and that SF selectivity of V1 neurons was sharpened when large gratings were used, according to the shifts of peak and high cut-off of SF tuning curves. Second, we measured area summation tuning curves under several SF grating conditions, and found that a higher SF stimulus caused a reduction of the receptive field size and an increase of the surround suppression. The same tendency was observed in LGN, which is the main source of excitatory input to V1. This implies that the relationship between SF and area summation properties observed in V1 has its origin in LGN and is modified in the intracortical network. These results suggest how neural circuit in early visual pathway changes its way of information processing and how it reduces redundancy in various visual environments; for small visual objects, neurons increase SF sensitivity to get fine resolution, on the other hand, for large ones, they sharpen SF selectivity to reduce redundancy.

P262 (2P1-021)

Surround suppression sharpens orientation tuning in cat primary visual cortex

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It is known that orientation tuning of neurons in the primary visual cortex (V1) becomes sharper as the size of stimulus increases beyond the classical receptive field (CRF) (Orban et al., 1979; Chen et al., 2005; Xing et al., 2005). We investigated relationships between the strength of the orientation selectivity and that of the surround suppression in 74 V1 neurons recorded from anesthetized and paralyzed cats. The orientation selectivity became stronger as an increment of surround suppression due to an enlargement of stimulus that covered the CRF and the receptive field surround (SRF). However, the preferred orientation of the neurons was independent of stimulus sizes and did not vary according to a change in stimulus size. Also, both the surround suppression and the orientation selectivity of responses to the stimulus larger than the CRF were significantly stronger in layers II/III than in layers V/VI. We compared our results with predictions of a simple iceberg model where a large stimulus that covered the CRF and SRF equally and linearly suppressed responses to all stimulus orientations. The model, however, overestimated the sharpening of the orientation tuning by large stimulus. These results suggest that surround suppression in V1 exhibits similar to but less-selective orientation tuning than the response to CRF stimulation. Such an effect of surround suppression can be a reason why the stronger orientation selectivity is observed for large stimulus.

P263 (2P1-022)

All-trans retinal acts as a photosensitizer in frog rod photoreceptors.

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It has been suggested that oxidation of rod outer segment (ROS) membrane lipids of photoreceptor cells may be involved in light-induced retinal degeneration. All-*trans* retinal is a potent photosensitizer and its role in mediating photodamage, mainly lipid oxidation by producing singlet oxygen, has been suspected for over two decades as shown in studies using ROS membranes or liposomes. Here we demonstrate that all-*trans* retinal may actually act as a photosensitizer and produce photooxidation in living cells. Exogenous all-*trans* retinal caused lipid oxidation under UV light (365nm) in bleached bovine ROS membranes in proportion to either the duration of UV radiation or the concentration of retinal. Similar results were obtained from dark-adapted ROS membranes with retinal released from rhodopsin after irradiation with long wavelength light (530nm). Finally, consistent with previous studies which have implicated that accumulated all-*trans* retinal may cause light-induced oxidation in photoreceptor cells, intact ellipsoid cells, which are able to quantitatively convert all-*trans* retinal to all-*trans* retinol, showed significantly less oxidation compare to those without ellipsoid when exposed to UV light after releasing all-*trans* retinal with 530nm light. UV did not have much effect when there was no 530nm light treatment. These results indicate that all-*trans* retinal, which is released from rhodopsin by 530nm light, is photosensitizing component in living cells and causes membrane oxidation in living cells.

P264 (2P1-023)

Effects of mouth guard wearing on dynamic visual acuity

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To determine whether mouth guard (MG) wearing improves performance capacity of athletes, we examined the effects of MG wearing on dynamic visual acuity (DVA). Subjects were asked to read and answer 3 numerals presented sequentially at random order. The numerals moved left to right at 90 deg/sec on a CRT monitor under two conditions of head motion: 1) stationary state, and 2) voluntary rightward rotation. In each condition, thirty trials with and without MG were carried out and numbers of correct answers (full score; 3 x 30 = 90) were evaluated as DVA score. We measured DVA improvement indexes (= DVA scores with MG minus those without MG) and occlusal forces. In the condition 1, the correlation-coefficient between DVA improvement indexes and occlusal forces was negative (r=-0.74) for nine subjects (20-32 years), whose DVA improvement indexes were >0 in the condition 2. On the contrary, the correlation-coefficient was positive (r=0.76) in the condition 2. These results suggested that the effects of MG wearing on DVA strongly depended on the condition of head motion and the occlusal force. As we recorded simultaneously electromyographic activities of masseter and sternocleidmastoideus, eye movements and head angular velocities, these data will be described to discuss the effects of MG wearing on DVA.

P265 (2P1-024)

Early change of ocular dominance by brief monocular deprivation in the pharmacologically inhibited visual cortex of kittens.

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Monocular deprivation (MD) during an early postnatal period causes a loss of visual cortical response to the deprived eye (Ocular dominance (OD) shift). When MD is combined with inactivation of the visual cortex by muscimol infusion, cortical neurons lose their response to the open eye (reverse OD shift). Although these two forms of OD plasticity differ in direction, it is largely unknown whether their other characteristics are also different. Such information would be useful to clarify mechanisms underlying these two plasticity. The normal OD shift proceeds rapidly and the physiological effect of MD saturates after 48 hours in kittens. Therefore, we examined how rapidly the reverse OD shift proceeds and whether the shift could be induced in adults, to compare the dynamics and age-dependency of two forms of OD plasticity. We have inhibited the visual cortical neurons by infusing muscimol in four-week old kittens and adults. After 3-6 days of MD, OD of cortical neurons was evaluated by single-unit recording. The reverse OD shift was observed after 6-day MD as reported previously. After 3-day MD, however, the reverse OD shift was not observed and the OD distribution remained similar to that of normal animals. In adults, OD distribution in the inhibited cortex remained unchanged even after 7-day MD. These results suggest that the reverse OD shift might reflect a mechanism of developmental plasticity that has a slower time course than the normal OD shift.

P266 (2P1-025)

Functional mapping of macaque primary visual cortex with implanted multi-electrode array

Miyakawa, Naohisa[1]; Blake, David[2]; Merzenich, Michael[2]; Tanifuji, Manabu[1] (*[1]Integrative Neural Systems, RIKEN BSI, Wako, Japan; [2]Coleman Lab., KeckCenter for Integrative Neurosci., UCSF, San Francisco, USA*)

Functional mapping of macaque primary visual cortex with implanted multi-electrode array Naohisa Miyakawa1, David Blake2, Michael Merzenich2, Manabu Tanifuji1 1 Integrative Neural Systems, RIKEN BSI, 2 Coleman Lab., KeckCenter for Integrative Neurosci., UCSFIt has been known that in primates, different levels of visual information are processed at different areas within the cortical visual streams. Much remain unknown on how each level of information is processed within each area, but some evidence show possibility of information represented in a distributed manner within the local network of respective cortical areas (Tsunoda et al. 2001). To understand the function of this local network activity, we have developed a chronically implantable multi-electrode array that is laid out in high-density grid configuration with 350 μm spacing. We implanted the array to the primary visual cortex of macaque monkey, and detected multi-unit activity (MUA) from all electrodes for as long as 3 month. We reconstructed a two-dimension functional response map by visualizing the neuronal activity evoked with grating stimuli of different orientations. The map showed significant stability over the 3-month period, indicating that our array is capable of monitoring cortical network activity with minimum damage to the cortical tissue over this period.We will show our preliminary result of multi-electrode array recording of infero-temporal (IT) cortex neurons evoked by natural image stimuli.

P267 (2P1-026)

Spatiotemporal dynamics of surround suppression in cat V1: Stimulus-duration and orientation-contrast

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In the primary visual cortex (V1), a neuronal response to stimulation of the classical receptive field (CRF) is suppressively modulated by the stimulus presented at the receptive field surround (SRF). Using stationary flashes of sinusoidal grating as stimuli, we examined the dependency of effect of SRF stimulation on the orientation-contrast (OC) between CRF and SRF stimuli (Exp.1) and that on the presentation duration (50 ms vs. 500 ms) (Exp.2) in V1 neurons of anesthetized cats. In Exp.1, CRF was stimulated with a flash (500 ms) of the grating patch with optimal parameters and SRF was stimulated with a flash (50 ms) of the grating annulus that was either iso- or cross-oriented to the CRF stimulus orientation. The late (> 80 ms) component of response was suppressed specifically by iso-oriented SRF stimulus, while the early (40 - 80 ms) component of response was suppressed by SRF stimulus regardless of OC. In Exp.2, the suppressive effect of short (50 ms) SRF stimulation lasted up to the offset of CRF stimulus (500 ms), and its time course of the suppression was compatible with that of long (500 ms) SRF stimulation. In conclusion, the short (50 ms) presentation of SRF stimulus is sufficient to evoke both orientation-nonspecific fast component and orientation-specific slow component of suppressive effect.

P268 (2P1-027)

Activation of NK1 receptor of trigeminal root ganglion via substance P paracrine mechanism contributes to the mechanical allodynia in the temporomandibular joint inflammation in rats

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The aim of the present study was to investigate whether under in-vivo condition, temporomandibular joint (TMJ) inflammation alters the excitability of Aβ-trigeminal root ganglion (TRG) neuronal activity innervating the facial skin by using extracellular recording with multibarrel-electrodes. CFA was injected into the rat TMJ. A total of 36 Aβ-TRG neurons responding to electrical stimulation of the whisker pad was recorded in pentobarbital-anesthetized rats. The number of Aβ-TRG neurons with spontaneous firings and their firing rate in TMJ inflamed rats were significantly larger than those in control rats. The firing rates of their spontaneous activity in the Aβ-TRG neurons were current-dependently decreased by local iontophoretic application of a NK1 receptor antagonist (L-703,606) in inflamed, but not non-inflamed rats. The spontaneous activities were increased by iontophoretic application of substance P in both group of rats.The mechanical stimulation threshold of Aβ-TRG neurons in inflamed rats was significantly lower than that in control rats. There were no significant differences on the mechanical stimulation threshold between control and inflamed rats after iontophoretic application of L-703,606. These results suggest that TMJ inflammation can modulate the excitability of Aβ-TRG neurons innervating the facial skin via paracrine mechanism due to SP released from TRG neuronal cell body.

P269 (2P1-028)

Stress responses to the heelsticks in human infants were attenuated by their own mother's milk odor

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Newborn's attraction to the breast milk odors is one of the fundamental behavioral responses that human newborns share with other mammalian species, but little is known about the effects of the breast milk odors on stress response. In the present study, we examined whether breast milk odors affect the stress responses to a capillary puncture on the heel (heelsticks) during routine blood draws to screen for phenylketonuria in 5 days-old infants. Forty eight healthy infants were randomly assigned to the following four groups, 1) control group which was exposed to saline as a sham odor, 2) mother's milk group which was exposed to their own mother's milk odor, 3) other mother's milk group which was exposed to other mother's milk odor and 4) formula milk group which was exposed to formula milk odor. To assess the infant distress, their facial expression (grimacing) and crying were recorded by video camera and their body movements were recorded by actigraph. This study was approved by the ethics committee of Nagasaki University. As we expected, infants showed significantly more distress after heelsticks in all groups. However, stress responses to the heelsticks were significantly attenuated by their own mother's milk odor, but neither by other mother's milk nor formula milk odors. These results suggest that the mother's milk odor has not only an attractive effect but also a calming effect on their own infant.

P270 (2P1-029)

Suppressive effects of low-power laser irradiation on bradykinin evoked action potentials in cultured mouse dorsal root ganglion nuerons

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Mechanism of the pain relief effect of low-power laser (Ga-Al-As diode laser, 16.2mW) irradiation using cultured mouse dorsal root ganglion neurons associated with C-fiber was studied by patch-clamp technique. Bradykinin (BK) and laser stimulations were limited to the process or the cell body (soma) with a separator developed by us. The action potentials of the soma by BK application to the process were reversibly suppressed by the irradiation for 2 min to the process. After the irradiation to the soma was done without BK stimulation, resting potential was depolarized by potential of 2-3 mV, and the frequency of the spike evoked by a depolarization pulse was enhanced, except for the case in which the irradiation was limited to the process. K^+ channel openings elicited by BK were reversibly suppressed by the irradiation to the soma. Inward current evoked by a depolarization pulse was not suppressed by the irradiation to the soma. BK-evoked inward currents were suppressed by the irradiation to the process. When BK was applied to the process and the irradiation to the soma was done, the action potentials by BK were not suppressed. The results suggest that the increase in the spike frequency by the irradiation to the soma without BK is ascribed to depolarization due to inhibition of K^+ channel openings, and the suppressive effect of laser irradiation on BK-evoked action potential may come from suppression of the system of receptor-G protein in the process.

P271 (2P1-030)

ATP release from the muscle induced by the mechanical stimulation

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ATP is now known as a substance that is released from injured/stimulated cells to induce or augment pain. In the urinary bladder ATP is released from the endothelium when the bladder is stretched, and thus released ATP stimulates sensory nerve terminals to transmit the stretched state of the bladder to the central nervous system. We hypothesize that the same happens in the skeletal muscle, and measured ATP release upon mechanical stimulation (compression) of the muscle. The extensor digitorum longus muscle was excised from the deeply anesthetized rats, and superfused with Krebs-Henseleit solution. The superfusate was sampled at a rate of 0.8 ml/min and its ATP concentration was measured with Luciferin-Luciferase method. At first we examined ATP release to repetitive application of 20 g force/10 s stimulation, which was used for the study of nociceptor characteristics, with a servo-controlled mechanical stimulator with intervals of 30 min. ATP release was clearly decreased on repetition of the mechanical stimulus. Then we examined stimulus response relationship. The muscle was stimulated five times at 5, 10, 20 and 40 g forces at a rate of 10 g/s. ATP release was increased roughly stimulus-strength dependently in this range. Whether ATP thus released transmits mechanical event to nociceptors is to be studied.

P272 (2P1-031)

Sex difference in the response of DARPP-32 immunoreactive cells in the rat BST to formalin-induced nociceptive stimuli as revealed by the expression of the pCREB immunoreactivity

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It is widely accepted that females are more sensitive to nociceptive stimuli than males. In the previous study, we showed that there were sex differences in the response of CNS to formalin-induced nociceptive stimuli by checking the expression of phosphorylated cAMP response element-binding protein (pCREB) as a marker of neural activity. In the bed nucleus of the stria terminalis (BST), the number of cells expressing pCREB in female rats was increased 5 min after formalin injection but not in male rats. In the present study, we determined which cells expressed pCREB in response to formalin injection in adult male and female rats. Rats were injected with saline or 2% formalin into the planter surface of the right hindpaw, and they were killed 5 min after the injection. Their brains were served to immunocytochemistry and the number of cells expressing pCREB and dopamine- and cAMP-regulated phosphoprotein (DARPP-32) in the BST was counted. We found that, in females but not in males, the number of DARPP-32 cells expressing pCREB in formalin-injected rats was significantly greater than that in saline-injected rats. The present study suggests that DARPP-32 cells in the BST are at least in part responsible for sex difference in the response of CNS to formalin-induced nociceptive stimuli.

P273 (2P1-032)

Involvement of peripheral glutamate receptors on melittin-induced neurogenic inflammation

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Aim of Investigation: Melittin is the main toxin of bee venom. Previously, we have reported that intradermal injection of melittin into the volar aspect of forearm in humans produces a temporary pain and a subsequent sustained neurogenic-inflammation-skin temperature increase. Furthermore, not only subcutaneous melittin but also subcutaneous glutamate produced neurogenic inflammation on the rats' hindpaw. Aim of the present study was to confirm the involvement of glutamate receptors on melittin-induced neurogenic inflammation.

Methods: Melittin or glutamate was injected subcutaneously into the hindpaw of pentobarbital-anesthetized rats. NMDA receptor antagonist, MK-801 or AMPA receptor antagonist, CNQX was injected simultaneously with melittin/glutamate. Peripheral glutamate was collected by microdialysis and quantified using HPLC. Skin temperature increase was analyzed using the computer-assisted-thermography for the evaluation of neurogenic inflammation.

Results: Microdialysate concentrations of glutamate were increased significantly following subcutaneous melittin injection. Simultaneous MK-801 injection suppressed not only glutamate-induce-neurogenic inflammation but also melittin-induce-neurogenic inflammation. The suppression effects of CNQX were weak.

Conclusion: These data demonstrated that glutamate released following melittin injection partially contributed to neurogenic inflammation by activating NMDA receptors on nociceptors. Melittin-induced glutamate release suggested to prolong the melittin-induced neurogenic inflammation.

P274 (2P1-033)

In vivo patch-clamp analysis of inhibitory effects of baclofen on noxious synaptic transmission in substantia gelatinosa neurons of the rat spinal cord.

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Intrathecal administration of baclofen, a selective $GABA_B$ receptor agonist, is known to have an antinociceptive effect on various pain models. In the present study, we investigated effects of baclofen on modality-dependent excitatory synaptic responses of substantia gelatinosa (SG) neurons in the spinal dorsal horn using *in vivo* patch-clamp recording technique. Adult male Sprague-Dawley rats were anesthetized with urethane. After thoracolumbar laminectomy was performed, patch electrodes were inserted into the SG at the spinal level of L3-L5 and then whole-cell patch-clamp recordings were obtained from SG neurons. Under voltage-clamp conditions, SG neurons exhibited miniature EPSCs.Baclofen decreased the frequency but not amplitude of mEPSCs. Pinch and touch stimuli applied to the ipsilateral hindlimb evoked a barrage of large amplitude of EPSCs. Baclofen also inhibited the amplitude of large amplitude of EPSCs evoked by those stimuli in a dose-dependent manner. On the other hand, the frequency of large amplitude of EPSCs was not affected by baclofen. These inhibitory actions of baclofen were blocked in the presence of CGP55845, a selective antagonist of $GABA_B$ receptor. The present findings suggest that baclofen inhibits both noxious and innocuous mechanical excitatory transmission in the SG through activation of $GABA_B$ receptors on presynaptic terminals. This inhibition of mechanical inputs to the SG may be a possible mechanism for antinociception by baclofen.

P275 (2P1-034)

Comparison of the antinociceptive effects by conditioning stimulation of different amygdaloid nuclei in the rat.

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We have shown that conditioning stimulation of the amygdala has an inhibitory effect on nociceptive neurons of the medullary dorsal horn of the rat. The amygdala is considered to be a complex of anatomically different units. The aim of this study was to compare the inhibitory effects of different nucleus within the amygdala. The animals were anesthetized with N_2O-O_2 and 0.5% halothane and were immobilized with pancuronium bromide. A peripheral test stimulus (a single rectangular pulse of 2.0 msec in duration) was applied to the receptive field of nociceptive neurons, and ipsilateral amygdaloid conditioning stimuli to the recording site were trains of 33 pulses (100-300 μA) delivered at 330 Hz. Effective sites for inhibition were widely distributed throughout the amygdala except for the lateral nucleus. This finding is in contrast with our previous data that inhibitory sites were concentrated into the central nucleus in the cat. There were no significant differences in the mean inhibitory effects by the different amygdaloid nuclei, and the inhibitory effect was between 56.6 and 62.4% of the control (n=32). The present results suggest that there are marked species differences with regard to the antinociceptive mechanisms within the amygdala and support the anatomical observations that the efferent fibers of the amygdala originate mostly from the central nucleus that is projection focus from the other amygdaloid nuclei in the rat.

P276 (2P1-035)

Responsible receptor and afferent fiber for Moxibustion in human subject

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OBJECTIVE: Increments of local blood flow and vasodilatation induced by acupuncture and moxibustion have been known as axon reflex of unmyelinated afferent fiber receptors such as polymodal receptors. In this study, effects of application of local anesthetic patch on pain threshold of the skin and axon reflex induced by moxibustion were examined. **METHODS:** Five healthy volunteers with informed consent (ten forearms allocated anesthetic or sham group, double blinded manner) were used and pin-prick pain and heat pain thresholds were measured. The flare reactions were induced by the Kamaya-mini (Kamaya Co.Ltd, Japan), a kind of indirect moxibustion (peak temperature was about 50°C), and the vasodilatation was measured by blood flow meter. A piece of anesthetic (lidocaine 18mg) or sham patch (30.5 x 50 mm) was applied to the skin surface where pain tests and blood flow measurements were done. **RESULTS:** The mechanical pain threshold (pin-prick) and local blood flow response were significantly reduced ($P<0.05$, Wilcoxon t-test) 60 min after the application of anesthetic patch, but the heat pain threshold did not change. **CONCLUSION:** In this study, the flare reaction evoked by moxibustion was blocked by local anesthetic patch without changing heat pain threshold. These results suggest that lidocaine insensitive type receptors might be involved in the signal transduction mechanism of moxibustion, and also suggest that the thermal sensation provoked by moxibustion was conducted through unmyelinated peptidergic fibers such as the polymodal type receptors.

P277 (2P1-036)

Effects of propofol on nociceptive transmission of rat spinal dorsal horn neurons revealed by in vitro and in vivo patch-clamp recordings

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Spinal actions of intravenous anesthetics such as propofol are less clear. The aim of this study was to investigate the spinal effects of propofol on nociceptive transmission. Adult male rats were used in this study. For *in vitro* patch-clamp study, a transverse slice of the spinal cord was cut and blind whole-cell patch-clamp recordings were made from substantia gelatinosa (SG) neurons. The half decay time of GABAergic evoked inhibitory postsynaptic currents (eIPSCs) was increased by bath-applied propofol dose dependently. Furthermore, for *in vivo* patch-clamp study, a rat was fixed in a stereotaxic apparatus after the lumbar spinal cord was exposed under urethane anesthesia. Propofol was systemically injected from left femoral vein, and its effect was evaluated before and after injection of 5 mg/kg propofol under the voltage-clamp mode. Propofol reversibly prolonged decay time of GABAergic spontaneous IPSC in all neurons tested. In the current clamp mode, pinch stimuli applied to the hindlimb elicited a barrage of excitatory postsynaptic potentials, some of which initiated an action potential (AP). Number of APs decreased after injection of propofol in most of neurons tested. These results suggest that systemically bolus injected propofol in clinical dosage reversibly depress noxious transmission at least in part by enhancing postsynaptic GABA receptors in the SG of the spinal cord.

P278 (2P1-037)

Actions of local anesthetics on noxious and innocuous transmission to the rat spinal dorsal horn

Uta, Daisuke; Furue, Hidemasa; Rashid, Harunor; Koga, Kohei; Yoshimura, Megumu (*Dept. Integrative Physiol., Grad. Sch. Med, Sci., Uni. Kyushu, Fukuoka, Japan*)

Previous research has shown that the toxicities of single S (-) enantiomers levobupivacaine and ropivacaine to the cardiovascular and central nervous systems are weaker than a racemic mixture of S (-) and R (+) enantiomers such as bupivacaine. In this study, we investigated effects of levobupivacaine, ropivacaine, bupivacaine and R (+) bupivacaine on excitatory synaptic inputs to spinal dorsal horn neurons evoked by dorsal root stimulation, and on action potentials (APs) in dorsal root ganglion (DRG) neurons generated by the dorsal root stimulation. In the spinal dorsal horn, levobupivacaine reversibly suppressed the amplitude of monosynaptic Aδ and C fiber-evoked EPSCs. However, Aβ fiber-evoked EPSCs were slightly inhibited in amplitude at the same concentration. On the other hand, bupivacaine equally suppressed those of the three fiber-evoked EPSCs. These local anesthetics did not change the frequency and amplitude of miniature EPSCs. In DRG neurons, APs were reversibly inhibited in amplitude by the local anesthetics. Half-maximum inhibitory concentrations (IC_{50}) of bupivacaine and R (+) bupivacaine were almost equal on Aβ , Aδ and C neurons. On the other hand, IC_{50} of levobupivacaine and ropivacaine on Aδ and C neurons were lower than that on Aβ neurons. The present results suggest that pure S (-) enantiomers especially levobupivacaine effectively inhibits noxious transmission to the spinal dorsal horn by the blockade of AP conduction through C and Aδ fibers.

P279 (2P1-038)

Green Tea Extract could suppress heat-induced pain.

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BACKGROUND For millenniums, medicinal herbs have been used to alleviate pain because pain control is human eternal issue. We have also tested various herb extracts in search of active ingredients capable of relieving pain, particularly when it is heat-induced. Simultaneously as pain perception is very complex and widely varies among individuals, we have originally developed an experimental protocol to be able to induce equivalent pain intensity. **METHODS** A double-blind, placebo controlled, cross-over study was performed on volunteers to compare the pain felt when a heated copper coin was placed on their skin 10 minutes after the application of green tea extract, that have never been tested, or placebo transdermally. The degree of pain that each volunteer felt was then measured on a Visual Analogue Scale <VAS >. **RESULTS** Heat-induced pain was significantly reduced by the application of green tea extract. Particularly, the pain suppressive effect was more visible with increasing pain though green tea extract never cause numbness at the applied area. **CONCLUSION** Our results suggested that green tea extract has original functions to ease heat-induced pain, even when applied for a short period prior to the painful experience. The extract can be broadly and safely used in the pain-control or aesthetic business, differently from local anesthesia.

P280 (2P1-039)

The pERK in the spinal trigeminal nucleus of the aged rats with acute facial inflammation

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<Objectives> The aim of present study is to elucidate the underlying neuronal mechanism of the change in trigeminal nociceptive transmission with advancing age.

<Methods> The adult (8-12 months old) and aged (30-12 months old) rats were injected with 10 mM capsaicin into the right whisker pat under adequate anesthesia. In addition, other adult and aged rats were injected capsaicin into the right whisker pat at 20 min after the naloxone administration (1.2 mg/kg, i.v.) in order to study the involvement of descending modulation system on trigeminal nociception in aged rats. They were perfused at 5 min after capsaicin injection. The whole brain was removed, and then 30 μm thick serial sections were made. We analyzed the change in pERK-LI expression in the medulla and the upper spinal cord of adult and aged rats.

<Results> A large number of pERK-LI cells were expressed in the superficial laminae of the ipsilateral trigeminal subnucleus caudalis (Vc) and C1 of the spinal cord in each rats. A few pERK-LI cells were observed in the paratrigeminal nucleus bilaterally in the adult and aged rats. The expression pattern of pERK-LI cells in Vc/C1 was not difference between adult and aged rats. After the naloxone administration, the number of pERK LI cells in the ipsilateral Vc/C1 in the adult rats was significantly larger than that of aged rats.

<Conclusions> These findings suggest that the advancing age may lead to the dysfunction of descending pain modulation system as well as ascending system, resulting in the abnormal pain sensation in aged rats.

P281 (2P1-040)

Differences of chronic pain behaviors between child and adult rats

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Clinically, it is known that the incidence of chronic pain is much lower in children than in adults. This low-incidence may disclose some important factors to develop chronic pain in the adulthood. In this study, we examined whether chronic pain would be induced in child rats, using a chronic pain animal model we previously developed. Lipopolysaccharide (LPS: 2 mg/kg) and 6% hypertonic saline were injected into the unilateral gastrocnemius muscle of rats. The treatments were done in 3-week-old rats (T-3w) and 9-week-old rats (T-adult). We measured changes in the pain behaviors (von Frey test: VFT) at the bilateral plantar surface, the circumference of the calves, and the body weights. Non-treated age-matched control group was also measured. Pain behavior increased and lasted over 10 weeks in T-adult. On the contrary, in T-3w, pain behaviors did not last and decayed after post-treated two weeks. The responses to LPS were smaller in T-3w than in T-adult. In the normal condition, sensitivity to VFT was higher in 3-week-old rats than in 9-week-old rats. In child rats, it was indicated that chronic pain was hard to occur. It is suggested that the developments of nervous and immune systems may be important in onset and maintenance of chronic pain.

P282 (2P1-041)

Behavioral discrimination of the taste qualities in zinc deficient rats

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Some clinical reports demonstrate that zinc deficiency brings about taste disturbance. But there are very few reports about behavioral responses in zinc deficient animals. In the present study, therefore, we investigated whether or not zinc deficient rats could discriminate qualities of four basic taste stimuli by using the conditioned taste aversion paradigm. As the zinc deficient animals, male Wistar rats fed zinc deficient diet during 5 weeks after the weaning period were used. When these rats were subjected to aversive conditioned to one of the basic taste stimuli, any animals could acquire the conditioned taste aversion, and they never generalized to other stimuli. This result suggests that zinc deficient rats have the ability of the discrimination of the taste qualities.

P283 (2P1-042)

Characterization of outward currents of morphologically identified frog taste cells

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Frog taste discs consist of morphologically and physiologically diverse types of cells. Recently, Suwabe and Kitada have demonstrated the properties of voltage-gated inward currents of type Ib, type II and type III cells in frog taste discs. However, little is known about the properties of outward currents of these cells. To investigate the properties of outward currents of morphologically identified cells, patch clamp technique was used to make recordings from taste cells in vertical slices of taste disc. Cell types were identified by staining with Alexa Fluor 488 hydrazide in a pipette. We recorded voltage-gated potassium outward currents from all recorded type Ib, II and III cells under the sodium-free Ringer perfusion. Peak amplitudes of outward currents of type Ib and III cells were significantly larger than that of type II cells. Outward currents were composed of delayed rectifier current, transient potassium currents and calcium activated potassium currents. Type Ib and II cells exhibited delayed rectifying potassium currents and transient potassium currents. Type III cells exhibited all three types of outward potassium currents. Calcium activated potassium currents of type III cells were sensitive to apamin and charybdotoxin. These observations demonstrate that the properties of potassium currents are different among cell types and suggest that the roles in taste reception and transduction of each cell types may differ.

P284 (2P1-043)

Multiple receptor sites for phasic taste responses of the glossopharyngeal nerve to bitter substances and salts revealed by cross-adaptation method in the frog.

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Application of bitter substances and some salts to the tongue elicits phasic taste responses in the frog glossopharyngeal nerve. However, it is not known whether there are multiple taste receptor sites in the phasic components. In the present study, cross-adaptation was carried out by varying concentrations of bitter substances (quinine-HCl, caffeine, theophylline and denatonium) and salts (NaCl and choline Cl) in a wide range. The peak magnitude of the integrated responses of the glossopharyngeal nerve to taste stimuli in the frog (*Rana catesbeiana*) were measured. The response to caffeine after quinine was decreased with increasing concentrations of quinine applied first and reached the spontaneous level, while that to theophylline and denatonium after quinine was decreased to 60-70% of the original level. Responses to NaCl and choline Cl were scarcely affected after adaptation of quinine. The results obtained suggest that quinine and caffeine stimulate the same receptor site and that theophylline and denatonium stimulate receptor sites that are different from the receptor site responsible for quinine response although there exist receptor sites stimulated commonly by bitter substances. It is also suggested that quinine and salts such as NaCl and choline Cl stimulate different receptor sites.

P285 (2P1-044)

Effect of taste stimulation of tongue on swallowing reflex in humans

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We have shown that distilled water (DW) applied to the pharyngolaryngeal region is effective for elicitation of swallowing reflex, but NaCl solutions reduced the effect of DW. However, little is known about role of taste in swallowing reflex in humans. In the present study, we examined how taste stimulation of the tongue is involved in swallowing reflex in humans. Each subject was instructed to repeat swallowing as fast as possible. In dry swallowing without a supply of fluid, the time between the first and 6th swallowing (the dry swallowing test time) was measured. Then, taste solutions were delivered to the anterior tongue through a fine tube at a slow rate (0.2 ml/min). In taste stimulation, the intervals between two consecutive swallowings in a test (swallowing intervals) were measured. The effect of Na salts taste stimulation on swallowing reflex appeared only in subjects who showed long dry swallowing test time (>60 sec). That is, swallowing intervals induced by 0.15 M NaCl and 0.15 M Na acetate were much shorter than those by DW, 0.15 M KCl and olive oil. While, the effect of Na salts taste stimulation on swallowing reflex did not appear in subjects who showed short dry swallowing test time (<55 sec). The present study suggests that salty taste is effective for swallowing initiated voluntarily. Since the effect of Na salts appeared only when swallowing intervals are long, it is also suggested that excitation of sodium-taste receptors affects swallowing center in brain stem slowly.

P286 (2P1-045)

Effects of oral capsaicin on the activity of gustatory neurons in the bulbar solitary nucleus and pontine parabrachial nucleus in rats

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In the present study, we investigated whether oral capsaicin affects the taste-evoked neuronal activities in the brainstem gustatory centers, the nucleus of the solitary tract of the medulla (NTS) and parabrachial nucleus of the pons (PBN), in the rat by using c-fos immunohistochemistry. Taste stimuli used were as follows: 0.2 M NaCl, 2 mM quinine-HCl, 0.2 M NaCl mixed with 330 μM capsaicin, and 2 mM quinine-HCl mixed with 330 μM capsaicin. Rats were chronically implanted with oral cannula and presented with these test stimuli for 10 minutes (500 μl/min). Application of either NaCl or quinine alone induced significant Fos-like immunoreacitivity (FLI) both in the waist and lateral areas of PBN. It has been reported that the neurons are responsive to NaCl or quinine in the waist and lateral areas, respectively. Combined application of capsaicin with NaCl or quinine, however, significantly reduced FLI in the waist area in PBN compared with FLI induced by NaCl or quinine by itself, whereas addition of capsaicin did not affect FLI in the lateral area of PBN. Furthermore, combined application of capsaicin with quinine reduced FLI in the gustatory portion of NTS compared with the FLI evoked by quinine alone. These results suggest that Capsaicin affects neuronal activity in subnuclei of the PBN differentially. These inhibitory effects of capsaicin on taste neuron can correlate with capsaicin-induced suppression of taste perception in humans.

P287 (2P1-046)

The relationship between individual differences in sweet taste sensitivity and single nucleotide polymorphisms of T1rs in human

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It is reported that T1r2/T1r3 heterodimer plays a role as a sweet taste receptor. Mice lacking T1r3 showed no preference for artificial sweeteners and had diminished but not abolished behavioral and nerve responses to sugars, suggesting that T1r3-independent sweetener binding site also exist in taste cells in mice. However, the numbers and functions of ligand binding sites on T1r2/T1r3 (and/or other sweet receptor) remain largely unknown. In this study, in order to predict the number of sweetener binding site in human, we measured sensitivity thresholds to various sweet taste substances [sucrose, glucose, fructose, saccharin, aspartame, acesulfame-K, glycine, D-phenylalanine, D-tryptophan and L-proline] in human subjects and examined the qualitative similarities among these sweeteners by using a hierarchical cluster analysis. We also used Gymnemic acid and γ-cyclodextrin, which selectively inhibits sweet responses and reduces the inhibitory action of it in human. The ten sweet compounds were classified into five groups [(1) sucrose, glcose and fructose, (2) saccharin, aspartame, acesulfame-K and glycine, (3) D-phenylalanine, (4) D- tryptophan, (5) L- proline]. Four and two single nucleotide polymorphisms with amino acid substitution were detected in T1r2 and T1r3, respectively. These results suggest that there may be at least five different binding sites in human sweet receptor system. The individual differences in sweet sensitivities may be due to these single nucleotide polymorphisms.

P288 (2P1-047)

The decreased amount of food intake related to the changes of gustatory and olfactory thresholds in patients with hematopoietic tumors and chemotherapy

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We investigated if the changes in gustatory and olfactory thresholds (G-th and O-th) in the process of chemotherapy affect food intake in patients with hematopoietic tumor. 32 patients were studied after obtaining informed consent. Measurements of G-th and O-th, the amount of food intake (AFI) and questionnaires were carried out before the initiation of chemotherapy (*before*), on the third day (*3rd*) and on the seventh day (*7th*) during chemotherapy, and 2 weeks after the termination of chemotherapy (*after*). AFI was remarkably decreased at *3rd* and was recovered at *after*. The patients, who experienced alterations of gustatory and olfactory sensations, were increased in number at *3rd*. The% change of G-th for salt and bitter as basis by that at *before* was significantly elevated at *3rd*, whereas that for sweet was significantly decreased at *after* ($P<0.05$). The% change of O-th for detection was significantly decreased at *7th* ; however, O-th for recognition was significantly increased in female ($P<0.05$). Multiple regression analysis revealed that the increase in G-th for salt significantly decreased AFI and that the decrease in O-th for detection of β-phenyl ethyl alcohol significantly decreased AFI. The averaged O-th for recognition reciprocally correlated with AFI in female patients ($r = -0.34$, $P<0.05$). Alterations in G-th and O-th in the process of chemotherapy affected AFI in the patients with hematopoietic tumor.

P289 (2P1-048)

Amiloride sensitivity of NaCl responses of mouse fungiform taste cells

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Previous electrophysiological studies have shown that the chorda tympani nerve contains two types of NaCl-responsive fibers. One, N-type fiber, narrowly responds to NaCl and the NaCl response is strongly inhibited by amiloride, a blocker of the epithelial sodium channel (ENaC). The other type (E- or H-type) has broad responsiveness to electrolytes and shows almost no amiloride sensitivity. These fibers may receive input from amiloride sensitive and insensitive taste receptor cells. In this study, we examined NaCl responses of mouse fungiform taste cells in isolated taste bud and amiloride sensitivity of them. In our experiments, taste stimuli were applied only to the pore side of an isolated taste bud, and responses of one single cell of the bud to the stimuli were recorded from its basolateral side of the membrane as increase in firing frequency. The response to apical NaCl stimulation was recorded in some fungiform taste cells. These responses were concentration dependent. Amiloride mixed with apical NaCl solution inhibited NaCl responses in some taste cells [amiloride sensitive (AS) cells] but not in others [amiloride insensitive (AI) cells]. AI cells responded to other electrolytes such as KCl and HCl. These results suggest the existence of at least two types of NaCl sensitive cells, AS and AI cells. N- or E-type fiber may selectively innervate AS or AI cells respectively.

P290 (2P1-049)

The effects of amiloride on salt responses of the chorda tympani nerve in 129X1/SvJ and 129P3/J mice

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The effect of amiloride on responses of the chorda tympani (CT) nerve to NaCl differs among mouse strains. For example, in C57BL mice, amiloride suppresses NaCl responses to about 50% of control, whereas no clear amiloride inhibition was observed in 129 mice. The 129 inbred strain, however, has a number of substrains derived mainly from two major parent stocks, 129/J and 129/SvJ. Recently, 129X1/SvJ (formerly 129/SvJ) mice are reported to differ from the 129P3/J (formerly 129/J) strain by 25% of sequence length polymorphisms. In the current study, therefore, we examined possible substrain difference between 129P3/J and 129X1/SvJ in the amiloride sensitivity of the CT response. The results suggest that amiloride is effective in 129X1/SvJ mice. CT responses to 0.3 M NaCl were significantly suppressed by amiloride at the concentration of 10 μM or more, and the inhibition reached the maximum (about 50% of control to 0.03-0.3 M NaCl) at 100 μM. In contrast, no such amiloride inhibition was evident in 129P3/J mice. These results suggest that amiloride-sensitivity of NaCl responses differ among 129 substrains.

P291 (2P1-050)

Licking responses to quinine in TRPM5 null mice

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TRPM5, a member of the transient receptor potential channel(TRP), is related with taste cell responses to sweet,umami and bitter compounds. Activation of TRPM5 occurs downstream activation of G-protein-coupled taste receptors and is proposed to generate a depolarizing potential in taste receptor cells. Mice with a partial deletion of the TRPM5 protein, which retained intact the amino terminal portion, have been shown to be unresponsive to bitter, sweet, umami tastes. To avoid any confounding effects of this amino terminal fragment, we generated knockout mice null for TRPM5 protein. In previous study, this TRPM5 knockout mice showed reduced, but not abolished, responses to quinine hydrochloride in both nerve recording and two-bottle preference test. In this study, in order to examine behavioral responses to quinine hydrochloride in TRPM5 knockout mice in further detail, we used a short-term (10s) lick test for measurement of consumption of its solutions. TRPM5 knockout mice showed significantly reduced responses to 0.1-10 mM quinine hydrochloride, but not abolished at high concentrations (3.0,10mM) of it, although no such difference was evident in response to DW. These results may be almost consistent with previous nerve recording and two-bottle preference test, suggesting that there may be TRPM5-dependent and independent pathways in signal transduction mechanism for quinine hydrochloride..

P292 (2P1-051)

Localization of TRPV2 in the Axon of Mouse Olfactory Cells

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TRPV2, a member of the transient receptor potential family, has been isolated as capsaicin-receptor homolog and is thought to respond to noxious heat. Here we show that TRPV2 mRNA is expressed in GAP43-positive immature and OMP-positive mature olfactory sensory neurons. Intensive TRPV2 immunostaining was observed at the olfactory axon bundles in olfactory mucosa. TRPV2-positive labeling was preferentially found in the olfactory nerve layer in the olfactory bulb. Furthermore, we demonstrated that cell bodies of olfactory sensory neurons settled in cell layer expressing TRPV2 mRNA are insulin-like growth factor (IGF)-I receptor-immunopositive. The increase in intracellular calcium levels in olfactory neurons isolated from adult olfactory mucosa was found to be induced by the application of IGF-I, and was not observed in the presence of SKF96365, an inhibitor of TRPV2. In embryonic stages, TRPV2 immunoreactivity was observed on axon bundles of developing olfactory neurons in the nasal region starting from 12.5 days of gestation and through fetal development. Observations in this study indicate that TRPV2 localizes to growing olfactory axons and contributes to the elevation of intracellular calcium levels in olfactory neurons in response to IGF-I.

P293 (2P1-052)

Acetylcholine enhances odorant sensitivity in newt olfactory receptor cells

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The olfactory epithelium is innervated by efferent neurites and the olfactory receptor cells express muscarinic receptors. These observations raise the possibility that acetylcholine could affect odor responses of the olfactory receptor cells. Here we investigated the effect of acetylcholine on newt olfactory receptor cells, using the whole-cell version of the patch-clamp technique. Under current clamp condition, bath-applied 100 μM carbachol, an agonist of acetylcholine receptor, lowered spike threshold from 5.3 ± 0.6 pA to 3.8 ± 0.5 pA. Furthermore, the maximum spike frequency was increased from 9.1 ± 1.4 spikes/s to 11.0 ± 1.3 spikes/s by carbachol. These results suggest carbachol directly modulates spike generation in ORCs. Under voltage clamp, condition carbachol increased the peak amplitude of a voltage-gated T-type calcium current by 39% and sodium current by 32%. However, carbachol did not change the amplitude of an L-type calcium current or a delayed rectifier potassium current significantly. An antagonist of muscarinic acetylcholine receptor, atropine, blocked the enhancement by carbachol of sodium current, suggesting that carbachol modulates sodium current via the muscarinic receptor. Because T-type calcium current is known to lower the threshold in olfactory receptor cells, we suggest that acetylcholine, which is released from efferent fibers, may enhance odorant sensitivity by lowering the threshold of spike generation in olfactory receptor cells.

P294 (2P1-053)

Functional maturation of vomeronasal neurons induced by the co-culture with accessory olfactory bulb neurons

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Pheromones are detected by the vomeronasal organ, but precise natures of detection at the cellular level are poorly understood. To characterize cellular aspects of receptivity to pheromones, we investigated responsiveness of the vomeronasal neuron (VRN) to pheromone - containing materials in the cell culture. As previously reported, VRNs in culture form a spherical structure with a central cavity, referred to as a vomeronasal pocket (VNP). We also reported the maturation of each VRN in the VNP was induced by co-culture with dissociated accessory olfactory bulb (AOB) neurons. Using this co-culture system, we applied charged compounds in mouse urine iontophoretically into the cavity of VNP using microelectrode and analyzed VNP response by a Ca^{2+} imaging method with or without cultured AOB cells. When urine compounds were ejected into the VNP co-cultured with AOB cells with a current of 1-2 μA, subpopulation of VRNs clearly showed long-lasting Ca^{2+} increases. Such Ca^{2+} increases were not observed without AOB neurons and injections of a current below 5 μA alone had no effect. Moreover, a western blotting analysis showed the expression of some putative pheromone receptors in the VNP was induced and increased with days in co-culture. These results indicate that VRNs result in expressing pheromone receptors and then acquire responsiveness to compounds in urine by interacting with AOB neurons in co-culture.

P295 (2P1-054)

Function and Distribution of HCN Channels in Olfactory Epithelium

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels contribute to physiological functions such as regulating cell excitability, eliciting rhythmic activities and so on. Four subtypes (HCN1-4) have ever been identified. We previously cloned HCN4, which shows the slowest activation kinetics and the profound modulation by cAMP. To investigate the physiological roles of HCN4, we have generated a transgenic (Tg) mouse, in which tetracycline repressor protein (TetR) is expressed under the control of HCN4 promoter. This mouse was crossbred with another Tg mouse, in which GFP expression is regulated by TetR. Thus generated Tg mouse expressed GFP in HCN4-expressing cells. We observed on this Tg mouse that GFP fluorescence and HCN4 immunoreactivity (HCN4-IR) were colocalized in the cerebrum, the sino-atrial node, the taste buds and the retina; all of which were previously confirmed to express HCN4. The olfactory receptor neurons (ORN) expressed GFP, where only HCN current (Ih) had been formerly reported, however, its physiological roles remain unknown. HCN4-IR was detected in the olfactory knobs, the soma and the axon bundles of the ORN. We have further performed immunohistochemistry to determine the expression patterns of the other HCN subtypes in the ORN. Also electrophysiological studies are in progress to analyze the physiological properties of HCN channels in the ORN.

P296 (2P1-055)

Intrinsic optical images to urinary stimulation in the rat accessory olfactory bulb

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It is generally believed that the main olfactory system processes common odors and the vomeronasal system is involved mainly in the detection of pheromones. The accessory olfactory bulb (AOB) is the first relay station in the vomeronasal system. To investigate how pheromonal information is processed in the rat AOB, we obtained high resolution mapping of pheromone-induced activation by optical imaging of intrinsic signals. Urine collected from male or female rats were used as test substances. Application of volatile components in the male urine (2-5%) with a syringe mainly activated the anterior AOB (aAOB), whereas female urine-induced activation was observed in both the aAOB and caudal part of the AOB in the male rats. In the female rats, urine-induced activation occurred mostly in the aAOB. Application of non-volatile urine components was performed by putting the nostrils contact with filter paper moistened with urine (3-5%). Either male or female urine induced mainly activation in the posterior AOB (pAOB) and to a lesser extent in the aAOB. In contrast, urinary responses were also observed in a few of glomeruli of the main olfactory bulb. The threshold for urine-induced activation in the glomeruli, however, was approximately 50%, which was higher than that obtained in the AOB (1%). These results provide the evidence that the aAOB is activated by volatile components in male or female urine, whereas the pAOB is activated by non-volatile components.

P297 (2P1-056)

Optical imaging of neural activities of the central field of the right and left guinea pig auditory cortices.

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The processing of spectral and temporal information in the core fields of the left and right auditory cortices of the guinea pig was investigated using optical imaging with a voltage-sensitive dye (RH795). 23 guinea pigs were anesthetized with ketamine (80mg/kg) and xylazine (40mg/kg). In order to compare the tonotopical organization between the left and the right auditory cortex, optical imaging patterns to tone stimulation at 2, 4, 8, 16 kHz were recorded from core auditory fields (primary (AI), dorsocaudal (DC) fields) of both sides and tonotopy maps were made. In the same animal, the tonotopic organization of AI and DC of one side was more clear than the other side. In 65% animals, the distance between 2kHz and 16kHz isofrequency bands in the left AI was longer than that of the right AI, whereas that of left DC was shorter than that of the right DC. To compare the temporal properties, click or noise trains were presented at different repetition rates (4-20 Hz). Repetition rate transfer functions (RRTF) in field AI were low-pass showing a sharp drop-off in evoked activity per click or noise above 10 Hz but RRTFs in field DC were band-pass with the peak of 8 or 10 Hz. The cut-off frequencies of RRTF in the left cortex were the same as those in the right cortex but the slopes of the RRTF in the left cortex were sharper. We discuss the functional difference between the left and right auditory cortices of guinea pigs.

P298 (2P1-057)

Multiple-site optical recording with optic fiber illumination and software for reducing pulsation artifact to detect neural response in a single sweep in the rat somatosensory cortex

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We have developed an optical recording system to detect neural activity for a long time from 1020 sites of the cerebral cortex. But its signal-to-noise (S/N) ratio was insufficient for analyzing the neural response from single sweep recordings. Since there is no way to remove shot noise, to improve the S/N ratio for detecting the neural activity without averaging, we had to increase the excitation light intensity and enlarge signal size. For this purpose, we introduced a new illumination device using optic fiber bundle. Light from a halogen-tungsten lamp was rendered quasi-monochromatic by a band pass filter, converged to an optic fiber bundle and illuminated the preparation, which had been stained with a voltage-sensitive dye (RH414) in advance. A tandem lens system formed a magnified real image of the preparation, and the fluorescent light intensity was detected with a photodiode array. Neural responses to the electrical foot stimulation were monitored optically in the rat somatosensory cortex with this improved system. Among a large artifact derived from pulsation, we could detect neural response in a single sweep. Furthermore, with using digital subtraction, aligned at ECG, of a control record without stimulation, we succeeded in greatly reducing the pulsation artifact leaving the optical signal related to neural activity fairly intact. In this way, we could apparently detect cortical neural responses *in vivo* with a sufficient S/N ratio for analyses on the basis of single sweep recordings.

P299 (2P1-058)

The morphological and electrophysiological study of the local circuits of mammalian inferior colliculus.

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Mammalian inferior colliculus(IC) is an integrative auditory processing center of the midbrain. IC has complex neural circuits and is majorly devided into two regions: central nucleus (CIC, tonotopic region) and dorsal and external cortices (non-tonotopic region). By the focal injection of neuronal tracers into IC and GAD immunohistochemical technique, we investivated the characteristics of the local circuits of IC.

The results show that the left and right ICs have characteristic connections; 1) the left and right CIC-CIC, cortices-cortices have strong connections(about 70% of total connections). 2) the CIC-CIC connections are symmetric and they are majorly excitatory (98%). We further analyzed the characteristics of the labeled local circuits by in vivo and in vitro electrophysiological techniques.

P300 (2P1-059)

Asphyxia-induced decrease in endocochlear potential is triggered by the activation of L-type Ca^{2+} channel

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A large positive potential in the endolymph, named the endocochlear potential (EP) has been considered to occur at cochlear stria vascularis and this DC potential is essential for the transduction of sound by hair cells. In this study, we examined the effect of the intracellular Ca^{2+} concentration ($[Ca]_i$) in the endolymphatic surface cells on EP by using conventional and Ca^{2+}-sensitive microelectrodes. 1) A large increase in the Ca^{2+} concentration in the endolymph up to 10^{-3} M with a decrease in EP from +70 mV to +20 mV was induced by transient asphyxia (100 sec). 2) The application of 300 μM EGTA-tetraacetoxymethyl ester (EGTA-AM) with 10 mM EGTA-containing solution or 3 μM nifedipine to the endolymph produced a slight increase in EP and suppressed significantly an initial decrease in EP induced by transient asphyxia. 3) The administration of 10 μM Bay K 8644, an activator of L-type Ca^{2+} channels, to the endolymph produced a gradual decrease in EP. 4) Perilymphatic administration of 1 mM EGTA-AM or 30 μM nifedipine caused no significant suppression of the asphyxia-induced decrease in EP. These results suggest that transient asphyxia-induced decrease in the EP is triggered by an increase in $[Ca]_i$ with the activation of L-type Ca^{2+} channel in endolymphatic surface cells.

P301 (2P1-060)

Contrast of Binaural Coincidence Detection in Nucleus Laminaris is likely improved through the activity of SON.

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Interaural time difference (ITD) is an essential cue for the sound source localization along the horizontal axis. In birds, ITDs are first calculated in neurons of nucleus laminaris (NL) by detecting the coincidence of binaural synaptic inputs. Previously, we recorded neurophonic-potentials (NPs) in NL of anesthetized chicken, and pharmacologically demonstrated that NPs originated from EPSCs and action potentials in NL. Because of the robust NPs, recording single unit from NL is difficult. However, occasionally recorded NL unit activities showed the same properties as NPs in the sensitivity to frequency and ITD of stimulus sound. Plot of NL activity against ITDs (ITD tuning curve) has peaks and troughs alternately, corresponding to the favorable and unfavorable ITDs. In the high- and middle-best frequency (BF) neurons, the increase of sound intensity shifted the whole ITD tuning curve upward, but in the low-BF neurons, the troughs of ITD tuning curve was depressed compared to the peaks, creating a large contrast between peaks and troughs. This proposes the effect of inhibitory input from superior olivary nucleus (SON). For testing this hypothesis, we lesioned SON electrolytically, and recorded well-isolated and stable single-unit activities with a loose patch technique. After lesioning SON, the whole ITD tuning curve shifted upward even in the low BF neuron when sound intensity was increased. This might support the idea that inhibitory input from SON improves coincidence detection in NL.

P302 (2P1-061)

Electrophysiological analysis of the pressure-sensitive afferents from the submandibular salivary glands in the rat.

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Recent our histochemical study has shown that the sensory nerves travel in both the sympathetic and parasympathetic nerve routes supplying to the submandibular gland in the rat. In the present study we analyze afferent neural activities in the peripheral cut ends of the sympathetic and parasympathetic nerve branches innervating the submandibular gland in urethane-anesthetized rats. The following results were obtained: 1) The afferent activity could be recorded from both sympathetic and parasympathetic routes; 2) both afferents had no spontaneous activities, but responded to mechanical pressure applied onto the gland; 3) when back pressure was applied from the main excretory duct by infusion of saline, both afferents showed tonic impulse discharges at pressure of higher than 100 mmHg; 4) the threshold pressure was little lower than the maximal secretory pressure measured by electrical stimulation of the parasympathetic secretory nerve (the chorda tympani); 5) there is no differences in the threshold pressures between afferents in the sympathetic and parasympathetic nerve routes; 6) when Bradykinin was infused from the duct, the afferent nerve discharged vigorously. Previous histochemical studies show that substance P and CGRP-containing nerves considered as possible afferent fibers were identified frequently around small ducts and blood vessels. We speculate that the afferent activity may relate to monitoring of excessive pressure of the fluid secretion and blood flow, or to pain due to salivary stone.

P303 (2P1-062)

Hypotonicity increases paracellular ion permeability in renal epithelial A6 cells

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Epithelial tight junction forms the barrier with ion moving pathway. Paracellular ion permeability is modified by expression pattern of claudins, a component of tight junction, but the mechanism of its regulation and physiological implication is unknown. In renal epithelial A6 cells, hypotonicity induces Na^+ transport via the transcellular pathway. However, the effect of hypotonicity on paracellular ion permeability is unknown. To study the effect of hypotonicity on paracellular ion permeability, we measured currents in A6 cells. Basolateral but not apical hypotonicity markedly increased the paracellular conductance (Gp) and current (Ip) from apical to basolateral side, indicating that the basolateral hypotonicity increases the paracellular permeability to more Na^+ than Cl^+. Furthermore, replacement of NaCl with sucrose in the basolateral solution did not increase Gp or Ip. Furthermore, under the condition that Na^+ replacement with NMDG, basolateral hypotonicity increased Gp but not Ip. Under the condition that Cl^+ replacement with gluconate, basolateral hypotonicity did not increase Gp or Ip. These observations indicate that basolateral hypotonicity and paracellular Cl^+ movement increase paracellular permeability and induce more conductance for Na^+ than Cl^+ and NMDG. Based on these observations, we conclude that the hypotonicity-induced changes of paracellular ion conductances stimulate Na^+ reabsorption in the renal A6 cells via not only transcellular but also paracellular pathway.

P304 (2P2-063)

Saccade-related inhibitory burst neurons (IBNs) alter their activity in relation to amplitude-decreasing adaptation in the monkey.

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Saccade accuracy, a prerequisite for efficient collection of visual information, is maintained by an adaptive mechanism that modifies saccade amplitude. Previous studies show that adapting saccade amplitude induces correlated changes in neuronal activity in the cerebellar fastigial oculomotor region (FOR). In the present study we recorded the activity of saccade-related inhibitory burst neurons, IBNs, which may relay FOR signals to motoneurons. We examined if IBN activity changed during adaptation and could contribute to it. We tested 44 IBNs during amplitude-decreasing adaptation of contraversive (off-direction) saccades. Thirty-seven IBNs fired, at least occasionally, for off-direction saccades. The remaining 7 exhibited no spikes for off-direction saccades. We used intrasaccadic target steps to adapt the gain of saccades to 10° target steps in the off-direction. Many IBNs which exhibited off-direction spikes showed an increasing number of spikes (23/37), a shortening burst lag (13/37) or both (12/37) as adaptation progressed. IBNs with no off-direction spikes showed no spike activity after adaptation. These results suggest that the adaptation-related changes in IBN activity decrease the size of contraversive saccades by decreasing the size of the burst in motoneurons.

POSTERS
Motor functions

P305 (2P2-064)

Coordinate frames in representing pursuit signals in simian frontal eye fields (FEF)

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To examine coordinate frames for FEF pursuit signals, we first compared preferred directions during head-fixed upright position and static 40° roll tilt. Preferred directions of 30 pursuit neurons were shifted minimally during static tilt, indicating that their coordinate frames are not world-centered. In the head-fixed condition, head-centered- and body-centered- coordinates are not dissociated. To dissociate them, the monkeys were allowed to rotate the heads horizontally on the upright stationary body for pursuit of a reward feeder and laser spot. Responding neurons during gaze (eye-in-space)-pursuit were tested for eye-pursuit of the spot while the feeder was stationary and head-pursuit of the feeder while the spot was stationary. Majority (61%) of 99 responsive neurons fired strongly for both eye- and head-pursuit even when gaze was stationary; the modulation during gaze-pursuit was linear sum of modulation during eye- and head-pursuit. Modulation during VOR cancellation induced by passive whole body rotation was not correlated with eye-pursuit modulation. These results suggest that these cells fired for body-centered coordinates. Minority (24%) was modulated during gaze- and eye- pursuit similarly but minimally during head-pursuit when gaze was stationary; modulation during VOR cancellation was correlated well with eye-pursuit modulation, suggesting that these cells fired for head-centered coordinates. Our results suggest that both body-centered and head-centered coordinates are present in FEF for processing eye-, head-, and gaze-pursuit signals.

P306 (2P2-065)

Symmetry of the convergence eye movement - anticipatory and visually-evoked

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The convergence eye movement is known as a disjunctive eye movement in which, typically, both eyes adduct symmetrically in the same time. But asymmetrical convergence also found in the natural condition. These asymmetrical convergence may reflect asymmetries of central control of convergence eye movement. The lateral suprasylvian (LS) areas are extrastriate cortices which receive visual information from V1. The LS has contralateral dominant receptive fields. The cat has convergence-related areas in the LS of both hemispheres. From the short latency regions of convergence-related area, symmetrical convergence was evoked but from convergence eye movements evoked from the long latency regions were asymmetrical. The convergence-related areas have neurons respond to approaching movement of a visual target. Cats (n=7) were trained to start convergence by an alarm signal (buzzer sound or combination of buzz and blinking of LED), preceding target movement by 4s. After training, ocular convergence was elicited by the alarm signal before target movement (predictive open-loop convergence) in 60% of trials. In two cats, we used training with obliquely approaching target. After training, asymmetrical anticipatory eye movements were observed. Based on these findings, related LS neuronal activities and results from lesion study, we will discuss the role of LS in asymmetry of anticipatory and visually-evoked convergence eye movement.

P307 (2P2-066)

Involvement of cerebellar NMDA receptors in adaptive modification of optokinetic response

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N-methyl-D-aspartate (NMDA) receptors are implicated in synaptic plasticity and learning in the hippocampus. In the cerebellar cortex, NMDA receptors are expressed in glanule cells and inhibitory interneurons, and the NMDA receptor dependent plasticity has been reported. However, physiological role of such synaptic plasticity remains to be investigated. Here we examined the role of NMDA receptors in the adaptive modification of optokinetic response (OKR adaptaion) as a model of cerebellar-dependent motor learning in mice. OKR adaptation was induced by sustained sinusoidal oscillation (0.4Hz, 6deg peak-to-peak) of a vertically striped screen around a mouse. The mutant mice deficient in NR2A/NR2C subunit failed to show the adaptive increase of OKR gain. Further, iontophoretic application of NMDA receptor antagonist (D-AP5) into the flocculus (the cerebellar cortical region implicated in the control of OKR) in wild type mice, suppressed the adaptive increase in the OKR gain. These results suggest that NMDA receptor dependent mechanisms in the cerebellar cortex are involved in motor learning.

P308 (2P2-067)

Integration of a target position and eye-movements during pointing movements

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On pointing or reaching movements, a target is usually in the peripheral visual field and a hand reaches toward the target after eyes moved to foveate the target. It has been suggested that a position of a remembered target for these hand movements is remapped in gaze-centered coordinates during eye movements. To investigate this remapping mechanism, we asked subjects to point a remembered target with eye-fixations. The subjects remembered a target with gazing at an initial fixation point (FP1) and moved their eyes to a second fixation point (FP2) after the target had been turned off, and pointed to the remembered target with gazing at the FP2. The following three task conditions were used: 1) the single fixation condition (SF), the FP2 appeared at the same position of the FP1 and a target was presented at the peripheral visual field; 2) the center of visual field condition (CVF), the FP2 appeared at the different position from the FP1 and a target was presented at the FP1; 3) the peripheral visual field condition (PVF), the FP2 appeared at the different position from the FP1 and a target was presented at the peripheral visual field. We assumed that the subjects calculated a sum of a FP1-target vector and a FP2-FP1 vector as a FP2-target vector (i.e., a gaze-centered remembered target position) and they had to estimate the target-FP1 and FP1-FP2 vector. The rates of miss-estimation of these vectors determined by results of the SF and CVF condition well explained results of the PVF condition. This study provides a plausible model of localization of a remembered target.

P309 (2P2-068)

Effects of reversible inactivation of unilateral frontal pursuit areas on the adaptation of post-saccadic smooth pursuit velocity in the monkeys

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Repetitive exposures to the acceleration of the target velocity for a brief period immediately after the onsets of the eye movements adaptively modify the velocity of smooth pursuit. After we located monkey frontal pursuit areas by unit recording and microstimulation, we injected $GABA_A$-receptor agonist muscimol locally. Inactivation of frontal pursuit areas reduced velocities of smooth pursuit in both ipsi and contra - versive directions to the inactivated frontal eye field by 70%, and significantly depressed the adaptation of smooth pursuit velocity dependent on the sites of inactivation. These results suggest that the frontal pursuit areas are involved in the adaptation of smooth pursuit.

P310 (2P2-069)

Firing pattern of the cat superior colliculus neurons during head unrestrained gaze shifts and their axonal projections.

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An important structure in the neural circuitry for saccadic gaze shift control is the midbrain's superior colliculus (SC). It has been proposed that the SC intermediate layer lies within a gaze feedback loop and generates an error signal specifying gaze position-error (GPE), the distance between target and current gaze positions. We investigated previously this feedback hypothesis, in cat, by briefly stopping head motion during large gaze saccades made in the dark. Firing frequency of a cell gradually increased to a maximum that just preceded the optimal gaze saccade encoded by the cell's position in the caudal SC. In "brake" trials, we demonstrated that the activity-level just preceding a brake-induced gaze plateau continued steadily during the plateau and waned to zero only near the end of the corrective saccade. In the present experiments, we tested descending axonal projections of the SC neurons on the motor map to the brain stem, using antidromic mapping technique. Stimulus currents were usually restricted to less than 30μ A. Some neurons were antidromically activated by the dorsal part of the midbrain and the reticular formation. The neural activity-level continued during the brake-induced gaze plateau. Discharge pattern reflected gaze trajectory perturbations. The data suggest that the cat's tecto-reticular cell probably lies in a gaze feedback loop.

P311 (2P2-070)

Effects of a thickening agent on the swallowing threshold.

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To investigate the effects of thickening agents, which may reduce the risk of aspiration, on the swallowing threshold during food mastication, the physical properties of bolus immediately prior to swallowing during mastication of test food containing a thickening agent (xanthan gum) were measured in healthy ten adult participants (mean age 31 yrs). Two kinds of test food (test food C, boiled Koya-dofu as a control and test food X, boiled Koya-dofu containing 1% xanthan gum) were prepared. Each test food has a cubic shape (15×15×15mm). Physical properties of test food and the test food bolus collected from the oral cavity immediately prior to swallowing were measured by method of the texture profile analysis (double bite test). Texture parameters (hardness, adhesiveness, cohesiveness, gumminess) were obtained from the stress strain curve. The number of chewing strokes until swallowing during mastication of the X was significantly smaller than that during mastication of the C. Hardness, cohesiveness and gumminess of both the C and X boluses were significantly decreased during mastication. Adhesiveness of the X bolus was also significantly ($p<0.05$) decreased during mastication, but the adhesiveness of the C bolus was significantly ($p<0.01$) increased. Adhesiveness of the X bolus immediately prior to swallowing was significantly ($p<0.001$) higher than that of the C bolus. These results suggest that the decrease in adhesiveness of bolus may be a main factor in initiating the swallowing during mastication of solid food containing the thickening agent.

P312 (2P2-071)

Pattern of modulation of the jaw-opening reflex during mastication

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Effects of mastication on the jaw-opening reflex (JOR) were studied in awake rabbits. The JOR was evoked by unilateral low-intensity electrical stimulation of the inferior alveolar nerve either chewing or non-chewing side before (control) and during mastication. The entire masticatory sequence (from food intake to just before swallow) was divided into three functionally different masticatory periods (preparatory, rhythmic-chewing and preswallow periods) based on the jaw movements and the related activity pattern of the jaw muscles, and each chewing cycle in each period was further divided into jaw-closing and jaw-opening phases. Then amplitude of the JOR was compared 1) among the masticatory periods and 2) between the stimulation sides (i.e. chewing or non-chewing sides). Overall, the JOR was suppressed during mastication. Although the suppressive effect on the JOR did not differ between the stimulation sides, considerable differences were noted in the suppressive effect among the masticatory periods. During rhythmic-chewing and preswallow periods, the JOR was strongly suppressed in the jaw-closing phase (8 to 20% of the control), but the suppressive effect was phasically weakened (30 to 61% of the control) in the late part of the jaw-opening phase. On the other hand, such phase-dependent fluctuation in the suppressive effect was not observed during the preparatory period. The findings suggest that neural mechanisms modulating the JOR during the preparatory period may be different from other masticatory periods.

P313 (2P2-072)

Induction of swallowing in in vitro brainstem preparations from newborn rats using electrical stimulation of the superior laryngeal nerve

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We examined whether the electrical stimulation of the superior laryngeal nerve (SLN) induces swallowing in an isolated brainstem preparation. We used in vitro brainstem preparations isolated either with or without the oro-facial and neck structure from 0-4 day-old Wistar rats. Neural activities were monitored from XII nerves as well as C4 ventral roots (C4 vr) with suction electrodes. Tongue EMG was monitored with wire electrodes. Movements of the peripheral structure were observed with a CCD camera system. Electrical stimulation of the SLN was applied through a suction electrode. In the preparation with peripheral structure, SLN stimulation induced the swallowing-like movements of the tongue and the larynx, involving the elevation of the tongue tip and anterior movement of the larynx. They corresponded to the tongue EMG with no concurrent activity in C4 vr, and were distinct from the spontaneous inspiratory activities in a burst shape. In the preparation without peripheral structure, SLN stimulation induced the short latency XII bursts whose shape was indistinguishable from the tongue EMG in the preparation with peripheral structure. The results demonstrate that the electrical stimulation of the SLN induces fictive swallowing in an isolated brainstem preparation as in vivo preparations. These preparations will be useful for the investigation of mechanisms underlying the central pattern generation of swallowing.

P314 (2P2-073)

Membrane properties of neurons in the rat nucleus prepositus hypoglossi

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Nucleus prepositus hypoglossi (NPH) is involved in the velocity-position integration for horizontal eye movements. To clarify which types of neurons compose the NPH, we investigated membrane properties, such as afterhyperpolarization (AHP), responses to hyperpolarizing currents, and firing patterns, of NPH neurons using whole-cell patch clamp technique in rat brainstem slices. AHPs were classified into three types: AHP without a slow component, AHP with a slow component, and AHP with a slow component and an afterdepolarization. Responses to hyperpolarizing current pulses were classified into three types: time-dependent inward rectification, time-independent inward rectification, and no inward rectification. Firing patterns were classified into continuous-spiking neurons that exhibited repetitive firings with relatively constant interspike intervals (ISIs), late-spiking neurons that exhibited a delay in the generation of the first spike, and neurons exhibiting an extremely low firing rate. Continuous-spiking neurons were further divided into two types according to whether the first ISI was markedly longer than the second. Neurons exhibiting the long first ISI were designated as FIL neurons. Application of 100μM apamin abolished the long ISI of FIL neurons, suggesting that the long ISI is attributed to activation of SK-type Ca^{2+} activated K^{+} conductances. All these results suggest that the NPH consists of heterogeneous neuronal population with various membrane properties and these neurons contribute to the neural integrator.

P315 (2P2-074)

The role of neck muscles on the bite force production in man

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I have previously reported that the subjects who did not move their head at all during chewing could always increase the maximum bite force (BF) by supporting their head. The purpose of this study is to investigate the functional role of lateral and dorsal neck muscles on the bite force production by the electromyographic (EMG) method. Fifteen adult volunteers participated in this study. The EMGs of the masticatory (MAS), the lateral neck (SCM) and the dorsal neck (TPZ) muscles were recorded with a pair of surface electrode. A bite stick with a strain-gauge transducer was used to measure the BF. The subject was asked to produce and maintain the BF at about 40% level of the voluntary maximum BF. There were close relationships between the BF and the EMGs of the MAS and the SCM. The dorsal head flexion elicited by contracting the TPZ decreases the BF as well as the EMGs of the MAS and the SCM. The tap stimulus to the muscle belly of the TPZ also decreases the BF. These results suggest that the lateral neck muscles as well as the masticatory muscles play an important role in the bite force production, and that the dorsal neck muscles, which function as the head stabilizer in the upright posture, control the above bite force producing muscles.

P316 (2P2-075)

Phosphorylation of Extracellular Signal-Regulated Kinase in trigeminal spinal nucleus neurons following passive jaw movement in rats with chronic TMJ inflammation

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We studied pERK expression in the trigeminal spinal nucleus caudalis (Vc) of the rats with TMJ inflammation, in order to elucidate the underlying neuronal mechanism of the trigeminal chronic pain. CFA was injected in the TMJ under pentobarbital anesthesia (50 mg/kg, i.p.). Two weeks after CFA injection, passive jaw opening (JO) was applied to the TMJ inflamed rats for 5-30 min (JO distance: 4, 6 and 15 mm). The pERK expression was studied in the medulla and upper cervical spinal cord after JO. The face temperature was significantly increased 2-3 days after CFA injection and returned to the preoperative level 7 days after that. The pERK-like immunoreactive (LI) cells were observed in the dorsal portion of the trigeminal spinal nucleus caudalis (Vc) of the TMJ inflamed rats after JO. On the other hand, we could not observe any pERK-LI cells in naive rats after 4, 6 and 15 mm JO. The number of pERK-LI cells was increased following increase in the JO duration (5, 15 and 30 min) and JO distance (4, 6 and 15 mm). The increment ratio of pERK-LI cells in Vc was significantly larger in 15 mm JO group than 4 and 6 mm JO groups following different JO duration. These findings suggest that the hyperexcitability of nociceptive neurons in the dorsal portion of the Vc would be involved in the TMJ chronic pain following TMJ inflammation through the activation of the intracellular ERK cascade.

P317 (2P2-076)

Handedness-related asymmetries in indirect corticomotoneuronal pathways of humans

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For arm movements, it is suggested that indirect corticomotoneuronal pathways, which are partly mediated by C3-C4 propriospinal neurons (PNs), have important roles as well as direct pathways. The purposes of the present study were to establish a procedure to quantify functions of PNs, and to examine left-right differences of the pathways by using the procedure. Surface electromyograms were recorded from the right or left biceps (Bi) muscle of right-handed normal human subjects (n=11), who all gave informed consent. During weak tonic voluntary contraction of the muscles, 1) electric stimulation of the ipsilateral ulnar nerve (wrist) alone, 2) transcranial magnetic stimulation (TMS) of the contralateral primary motor cortex alone, and 3) combined stimulation of both were delivered in pseudo-random order. Interstimulus intervals for the combined stimulation were set to 8.0-9.0ms (ulnar stimulation ahead). When appropriate stimulus strengths were selected, TMS-induced potentials in Bi were facilitated by combined ulnar stimulation. However, the facilitation was disappeared when strengthening ulnar stimulation and/or TMS. These were the case on both sides of all subjects, though appropriate stimulus strengths were variable among the subjects. When comparing the maximum facilitations on both sides, they tended to be stronger on the dominant (right) side. We conclude that the current procedure is valuable to quantify functions of PNs, though stimulus condition and handedness have to be considered.

P318 (2P2-077)

Functional difference between dominant and non-dominant limbs in dynamic postural control of human upright standing

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To understand the functional difference between dominant and non-dominant limbs in human upright standing, we analyzed electromyographic (EMG) activities while subjects stood on rocking platforms with various sizes. EMG activities were recorded from muscles of ankle and knee joints of both limbs. Subjects were instructed to stand on the rocking platforms so that they rocked in the saggital plane. Movements of the platforms, equivalent to fore and back movements of the subjects, were recorded by a force plate (center of pressure, COP).

Mean EMG amplitudes of ankle extensors (m. triceps surae, GS) and flexor (m. tibialis anterior, TA) were higher in the non-dominant side than in the dominant side. Nevertheless mean EMG amplitudes of knee joint muscles were rather complicated; some muscles of the dominant side could show lower amplitude than those of the non-dominant side. As the radius of the rocking platform decreased, the ratio of mean EMG amplitudes of dominant and non-dominant muscle pairs (dominant-ratio) increased, and the correlation coefficient of these muscle pairs increased. Cross correlation analysis of the COP and EMG changes showed that non-dominant side muscles strongly correlated with the COP when subjects stood on the rocking platform with the largest radius. It was supposed that in a condition where subjects easily kept standing, non-dominant limb muscles were primarily used to control the COP, but dominant and non-dominant muscle pairs were used synchronously when it was difficult to keep standing.

P319 (2P2-078)

Roles of the prefrontal cortex dopamine transmission in behavioral activation via the subthalamic nucleus

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Dopaminergic (DAergic) system in the medial prefrontal cortex (mPFC) is involved in cognition, memory, and behavioral control. Although the dysfunction of the DAergic system is suggested to cause several mood disorder and schizophrenia, the neural mechanisms for behavioral control through the interaction between the mPFC and the basal ganglia remain to be unclear. To elucidate this issue, we examined the effects of blockade of DAergic transmission in the mPFC of C57/BL6J mice during methamphetamine (METH)-induced hyperlocomotion. When mice received intra-mPFC infusions of muscimol, a $GABA_A$ receptor agonist, METH-induced hyperlocomotion was significantly reduced. Infusions of SCH23390, a D1 DA receptor antagonist, and sulpiride, a D2 DA receptor antagonist, also decreased their locomotion. Although METH induced *c-fos* expression in the subthalamic nucleus (STN), these drugs infused into the mPFC significantly suppressed METH-induced STN activation. These results indicate that DAergic transmission in the mPFC during DA-induced locomotion facilitates their behavioral activity through the activation of the STN, implying that the mPFC-STN pathway contributes to DA-induced behavioral activation.

P320 (2P2-079)

Characteristics of fore- and hindlimb movements during the midbrain stimulus-evoked locomotion in decerebrated rabbits

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This study aimed to investigate characteristics of fore- and hindlimb locomotor movements evoked by stimulation to the midbrain in decerebrate rabbits. Under halothane anesthesia, adult rabbits (NZW, 2-3 kg) were surgically decerebrated at the precollicular-postmammillary level. The head was then fixed in a stereotaxic apparatus, and the body was supported by rubber belts. To evoke locomotion, 50-Hz stimuli (10-100 μA, 0.2 ms duration, 5-10 sec) were applied to the midbrain cuneiform nucleus, which corresponds to the mesencephalic locomotor region in cats, with Wood's-metal-filled glass microelectrodes. When the midbrain was stimulated, the left and right hindlimbs consistently exhibited nonalternating, rhythmic hopping movements. Even after the spinal hemisection at the lower thoracic level, the stimuli still evoked in phase rhythmic movements of bilateral hindlimbs. In contrast, left and right forelimbs basically displayed alternating locomotor movements when the midbrain was stimulated. In particular, after complete transection of the lower thoracic cord, the stimuli evoked purely reciprocal movements of bilateral forelimbs at any stimulus intensity. Despite such differences in fore- and hindlimb movement patterns, locomotor cycle frequency of fore- and hindlimbs was usually equal when the full hindlimb hopping was evoked. These findings suggest that spinal neuronal circuits involved in the generation of fore- and hindlimb locomotor patterns are tightly coupled, although they are constituted in a different manner.

P321 (2P2-080)

Comparison of information representation in the primary motor and dorsocaudal premotor cortices

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In our previous study, temporal activity of single neurons of the primary motor cortex (MI) was correlated with kinematics or dynamics variables of arm movements using a multiple linear regression analysis. The present study extended the analysis to the dorsocaudal premotor cortex (PMdc) and compared the results with those of MI. As explanation variables, we used the following four types of variables sets (or models); 1) kinematics variables represented in Cartesian coordinates (model Kc), 2) those in joint coordinates (model Kj), 3) dynamics variables in Cartesian coordinates (model Dc) and 4) those in joint coordinates (model Dj). The analysis was applied to 189 MI and 52 PMdc units that responded to passive movements of the shoulder and/or elbow joints or maneuver manipulations of the muscles controlling these joints. Each unit was classified into one of the four types according to the model that best fitted the unit. About 85% neurons belonged to either of the dynamics types (i.e., type Dc or Dj) with predominance of type Dj, and the ratio of each type of neurons was similar in MI and PMdc. Moreover, activity of about 80% of the neurons preceded the arm movements, and this was observed commonly for MI and PMdc neurons. These results suggest that MI and PMdc neurons receiving proprioceptive information share similar roles in the execution of manual reaching movement.

P322 (2P2-081)

Suppression of activity of monkey subthalamic nucleus (STN) neurons induced by single-pulse electrical stimulation in STN

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The STN is a target for 'deep brain' stimulation (DBS) in the treatment of Parkinson's disease. DBS effects are likely to be a mixture of multiple effects, affecting both axons and somata within the STN. We here report on experiments studying one key component of the overall response to DBS, i.e., the effect of stimulation on the activity of neurons within the STN. Two monkeys received two chronic recording chambers each. The two chambers were directed at the same STN. One of them was used to carry out electrical stimulation of the STN with a microelectrode (using monophasic stimulation at 1/s, pulse width 50 μs, amplitude ~ 300 μA), and the other to simultaneously record the neuronal activity in STN with standard extracellular single-unit recording techniques. Peristimulus histograms were calculated to determine the latency and duration of stimulation-evoked responses.

In most cases, STN neurons responded to the nearby stimulation with a cessation of activity, typically starting immediately after the stimulation and lasting for 37.2 ± 20.7 ms (n = 161). In some neurons, the inhibition was followed by an excitation.

The results suggest that electrical stimulation of the STN has prominent and complex effects on the neuronal activity within the STN. Given the high frequency of therapeutic DBS (130 Hz), it is unlikely that the excitatory effects will manifest themselves. The inhibitory responses may result from activation of GPe axons which then would inhibit STN neurons.

POSTERS
Higher CNS functions

P323 (2P2-082)

Local Field Potentials of Hippocampus under Urethane-chloralose Anesthesia in Piglets

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The pig hippocampus has a highly laminated dentate hilus, which clearly resemble to that of primates. However, few studies have been performed to investigate its neural activities. Therefore, in this study we examined local field potentials (LFPs) in the hippocampus of male Landrace piglets under general anesthesia. The anesthesia was maintained by halothane (1-2%) or by intravenous administration of urethane and chloralose with the minimal level of halothane (0.5%). A few spike-shaped waves in the LFPs were found in the hippocampus of the five piglets anesthetized with halothane. In contrast, spike-shaped waves in the LFPs were observed frequently in the three animals anesthetized with urethane and chloralose. Thus, the present study indicates that more spiking activities can be obtained in the hippocampus of the piglets anesthetized by with urethane-chloralose than by halothane. This suggests that the hippocampal neurons are more excitable in the piglets anesthetized with urethane and chloralose than with halothane only.

P324 (2P2-083)

Changes in rat hippocampal and prefrontal local field potential powers related to learning stage in operant reversal task

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To investigate whether local field potential (LFP) levels may change in association with the learning stage, we analyzed LFPs from rat hippocampus (Hip) and prefrontal cortex (PFC) during operant discrimination reversal trainings. Rats were trained with initial discrimination task using light and sound (one for S+ and another for S-) as the discrimination stimuli until a stable discriminative performance was achieved. Then the rats received the reversal training. In this training, responses to S+ signal increased within 2-4 training sessions (early learning phase), whereas those to S- decreased at the late phase (4-8th sessions). LFP gamma-band powers showed an increase during training, which was significantly attenuated at the first training session in the Hip, and in the late learning phase in the PFC. These results suggest that LFP gamma-band powers change during the learning stage in the Hip and PFC differently. First exposure to the reversal training may depress Hip gamma-band activity. The depression of PFC gamma-band activity may relate to inhibitory control in the late learning phase.

P325 (2P2-084)

Long-term potentiation and long-term depression in the monkey hippocampus

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Long-term potentiation (LTP) and long-term depression (LTD) are both forms of synaptic plasticity, which have been studied extensively in rodents. However, information about LTP and LTD in the primate brain is limited, especially when using *in vivo* models. This limitation is primarily due to difficulties in the precise implantation of electrodes within deep telencephalic structures of the primate brain. Using MRI and local field potentials to guide the implantation procedure, we inserted a stimulation electrode in the perforant pathway and a recording electrode in the dentate gyrus of the monkey. Correct implantation was confirmed by the appearance of evoked potentials in the dentate gyrus following stimulation of the perforant pathway. Evoked potentials changed systematically according to the depth of the electrodes. The effects of high-frequency stimulation (HFS; 100, 200 or 400 Hz) or low-frequency stimulation (LFS; 1, 2 or 5 Hz) on evoked potentials were tested in an awake condition. We used HFS or LFS parameters that have been demonstrated to produce LTP or LTD in rodents. In the monkey, however, we found that only the HFS with the highest frequency induced LTP, while all LFS levels failed to induce LTD. These data suggest that 1) the present animal model is suitable for testing long-term synaptic plasticity in the primate brain, and 2) the primate hippocampus is more resistant to the induction of long-term synaptic plasticity than the rodent hippocampus.

P326 (2P2-085)

Effects of exercise on neurogenesis in the dentate gyrus and ability of learning and memory after hippocampus lesion in adult rats

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Present experiments were undertaken to explore the effects of exercise on dentate gyrus (DG) neurogenesis and the ability of learning and memory in hippocampus-lesioned adult rats. Hippocampus lesion was produced by intrahippocampal microinjection of kainic acid (KA). Bromodeoxyuridine (BrdU) was used to label dividing cells. Y maze test was used to evaluate the ability to learning and memory. Exercise was conducted in the form of forced running in a motor-driven running wheel. The speed of wheel revolution was regulated at 3 kinds of intensity: lightly running, moderately running, or heavily running. Hippocampus lesion could increase the number of BrdU-labeled DG cells, moderately running after lesion could further enhance the number of BrdU-labeled cells and decrease the error number (EN) in Y maze test, while neither lightly running, nor heavily running had such effects. There was a negative correlation between the number of DG BrdU-labeled cells and the EN in the Y maze test after running. The results suggested that moderate exercise could enhance the DG neurogenesis and ameliorate the ability to learning and memory in hippocampus-lesioned rats.

P327 (2P2-086)

Forced running enhances neurogenesis in the hippocampal dentate gyrus of adult rats and improves spatial learning ability

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To investigate the effect of forced running on neurogenesis in the hippocampal dentate gyrus (DG) of adult rats, 5-bromo-2-deoxyuridine (BrdU), the thymidine analog, that can be incorporated into the DNA during the S phase of cell cycle, was applied to mark cell proliferation. Neuroepthelial stem cell protein (nestin) expression was used to identify neural stem cell/precursor cells. The BrdU- and nestin-positive cells were examined by immunocytochemical technique. The ability of spatial learning and memory was evaluated by Y maze and Morris water maze tests to explore the functional role of the newly born cells in DG after running. It was found that the number of BrdU- and nestin-positive cells in the dentate gyrus in running groups was significantly increased as compared to that of control group. The effect of forced running on neurogenesis was intensity-dependent. In addition, an improvement of spatial learning ability was observed after forced running in Y maze, as well as in Morris water maze tests. These findings demonstrated that forced running could enhance neurogenesis in the hippocampal dentate gyrus of adult rats and facilitate acquisition of a hippocampus-related spatial learning task.

P328 (2P2-087)

Central nervous mechanisms in connection with spatial recognition in monkeys

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In order to study central nervous mechanisms on spatial recognition, monkeys were trained for go/nogo hand movements with discrimination of two different loci in a space. Two yellow LEDs were used as go and nogo stimuli. Cortical field potentials were recorded by electrodes implanted on the surface and at a 2.0-3.0 mm depth in various cortices in monkeys performing the movements. The potentials were usually averaged 50 times by the onset pulses of go and nogo stimuli. In task (I) with right LED as go stimulus, and left LED as nogo stimulus, a surface-negative, depth-positive potential at a peak latency of about 100 ms in the left A7 was bigger in amplitude in go trials than in nogo trials, and a surface-positive, depth-negative potential at a peak latency of about 100 ms in the right A7 was bigger in amplitude in nogo trials than in go trials. On the other hand, the data in A7 in task (II) with left LED as go stimulus, and right LED as nogo stimulus were the opposite of that in I. Nogo potential in the prefrontal cortex was recorded in nogo trials only in I and II. This suggests that A7 on both sides is related to spatial recognition. Influences of electrical perturbation on the movement were also studied. A train of electric pulses was delivered 60 and 270 ms after stimulus onset to the electrodes in A7 in a monkey performing the movements. Only the electric pulses at 60 ms delay decreased the number of appropriate responses to stimuli. This indicates that A7 was significant for spatial recognition in monkeys.

P329 (2P2-088)

Assessment of exhilarating levels during watching amusing films and comical picutures

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To evaluate human feeling objectively, we analysed electroencephalogram (EEG), electrocardiogram (ECG) and pneumogram. Amusing films (Japanese comedians;Down-town or Mr. Beans) or comical pictures were shown to healthy male subjects. EEG was recorded almost 30 min (5 min control, 10 min watching VTRs or 20 pictures for 15 sec each, and 15 min recovery) by Neurofax EEG-1100 and analyzed by data analysis software, Focus/QP-211A (Nihonkohden). Electrode configuration of 25 points on scalp of subjects was based on the 10-10 electrode method by the IEF, International Encephalogram Federation. EEG was analyzed by mean FFT (Fast Fourier transform). We detected that amplitudes of beta (13-30 Hz) and gamma (30-50 Hz) bandson electrode positions, T7 and T8 in temporal and parietal regions were greatly enhanced during watching the amusing VTRs or comical pictures. The amplitude of an enhancement was larger when watching more amusing VTR. If the quantity of amusing level to each subject on individual VTR was small, the amplitude of beta and gamma amplitudes on mean FFT was small. On the other hand, intervals of respiratory ventilation were decreased during watching the amusing VTR or comical pictures. When subject felt that the VTR or comical pictures was so funny, the heart rate was transiently increased and continued to be increased after watching the VTR or pictures. Present results suggested that EEG, pneumogram and ECG were changed specifically in accompanying with emotional changes in response to exhilarating level of the subjects to amusing VTRs or comical pictures.

P330 (2P2-089)

Categorization of actions in monkeys as revealed in tasks of retrieving food from a tube of varied directions using tweezers

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Monkeys had learned a task of sequential movements of grasping tweezers in front, bringing it to a piece of food on a plate, and picking it up to eat. We tested a new task of picking food from inside a vertical tube (R15,H20mm), which required pronation of the forearm so that the tweezers are directed properly for insertion into the tube. Monkeys could not learn such pronation and failed to take food even after many repeated trials. Thus, we used a horizontal tube instead that requires little pronation. Initially, they brought the tweezers to the opening of the tube, but failed to grasp food, only hitting the tube wall or otherwise. They gradually became better at handling the tweezers and able to pick up the food within 100 trials. Next, a small stepwise change (less than 10 deg) in the direction of tube toward the upright position was tested and showed that the monkeys had to learn the task for each new direction of the tube. Thus, learning was specific for each direction. The smaller the change in tube direction, the faster they learned, suggesting that they started to understand the similarities between the task movements. Finally, when the direction was changed in a large step from learned 60 to 90 deg (vertical), they adapted to the latter task quickly. These results suggested that monkeys can group task actions of various tube angles into a single category through practice.

P331 (2P2-090)

c-Fos expression in the primary sensory cortex during temporal order judgment in mice.

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To study how somatosensory stimuli are ordered in the brain, the expression of c-Fos was examined in C57BL/6N mice (male, n = 8) that were trained to judge the order of two air-puff stimuli delivered bilaterally to the right and left whiskers with stimulation intervals of 50-750 ms. The mice were rewarded with a food pellet when they responded by orienting their head toward the first stimulus (n = 2) or toward the second stimulus (n = 6) after a visual "go" signal. c-Fos-stained cell densities of these mice (test group) were compared with those of two control groups in coronal brain sections by applying statistical parametric mapping to the c-Fos immuno-stained sections. The expression of c-Fos was significantly higher in the test group than in the other groups in the bilateral barrel fields of the primary somatosensory cortex. Laminar analyses revealed that c-Fos expression in the test group was most evident in layers II and III, where callosal fibers project. Using double-labeling methods, we further found that c-Fos positive cells in the primary somatosensory cortex were mainly excitatory but that some of them were GABAergic. The results suggest that temporal order judgment involves processing bilateral somatosensory signals through the excitatory and inhibitory neurons in the supragranular layers of the primary sensory cortex.

P332 (2P2-091)

Neuronal activity coding visual category of foods in the monkey orbitofrontal cortex

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The visual performance of macaque monkeys is highly evolved comparable to human. Because acquiring adequate foods is important to survive for monkeys, they could distinguish foods from many objects using visual information. To elucidate neural mechanism of visual categorization of food objects, we conducted (1) behavioral analysis of visual discrimination task for food and (2) analysis of neuronal activity during the task in orbitofrontal cortex (OBF), the region related to foods recognition and vision information processes. In the food discrimination task, a picture of either a food or a non-food object was randomly presented on a compute monitor. Geometrically simple figures and alphabetical letters were also used as a comparison. Monkeys were trained to press a lever to get water reward when a picture that belonged to pre-determined "target" category, such as foods, was presented. As a result, monkeys were able to answer the paradigm correctly over 80% in each session, indicating that they can distinguish foods from other than foods using only visual information. Extracellular recoding of neurons during the task showed variety of responses. Although many neurons responded to all or some pictures in non-specific manner, some neurons responded in a category-specific manner, which responded to the pictures that belonged to food category. In addition, expectation- or reward-related activities were also recorded. In conclusion, OBF could be involved in associated learning of the food category and reward including expectation.

P333 (2P2-092)

Behavioral and neuronal analysis of visually-guided categorization of sex in macaque monkeys

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Visual information plays roles in social communication in monkeys. In this study, to elucidate visual function to categorize sexes, (1) performance of visually-guided sex discrimination task and (2) analysis of neuronal activity during the task in orbitofrontal cortex (OBF), the region could be related to sex recognition and vision processes, were investigated. Additionally, (3) visual preferences for sexuality were also studied. In the sex discrimination task, monkeys were trained to discriminate sex of a monkey shown in a picture that was randomly selected from photographs that was presented on the display. The monkeys pressed the right bar for males and the left for females to get water reward. In this task, the monkeys were able to discriminate the sexes of monkeys shown in pictures. Extracellular recordings in OBF neurons during the task showed that some cells responded to the pictures in a sex-specific fashion. In the sex preference task, monkeys voluntarily pressed a bar to watch the video movie showing either males or females. The total duration of the responses was measured. In the task, four out of six monkeys showed sex preference. Three monkeys preferred the video of males that was taken in breeding season. Another two did not show the preference. The other youngest one preferred the female movie that was taken in non-breeding season. The present results suggest that visual information alone is sufficient to discriminate sex and produce sex preference in some monkeys. OBF could be involved in visual categorization of sex.

P334 (2P2-093)

Single neuronal responses in monkey anterior insular cortex code the reward expectancy both during uncertain and certain reward condition as occurs in gambling

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Gambling is a form of risk-taking that probably occurs normally so that some exploration of the environment takes place when it is done in moderation. Recent neuroimaging studies revealed gambling or risk-taking related activity in several brain areas. Among these, the anterior insula has attracted interest because of its frequent activation during those experiments, though the reported results are not always consistent. Here we have used single neuronal recording in monkeys to refine the interpretation of imaging data related to motivation and reward expectancy. 120 neurons were recorded while the monkeys performed multi-trial reward schedule task. To earn juice reward, the monkeys were required to complete 1 - 4 simple visual discriminations. These four schedules were randomly interleaved. We used two conditions: (1) the cue indicates the progress toward the reward (cued condition : certain reward), (2) the cue was randomly chosen, making the reward availability to be unknown (random condition: uncertain reward). The monkeys showed higher performances in the random condition, suggesting higher motivation in expectation of possible reward. 84/120 neurons responded in all trials of the random condition. However, in the cued condition, 50/84 showed responses only in rewarded trials. The results suggest that reward expectancy signals coded by anterior insular neurons might underlie the activation related to gambling in imaging studies.

P335 (2P2-094)

Analysis of locomotor activity influenced by learning, memory, and emotion: Involvement of dopamine transmission in the prefrontal cortex

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The medial prefrontal cortex (mPFC) and dopamine (DA) reportedly control locomotor activity, which is an essential index in standardized animal tests for several neurotoxicity studies. To investigate the relationship between locomotor control and mPFC DA, we measured locomotor activity twice at a 24-h interval by applying footshock (0, 0.3, or 1.0 mA) at the first measurement, and simultaneously measured locomotor activity and mPFC DA using an HPLC-ECD-equipped microdialysis method. The 1.0-mA group tended to show greater DA release than the others on first moving to an open-field cage (45 cm square, equipped with shock grids), returning to the home cage, during the measurement period of the 24-h interval basal level, and during the second move. Remarkably, the DA basal level before the second move was higher than that before the first move in the 1.0-mA group, while it was lower in the 0-mA group. We report on an autocorrelation analysis of either DA or locomotion and a cross-correlation analysis between DA and locomotion, and discuss the fundamental roles of dopamine transmission in the prefrontal cortex with respect to behavioral control.

P336 (2P2-095)

Dopamine but not norepinephrine regulates glutamate release in the prefrontal cortex of the monkey

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It has been well documented that the prefrontal cortex (PFC) plays important roles in performance of working memory (WM) tasks. Furthermore, dopamine (DA) and glutamate in the PFC are shown to be indispensable for WM task performance both in the rat and monkey. We previously reported that there was a double dissociation in changes in glutamate and dopamine between a WM (spatial delayed alternation) task and a non-WM (sensory guided control) task i.e., a significant increase in dopamine but no change in glutamate during the WM task and a significant increase in glutamate but no change in dopamine during the sensory guided task. In order to clarify the mechanism of this dissociation, we investigated a DA-glutamate interaction in the PFC in the unanesthetized condition, using reverse dialysis method.

As the application of 1 mM dopamine itself(78.87 ± 12.17, $p = 0.049$, $n = 8$), the simultaneous application of dopamine D_1 (SKF38393; 1.0 mM) and D_2 (quinelorane; 1.0 mM) receptor agonists significantly decreased glutamate levels in the PFC ($85.01 \pm 4.33\%$, $p = 0.019$, $n = 8$). On the contrary, neither norepinephrine (1 mM) nor clonidine (α_2 agonist)(0.1 mM, 0.5 mM, 1.0 mM, 2.0 mM) affected glutamate releases significantly. After a solo application of D_2 agonists, glutamate levels decreased in a dose dependent manner. Furthermore, this decrease of glutamate by quinelorane was not antagonized by 0.5 mM muscimol.

The results suggest that dopamine D_2 receptors, but not α_2 receptors, have disfacilitatory roles for the WM.

P337 (2P2-096)

Dopamine neurons code reward values and their errors expected through multiple voluntary choices of actions

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Midbrain dopamine (DA) neurons have been reported to code reward value (probability and/or volume) of external cues and errors of the expected value. In the behavioral tasks used in most previous studies, reward value was assigned in single trials but not in consecutive multiple trials. Thus, an important and unresolved question is whether DA neuron activity represents value and its error of reward expected not in single trials but in consecutive multiple trials. In this experiment, we recorded activity of DA neurons in two monkeys performing a multiple free choice task to find a correct, rewarding target among three alternatives in trial-and-error manner. Monkeys made first (T1), second (T2) and third (T3) choices. Average reward probabilities of T1, T2 and T3 trials were about 30, 50 and 80%, respectively. After a correct target was hit, monkeys got water reward once more by choosing the same known target in a succeeding trial (R1) at 95% probability. DA neurons responded to the task start cue (49/62 cells) and/or reinforcer beep (58/62) after the choices. Magnitude of cue responses monotonically increased from T1 to T2 and to T3 trials in accordance with reward probability. But the magnitudes of responses in R1 trials were much smaller than those in T3 trials in spite of their highest reward probability. The smaller R1 responses occurred in a control task in which monkeys chose a visually instructed target. These results supported the view that DA neurons code reward values and their errors expected through a series of voluntary choices of actions.

P338 (2P2-097)

The 24-h dopamine release profile in the medial prefrontal cortex in male and female rats

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The dopaminergic neurons in the ventral tegmental area have major projection to the following areas: the nucleus accumbens, the basolateral amygdala, and the medial prefrontal cortex (mPFC). The dopaminergic projection is involved in the emotional states as well as motivational and cognitive processes. In the present study, to examine the 24-h profile of dopamine (DA) release in the mPFC (prelimbic cortex), in vivo microdialysis study was performed in intact male and cycling female rats. Dailysates were automatically collected via a microdialysis probe from the mPFC every 30 min for more than 24 h under the freely moving condition. DA concentrations in dialysates were quantified by high performance liquid chromatography system with electrochemical detection. Although all rats showed an episodic release profile throughout the day, significant diurnal variation was observed in both sexes of rats: the DA release was high from the late night through the wee hours of the morning, but low in the afternoon. Moreover, the amount of DA released in female rats was significantly greater than male rats ($p<0.01$). These results suggest that inherent sex and/or gonadal steroid enviroment can affect the dopamine release in medial prefrontal cortex in rats. This is the first report showing the 24 h profile of DA release in the mPFC in both sexes of rats.

P339 (2P2-098)

Interaction of neural activities in rat somatosensory cortex evoked by stimulation of the ipsi- and contra-lateral whiskers

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Neural activities in the rat somatosensory cortex are evoked mainly from contralateral side of the periphery, but stimulation of the ipsilateral side is sometimes also effective even if their responses are small. In case of whisker stimulation if the ipsilateral side precedes responses from the contralateral side is suppressed completely for 100 ms, and it returns to normal level within 150-200 ms. To test the hypothesis that this interaction occurs through direct communication between both hemispheres, we preceded somatosensory cortex stimulation(0.1-0.5 mA, 200Hz, x11) to that of the periphery, and observed neural activities in the opposite cortex with imaging techniques of intrinsic signals of deoxyhemoglobin. In case of the contralateral periphery stimulation their responses were suppressed for 50-100 ms, whereas in case of the ipsilateral side strong facilitation of their responses were observed for 100 ms before and 3 sec even after the periphery stimulation. It is interpreted that interaction of the ipsi- and contralateral periphery inputs is, at least partly, owe to direct communication between both hemispheres, and the preceding cortex stimulation have facilitatory effects on the periphery input, so that the ipsilateral periphery evokes localized circulation changes to larger extent in the opposite hemisphere.

P340 (2P2-099)

The distinct modes of attention modulate binding color and motion in the monkey visual system

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Natural scene is crowded with many objects containing the various visual features, such as color and motion. Combining the multiple visual features are powerful clues to select and specify the target in the crowded visual scene. The process of target selection includes bottom-up and top-down attention. We examined the effects of bottom-up and top-down attention on the visual system, which selects the target among the competitive visual arrays. We used the random dots which contained the two colors (red and green) and motions (up and down). The target color was instructed as the red or green cue-spot. The two monkeys were trained to report whether the majority of random dots with the target color moved upward or downward by touching right or left bar, respectively (Choice condition). In the other condition, the monkeys were not required to choose the left or right bar, and had only to touch the middle bar, regardless of which colors and motions were combined (Ignoring condition). This manipulation dissociated the perceptual process with and without top-down attention. Bottom-up attention was manipulated by changing stimulus discriminability as a function of binding correlation of color and motion. Under these distinct modes of bottom-up and top-down attention, we compared the behavioral markers, such as accuracy, reaction time and eye movements.

P341 (2P2-100)

Laterality of Human Gustatory Pathway to Primary Cortices Studied by functional MRI

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Previous studies with MEG and fMRI located the human primary gustatory cortices at the ventral end of the central sulcus (cs), the rolandic operculum (Fop) and area G consisting of buried parietal operculum and posterior superior insula. In the present study, to examine the laterality of the gustatory pathway to these cortices, we stimulated at right or left side of the tongue with 1M NaCl in human to detect activated cortical regions with fMRI. Single subject analysis disclosed activations at area G bilaterally or ipsilaterally to stimulation sides, but not contralaterally only ($p<0.05$ FDR corrected, across entire volume). Group analysis with random effect models reveled activations at bilateral area G with stimulation of either side, but at the Rop and cs ipsilaterally with left side stimulation only ($p<0.05$ FWE corrected, ROI analysis).

P342 (2P2-101)

Olfaction- and pain-related activations estimated by a dipole tracing method.

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The aims of the study were to use the EEG dipole tracing method of the scalp-skull-brain head model (SSB/DT) to determine pain-related regions, and to examine whether simultaneous stimulus of pain and olfaction activated the same regions as observed in the sole stimulation of pain. All subjects were tested for (1) pain, (2) pain+detection level of odor, and (3) pain+recognition level of odor in randomized order. Level of painfulness and pain unpleasantness were reduced at condition 3. Pain-related somatosensory evoked potentials were identified in the averaged waveforms of all conditions; however, in condition 3, amplitudes of P2 were smaller than condition 1. SSB/DT estimated dipole localization in the contralateral somatosensory areas at N1 and in the secondary somatosensory areas at N2 in all conditions. At P2 component in condition 1, dipoles were converged in the anterior cingulated gyrus. In conditions 2 and 3, dipoles were localized in the insula and superior frontal gyrus at P2 component. The results suggest that activations of the olfactory-related areas including the thalamus could affect the pain-related process to the anterior cingulated gyrus. This study was approved by the Committee of Showa University School of Medicine.

P343 (2P2-102)

Decrease of auditory evoked potentials and appearance of high frequency oscillation in the auditory cortex associated with thalamic neurodegeneration.

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To elucidate the electrophygiological changes associated with thalamic degenerations in Purkinje cell degeneration (pcd) mice, chronic recording electrodes were implanted on the surface and at a depth of 1 mm from the surface of the auditory and visual cortices. In pcd mice, auditory evoked potentials (AEP), composed of surface-positive and depth-negative potentials at a peak latency of 20 ms, began to decrease gradually from postnatal day 45. Analysis of spontaneous transcortical potentials (surface minus depth potentials) by fast Fourier transform, revealed that high frequency oscillation (HFO) around 25 Hz appeared prominently in the auditory cortex but not in the visual cortex. Administration of MK801, an NMDA receptor antagonist, decreased the HFO, suggesting that the NMDA receptor might be involved in the generation of the HFO. The morphological investigation by using Fluoro-Jade, a fluorescent dye sensitive to degenerating neurons, located the distribution of the degeneration. Thalamic degenerations were conspicuous during postnatal days 50-70. Degenerating neurons were localized in particular thalamic nuclei including the ventral medial geniculate nucleus. These degenerating thalamic neurons were labeled by TUNEL staining, indicating that these neurons fell into apoptosis. Degenerating neurons were rarely observed in the lateral genicualte nucleus and cerebral cortex. These results suggested dynamic changes occurred in the thalamo-cortical system after thalamic degeneration.

P344 (2P2-103)

Striatal and thalamic beta-band activities are exaggerated in Parkinson's disease but not in essential tremor

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Background: During the stereotaxic thalamotomy for Parkinson's disease (PD), prominent beta-band oscillatory activities are recorded in the striatum, mostly within the caudate nucleus (Cd), and in the thalamic ventroanterior/ventrolateral nuclei (VA/VL). We tested the hypothesis that these activities were exaggerated to yield rigidity in PD, by comparing with those in essential tremor (ET) with tremor but without rigidity. Methods: 1) Six PD patients with rigidity, the other six PD patients with rigidity and tremor and six patients of ET gave their informed consent to undergo the thalamotomy. 2) Electrical activities were monitored via a bipolar concentric semi-microelectrode as filtered local field potentials and multiple unit spikes in exploring surgical targets for rigidity and tremor alleviation along a track passing the Cd through the thalamic VA/VL and ventralis intermedius nucleus (VIM). 3) To rate the occurrence of field wavelets at 13-27 Hz (beta-waves) and of those related with tremor at 3-7 Hz (tau-waves), we adopted their accumulated period (%) for 3-sec record sampled in depths. 4) Between the PD and ET cases, we compared the rated amounts of beta-waves in the Cd and VA/VL and of the tau-waves in the VIM. Results: Excess beta-waves occurred within the Cd and VA/VL in PD with rigidity and either with or without tremor, but not in ET. Conclusion: The results suggest that the excess Cd and VA/VL beta-band activities are pathological, being relevant to the generation of parkinsonian rigidity.

P345 (2P2-104)

Repair and regeneration processes of injured brain and spinal cord in infant rats: immunohistochemical studies.

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Repair of injured brain and spinal cord is not expected in adult mammals, however, repair and functional recovery after spinal cord injury has been reported in infant rats. In this study, we report on microglia/macrophage reactions and cell proliferation that occur in infant rats during the repair process. Hemi-section of the spinal cord or small cortical incision was made in 2-week-old infant rats. Repair processes after injury were examined using antibodies for microglia/macrophage (OX42), vimentin, GFAP, drebrin, MAP2, bFGF, and BrdU. OX42-positive round cells were found at the lesioned site of the brain and spinal cord 3-5 days after injury. Many vimentin-positive cells took the place of OX42-positive cells and occupied the lesioned site 5-7 days after injury. In the spinal cord, BrdU-labeled cells were concentrated at the lesioned site 5 days after injury. In the brain, enlargement of the subventricular zone (SVZ) was found 10-14 days after injury. Many cells in the SVZ and lesioned site were positive for bFGF. Repair of drebrin- and MAP2-immunoreactivity was found 3 months after injury in the brain and spinal cord. It is suggested that microglia/macrophage reaction in infant rats is supportive for repair processes and may induce cell proliferation and that proliferated immature astrocytes may support nerve regeneration.

P346 (2P2-105)

Adenosine A1 receptor is involved in the attenuation of motor coordination associated with anxiety.

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Many investigators have demonstrated an association between anxiety and motor coordination in humans. However, the neural mechanisms associated with the anxiety-motor linkage remain largely unknown. In this study, the existence of a correlation between anxiety and motor coordination was examined in mice. Anxiety was evaluated by an elevated plus-maze, and motor coordination using a rota-rod treadmill. Firstly, Anxiety and motor coordination were significantly correlated in healthy naive mice. Furthermore, the mechanisms of this linkage were investgated, with a focus on the adenosinergic system. The injection of a high dose of caffeine (100mg/kg) induced anxiety combined with impaired motor coordination but not no effect on locomotor activity. However, the low dose of caffeine (12.5mg/kg) caused only a behavioral stimulant effect but not a change in motor coordination. A grip test, which estimates neuromuscular strength, showed no significant differences among the conditions. The effects of an injection of CPT (adenosine A1 receptor selective antagonist) were similar to those observed in the case of a high dose of caffeine. The effects of an injection of SCH58261 (an adenosine A2A receptor selective antagonist) were also similar to those observed in the low dose of caffeine. These results suggest that the A1 receptor, but not the A2A receptor, is involved in the impaired motor coordination associated with anxiety.

P347 (2P2-106)

The role of glucose metabolism and Na^+/K^+ ATPase activity in the glucose-sensitive neuronal glucosensing of the hypothalamic arcuate nucleus

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The glucose-sensing mechanisms of glucose-sensitive neurons (GSNs) in the hypothalamic arcuate nucleus were analyzed by measuring intracellular Ca^{2+} ($[Ca^{2+}]_i$), Na^+ ($[Na^+]_i$), and NADPH concentration with fluorescent imaging. We asked whether lowering extracellular glucose is sensed by glucokinase (GK) to reduce glucose metabolism and energy production and whether these changes are linked to reduction in the electrogenic Na^+/K^+ ATPase activity, consequent activation of voltage-dependent Ca^{2+} channel (VDCC) and increase in $[Ca^{2+}]_i$. At high glucose (HG), in which $[Ca^{2+}]_i$ was low and silent, administration of a GK inhibitor, mannoheptulose, and a mitochondrial inhibitor, cyanide, increased $[Ca^{2+}]_i$ in a manner similar to low glucose (LG). LG decreased NAD(P)H and increased $[Ca^{2+}]_i$ in the same cell. Inhibition of Na^+/K^+ ATPase by ouabain and low K^+ at HG mimicked LG. Moreover, LG increased $[Na^+]_i$, suggesting inhibition of Na^+/K^+ ATPase. Blockers of VDCC attenuated LG-induced $[Ca^{2+}]_i$ increases. These data demonstrate that lowering glucose reduces glucose metabolism, energy production and probably electrogenic Na^+/K^+ ATPase activity, which in turn depolarizes the membrane to activate voltage-dependent Ca^{2+} influx and increase $[Ca^{2+}]_i$, thereby activating GSNs.

P348 (2P2-107)

The 24-h acetylcholine release profile in the medial prefrontal cortex in male and female rats

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We previously reported the sex difference in the acetylcholine (ACh) release in the hippocampus and the frontal cortex area 2 (precentral cortex). In the present study, to further examine the sex difference in the ACh release in the medial prefrontal cortex (prelimbic cortex), in vivo microdialysis study was performed in intact male and cycling female rats (9-12 weeks of age). The dialysate was automatically collected from the medial prefrontal cortex every 20 min for 24 h under freely moving conditions, and the spontaneous locomotor activity was simultaneously monitored in the same subjects. ACh was quantified by a combination of high performance liquid chromatography column, enzyme reaction, and electrochemical detection. No eserine was used in the present study. Although the ACh release in the medial prefrontal cortex and the spontaneous locomotor activity during the dark phase was significantly greater than during the light phase in both sexes of rats, female rats showed significantly greater mean ACh release and spontaneous locomotor activity than male rats ($P < 0.01$). Although both sexes of rats showed significant correlation between the ACh release and the spontaneous locomotor activity, female rats showed significantly greater correlation coefficient than male rats ($P < 0.01$). In the present study, we demonstrated the sex difference in the ACh release in the medial prefrontal cortex. It is possible that inherent sex and/or the hormonal environment affect the coupling between the ACh release in the medial prefrontal cortex and the spontaneous locomotor activity in rats.

P349 (2P2-108)

Short-term effect of gonadal steroid hormones to maintain the acetylcholine release in the hippocampus of male and female rats

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Septo-hippocampal cholinergic neurons play a significant role in the spatial learning and memory function. We found that corresponding sex steroid hormone is essential to maintain normal acetylcholine (ACh) release profile in the hippocampus of rats. In the present study, to further examine the short-term effect of sex hormones on the ACh release in the hippocampus, in vivo microdialysis study was performed in gonadectomized rats under the freely moving condition. The dialysate was collected from the dorsal hippocampus at 20-min intervals. Testosterone (2 mg) or 17β-estradiol (40 μg) was subcutaneously injected in gonadectmized male and female rats, respectively. The testosterone injection in gonadectomized male rats gradually but significantly increased the ACh release within the first few hours, and restored the ACh release in intact male rats within 10 hours. Similarly, the estrogen injection in gonadectomized female rats also increased the ACh release within the first few hours, and restored the ACh release in cycling female rats within 10 hours. Oil injection showed no significant effect on the ACh release. Present results demonstrated that testosterone in male rats as well as estrogen in female rats acutely increase the ACh release in the hippocampus. The short-term effect of gonadal steroid hormones may contribute to the enhancement of spatial mnemonic processing observed within the first few hours after the stroid treatemt.

POSTERS
Autonomic nervous functions

P350 (2P2-109)

Prenatal exposure to Bisphenol A enhances avoidance response to predator odor and affects sexual difference in excitatory neuronal response to predator odor in the medial amygdala

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We have reported that prenatal exposure (0.1ppm) to Bisphenol A (BPA) impairs the sexual differentiation of exploratory behavior and enhances depressive behavior (Fujimoto et al. 2005, 2006). In the present study, the effects of prenatal exposure to BPA on behavioral and neuronal responses to olfactory stimuli were examined. General motor activity and avoidance response to predator odor (fox) were measured in the odor avoidance test. Neuronal responses to various odors were examined in the medial amygdala using extracellular recording technique. The smell of fox predominantly suppressed locomotor activity and enhanced avoidance response in BPA exposure rats but not control rats. In the electrophysiological study, male medial amygdala neurons showed selective excitatory responses to odors of predators. This type of neurons did not respond to plant odors. In contrast female amygdala neurons did not show such selectivity in which excitatory neurons responding to predator odors also showed excitatory responses to plant odors. The sex difference in this neuronal response pattern was attenuated by prenatal exposure to BPA. These findings suggest that BPA impairs sexual differentiation of the medial amygdala affecting behavioral responses to the predator odors. Supported by COE program, Grants-in-Aid for Scientific Research and Health Sciences Research Grant.

P351 (1P1-093)

Cold stress and autonomic heart rate regulation in mice

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Ambient temperature was lowered from 30°C to 15°C in unrestrained mice with and without continuous presence of autonomic blockers, and the autonomic response to cold stress was analyzed. Time series of RR intervals were obtained from ECG recordings sampled continuously for 40 seconds every 2 minutes, and the mean RR interval and the mean respiratory interval were calculated for each time series. Frequency domain area of the amplitude Fourier spectrum of the variation of RR-intervals, LF (0.025-0.300 Hz) and HF (0.3-4.0 Hz), were calculated. After lowering the temperature to 15°C, the mean RR interval had decreased gradually and stabled at the minimum steady level with small variations in RR intervals in both control and pharmacologically treated mice. This minimum steady level of the mean RR-interval was not significantly different between the control mice and the atropine treated mice. These results suggested that the sympathetic activity was enhanced and the parasympathetic activity was eliminated by the cold stress. The tachycardia in cold accompanied the tachypnea. It was suggested that the increased metabolism was underlying the autonomic heart rate response to cold. The reason for inadequacy of the LF/HF ratio as an index for the autonomic balance in mice will be specified based on the present results.

P352 (1P1-094)

Autonomic heart rate regulation in unrestrained conscious mice

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Sympathetic and parasympathetic autonomic activities of unrestrained conscious mice were analyzed. The ambient temperature of animals was set to 30°C where significant variability of RR-interval could be recorded. Time series of RR-intervals were obtained from ECG recordings sampled continuously for 40 seconds every 2 minutes. LF (0.025-0.300 Hz) and HF (0.3-4.0 Hz) were calculated as areas of the Fourier amplitude spectrum of RR-intervals. The mean respiratory interval was calculated from the first derivative of ECG baseline fluctuations. The ratio of the mean respiratory interval to the mean RR interval stayed almost constant throughout experiments, which suggested an intimate relationship between the heart rate and the metabolism. After injection of atropine, both LF and HF suddenly decreased, whereas the mean RR-interval was not always significantly decreased. The respiratory-unsynchronized LF that remained in the presence of atropine was eliminated by an additional injection of propranolol, which indicated that the firing rate of pacemaker cells was exclusively determined by classical autonomic neurotransmitters in mice. LF/(LF+ HF) and HF/(LF+ HF) had no significant relationship with the mean RR interval that is presumed to be reflecting the autonomic balance. The possible reasons for this discrepancy will be discussed.

P353 (1P1-095)

Cross-correlation analysis between heart rate and firing rate of the single unit activity in the paraventricular nucleus region of hypothalamus in the conscious rat.

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To elucidate the integrative roles of the paraventricular nucleus (PVN) in the central regulation of autonomic and endocrine systems, we simultaneously recorded the single unit in the PVN, arterial blood pressure, electrocardiogram (ECG), electroencepharogram (EEG) and electromyogram (EMG) of cervical muscle in conscious freely-moving rats. The miniature drivable apparatus with eight microwires was implanted to the skull for the recording of single unit. The cross-correlation analysis between heart rate (HR) and firing rate of unit activity revealed three types of neurons. Type 1 neurons showed a positive correlation, its maximum value was at time lag of 2-4 sec. It means that the firing rate started to increase 2-4 sec prior to increasing HR. Type 2 neurons showed a negative correlation at similar lag. Both group did not showed a reflex changing in firing rate associated with perfusion of vasoactive drugs. However type 1 and 2 neurons increased and decrease in firing rate, respectively, in response to the moderate stressor. Sleep-wakefulness states were discriminated based on EEG and EMG. During REM sleep, both types of neuron increased their firing rate. Type 3 did not show a consistent relationship between HR and firing rate. Some of this type showed rhythmic phasic activity pattern. During REM sleep, these neurons were strongly inhibited. These findings suggested that type 1 and 2 neurons in the PVN contribute to reciprocal control of autonomic nervous system except for a period of REM sleep.

P354 (1P1-096)

Electrogastrography analysis at cool- and warm-water load

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Electrogastrography (EGG) is a non-invasive method but left unclear for clinical use. EGG was studied for 12 volunteers (22.8 +/- 2.1 years old). Nine silver disc electrodes were set on the abdomen in 3 by 3 matrix; referred to right shin. Electrocardiogram (ECG) and respiration were monitored. Signals were sampled at 250 Hz into 14 bits with a band-pass filter from 0.016 to 30 Hz (BIOTOP-6R12, San-ei, Japan). Subjects were sitting on a reclining chair with eyes-closed. First 2 min., the subject was asked to be at rest, and following 8 min., every 2 min. the subject was passively soaked the left hand in cool (17 deg) or warm (40 deg) water alternatively. The subjects were divided into 2 groups; Group-I was to load the cool water first, and Group-II was to load the warm water first. The power, analyzed by the fast Fourier transform, for Group-I showed little change, but the power for Group-II showed marked change following the loading. The initial thermal stimulus to hand affected on the following EGG, EGG changing was to be suspected by modified thermal stimulus. Therefore, the relation between EGG and the autonomic nerve system was left for further study.

P355 (1P1-097)

Nerve-mediated contractile response induced by orexin-A in the rat distal colon

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Orexins were discovered as neurotransmitters of hypothalamic neurons. However, orexins are not only expressed in the central nervous system but also in the enteric nervous system. In this study, effect of orexin-A on the frequency of spontaneous contractions was examined in longitudinal muscle strips of the rat distal colon. Then, involvement of enteric neurons in the orexin-A-evoked response was examined. The frequency of spontaneous contractions during 10 min was measured before and after addition of orexin-A. Orexin-A (3-300 nM) concentration-dependently increased the frequency of spontaneous contractions. The frequency was increased from 0.42 ± 0.05 min^{-1} to 0.61 ± 0.04 min^{-1} by 100 nM orexin-A ($n = 4$). This response was attenuated by the blocker of neural conduction, tetrodotoxin, and the muscarinic acetylcholine receptor antagonist, atropine. Muscarinic M3 receptor antagonist, 4-DAMP, also attenuated the orexin-A-evoked response while M1 and M2 receptor antagonists had no effect. The response was insensitive to the nicotinic receptor antagonist, hexamethonium. The orexin-A-evoked response was attenuated by the orexin-1 receptor antagonist, SB-334867-A, in a concentration-dependent manner. The results suggests that the putative neurotransmitter of enteric neurons, orexin-A, stimulates orexin-1 receptors on enteric neurons and activates enteric cholinergic neurons to increase the frequency of spontaneous contractions in the longitudinal muscle of the rat distal colon.

P356 (1P1-098)

Adrenagic stimulation of cultured rat urothelial cells releases ATP.

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Epithelial cells in the urinary bladder (urothelial cells) have been recognized to own neuronal like properties in addition to barrier function. Recently ATP released from urothelial cells could modify the afferent nerve activity from the bladder. The detection of adrenergic α 1 receptor in the bladder led us to study the role of urothelial adrenergic system in micturition reflex. We examined the adrenergic effect on ATP level in the primary cultured urothelial cells.

1.Urothelial cell culture from rat rapidly grew and reached to confluent within 10 days. 10 μM of noradrnarine, phenylephrine or isoproterenol increased ATP in culture medium. Prazocin inhibited the noradrenaline induced ATP release.

2.Immunohistochemical study demonstrated that dopamine β hydroxylase was detected in the urothelial cell culture, vesicular monoamine transporter 2 was detected in the urotelial cell layer of the rat bladder.

These results indicated that the urothelial cells in culture release ATP by adrenergic stimulation that are regulated through α and/or β receptors. Urothelial cells would release catecholamines. Therefore, catecholamines from urothelial cells might act on adrenergic receptors in the urothelial cells as autocrine signals. It should activate ATP release to facilitate the mechano-sensitive bladder afferent nerve and reflex voiding.

P357 (1P1-099)

Action of PACAP on submucosal neurons of guinea-pig caecum

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Pituitary adenylate cyclase activating polypeptide (PACAP) that shares high degrees of sequence homology with vasoactive intestinal polypeptide (VIP) is a member of the secretin/glucagon/VIP family of peptides. PACAP is known to regulate gastrointestinal secretion and motility. Whereas electrophysiological studies of myenteric plexus neurons of the guinea-pig ileum have shown that PACAP depolarized AH neurons (96%) and S neurons (36%), there is little information on the possible effects in submucosal plexus neurons. In the present study, we investigated action of PACAP on the postsynaptic membrane of submucosal neurons of the caecum. Intracellular recordings were made from submucosal neurons in vitro with glass microelectrodes. PACAP applied by superfusion (10-300 nM) caused membrane depolarizations in almost all the submucosal neurons, associated with a decreased conductance; the depolarization responses were decreased by membrane hyperpolarizations and could be observed in the presence of TTX (500 nM) which inhibits voltage-gated sodium channels. The PACAP responses sometimes triggered repetitive firings. It is concluded that PACAP increases excitability of submucosal neurons, possibly due to a decreased potassium conductance.

P358 (1P1-100)

Prostaglandin E_2 (PGE_2) facilitate micturition reflex; study from EP_1-receptor knockout mice

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We studied influence of intravesical administration of $PGE_2$and EP_1agonist(ONO-DI-004) for micturition reflex using EP_1 knockout (EP_1-KO) mice. Methods: Age matched male EP_1-KO mice (n=25) and male C57/BL6 wild type (WT) mice (n=25) were used. Cystometry without anesthesia was performed in a restrain cage. PGE_2 (30μM),EP_1 agonist (40μM), acetic acid (0.1%,AA) or capsaicin (0.3 mM,CAP) were administered intravesically.Mice urinary bladder Was use for EP_1-receptor immunohistochemical study. Results: ICI in EP_1-KO mice (624.3±58.5 sec) was longer than in WT mice(418.4±75.4 sec, $P<0.05$, n=9).In WT mice, PGE_2 shortened ICI to 198.9±40.3 sec (47.5% of control, $P<0.05$). However,PGE_2 did not altered ICI in EP_1-KO mice (744.9±73.6 sec, 119.3%). In WT mice, EP_1 agonist shortened ICI to 140.4±13.0 sec (41%, $P<0.05$).In contrast, EP_1 agonist did not altered ICI in EP_1-KO mice(101.7%). In WT mice, AA and CAP shortened ICI to 64% and 53% , respectively. In EP_1-KO mice AA and CAP shortened ICI to 63% and 55%, respectively. Conclusion: EP_1-receptor was presented in urothelium. ICI in EP_1-KO mice was larger than WT mice. Intravesical administration of PGE_2 or EP_1 agonist shortened ICI in WT mice, and not altered in EP_1-KO mice. In addition, AA or CAP shortened ICI in both WT mice and EP_1-KO mice. In summary, PGE_2 might activate EP_1-receptor in urothelium, and facilitate mechano-sensitive bladder afferent and reflex voiding.

P359 (1P1-101)

Sleep-related effects on gastrointestinal motility in young women

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To clarify the change in gastrointestinal motility of constipated young women during sleep, we assessed elecrogastrography (EGG) for 3 hours after they went to sleep in 10 college-age women. EGG was recorded in 1Hz sampling. The 3 cpm (2.5 to 3.5 cycle per minute) and 6cpm (4.5 to 5.5 cycle per minute) in the EGG power spectrum was a good indicator in designating spastic or flaccid constipation. To differentiate constipation, the ratio of [6 cpm power of EGG at 31 to 45 min segment] / [6 cpm power of EGG at 16 to 30 min segment] was defined as intestinal motility index (IMI). Sleep related change in EGG change has not been clarified. The 6cpm power during sleep was recorded and compared with appearance ration in class of normalized power. Power spectrum analysis by MemCalc (maximal entropy method) was used to assess EGG and auto sleep stager with EEG, EOG and EMG was used to determine sleep stage. The normalized power spectrum of EGG (divided into power in total frequency section) was classified with sleep stage (20 seconds interval) and the appearance rate was counted. The power spectrum of EGG is enhanced in high sleep stage. However, the power is most large in sleep stage 3 on some subjects. In the case of incomplete sleep, enhancement of the power did not be shown. We thus confirm that the sleep stage is effective factor in electrophysiological indices for assessing autonomic dysfunction during sleep.

P360 (1P1-102)

Acupuncture stimulation at the sacral segment affects the neuronal activuity in the locus coeruleus and the cortical electroencephalogram

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We have previously shown that acupuncture stimulation to the sacral segment induced suppression of urinary bladder activity which was accompanied by an increase in amplitude of electroencephalogram (EEG), meaning that the sacral stimulation affects the bladder activity or the vigilance state or both. It is well known that the noradrenergic neurons in the locus coeruleus (LC) play important roles in regulation of the vigilance state. Here, we examined the effect of acupuncture stimulation to the sacral segment on the EEG and activity of the LC neurons.Single neuronal activity and EEG were recorded from urethane anesthetized rats, under which condition the EEG showed a periodical alternation between slow wave with larger amplitude and fast wave with smaller amplitude. When EEG was fast and smaller, the acupuncture to the sacral segment increased the EEG amplitude. The response started from more than 70 s and continued for more than 82 s. When EEG was slow and large, the stimulation had no effect. Firing rate of the noradrenergic LC neuron, which was about 1-3 Hz before stimulation, decreased remarkably after stimulation. The decrease of neuronal activity had close relationship with increase of EEG amplitude. The result suggests that acupuncture stimulation changes the state of vigilance by affecting the noradremergic LC neurons.

P361 (1P1-103)

Origin and nerurotransmitter of parasympathetic postganglionic vasodilator fibers supplying the vessel of masseter muscles in rat

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We have recently reported that the vessels of the rat masseter muscle were innervated by the novel parasympathetic vasodilator fibers, and that these fibers were activated reflexly by the electrical stimulation of an afferent fiber of the trigeminal nerve such as the lingual nerve. These parasympathetic mediated vasodilatation was not abolished by a muscarinic receptor antagonist, atropine, suggest that there is a noncholinergic mechanism which mediate a parasympathetic vasodilatation in rat masseter muscle vessels. In the present study, we have investigated the origins of the parasympathetic vasodilator fibers and the noncholinergic neurotransmitters of these fibers with a use of methods of a combined retrograde tracings and transmitter immunohistochemistry. When fluoro-gold (FG), a retro grade neurotracer, injected in the masseter muscle, FG-labeling neurons were ipsilaterally found in the trigeminal motor nucleus, trigeminal mesencephalic nucleus, trigeminal ganglion, superior cervical ganglion and the otic ganglion but not in the pterygopalatine ganglion. In the otic ganglion, 59.6 ± 2.7% of FG-labeling neurons colocalized with the vasoactive intestinal polypeptide (VIP). In the master muscle, the VIP-immunoreactive parasympathetic fibers were distributed in an adventitia along the vessel wall. These results suggest that the postganglionic parasymapathetic vasodilatation fibers innervating the masseter vessels originate in the otic ganglion and one of that non-cholinergic neurotransmitter was VIP.

P362 (1P1-104)

Role of sympathetic nerve activation on the parasympathetic vasodilatation in the rat masseter muscle

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The sympathetic nerve (SN) has been reported to be involved in the development of jaw muscle dysfunctions because the masseter muscle pain is usually accompanied with the increase in the SN activity. We have recently reported that there are parasympathetic (PS) vasodilator fibers in the rat masseter muscle and that these fibers would be involved in the regulation of the hemodynamics of jaw muscles (J. Physiol. 569, 617-629, 2005). It is still unclear whether there is an interaction between the PS vasodilatation (PSV) in the masseter muscle and the SN activity. The present study was thus designed to examine 1) the effect of SN activation on the PSV in the masseter muscle, and 2) the neural mechanisms mediating the effect in anesthetized rats. The PS mediated masseter muscle blood flow (MBF) increases were evoked by electrical stimulation of the central cut end of the lingual nerve. The magnitudes of MBF increase were significantly reduced by ongoing electrical stimulation of the superior cervical sympathetic trunk in a frequency-dependent manner (0.5-10 Hz). Pretreatment with BIBP 3226, a neuropeptide Y (NPY) Y1 receptor antagonist, significantly reduced 30-40% in this inhibition, but phentolamine had no effect. The present study indicates that the excessive SN activation inhibits the PSV in the masseter muscle, suggesting a potential role in the etiology of jaw muscle dysfunctions. This inhibitory action may be due to an interaction of the PS vsodilator fibers and NPY that would be released from sympathetic fibers.

P363 (1P1-105)

Responses of spinal cord blood flow to noxious mechanical stimulation of the skin in anesthetized rats

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Recently, we have shown that in anesthetized rats innocuous mechanical stimulation increases spinal cord blood flow (SCBF), in part via alpha adrenoceptors. The present study documents responses of SCBF to noxious mechanical stimulation (pinching) in anesthetized rats. Regional SCBF was measured with a laser Doppler flowmeter probe placed on the dorsal surface of the L4 - 6 region of the spinal cord. Pinching of the forepaw or hindpaw increased SCBF, coincident with increases in arterial blood pressure. After treatment with phenoxybenzamine, an alpha-adrenoceptor blocker, the responses of arterial blood pressure to pinching of the forepaw or hindpaw became negligible; however, the responses of SCBF to pinching of the hindpaw were augmented. On the other hand, the response of SCBF to pinching of the forepaw disappeared after treatment with phenoxybenzamine, suggesting that the previously-observed SCBF response to forepaw pinching was due to an increase in arterial blood pressure. These results indicate that noxious mechanical stimulation of the skin can lead to a generalized increase in SCBF via elevation of blood pressure. In addition, it appears that there is a segmentally-organized increase in regional SCBF, which is inhibited, at least in part, by activation of alpha adrenergic receptors.

P364 (1P1-106)

Increase in peripheral blood flow and change of autonomic nervous activity by the application of heat- and steam-generating sheets to the lumbar or abdominal region

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The present study was undertaken to evaluate the effects of a heat- and steam-generating sheet (HSG sheet) on peripheral blood circulation and autonomic nerve activity when applied to the lumbar or abdominal regions. In females with high susceptibility to cold temperatures, the HSG sheet that increases the temperature between the sheet and skin to about 38.5°C was applied to the lumbar or abdominal region. Measurement items were; skin temperature at the lumbar and abdominal regions and the hand finger tip, total hemoglobin, pupillary light reflex, changes in ECG R-R interval and percutaneous electrogastrography (EGG). An application of the HSG sheet to either the lumbar or abdominal region significantly inhibited decreases in finger tip skin temperature, suggesting improvement of peripheral blood flow. In the pupillary light reflex, significant increases in the quantity and rate of miosis were noted. As for changes in the ECG R-R interval, the HF component increased, showing dominance of the parasympathetic nerve system. EGG slightly increased in the amplitude. Based on the above findings, we conclude that application of the HSG sheet to the lumbar or abdominal region may improve peripheral hemodynamics and make parasympathetic nerve activity dominant.

P365 (1P1-107)

Role of arterial baroreceptor in regulating renal and lumbar sympathetic nerve activity during normal daily activity in rats

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The purpose of the present study was to determine the role of arterial baroreceptor in regulating renal (RSNA) and lumbar (LSNA) sympathetic nerve activity and systemic arterial pressure (Pa) in freely moving rats. Wistar male rats were sinoaortic denervated (SAD) or sham operated (SO) and instrumented chronically with electrodes for the measurements of RSNA, LSNA, electroencephalogram, electrocardiogram and electromyogram, and catheters for the measurements of Pa and central venous pressure. At least two days after the surgery, these parameters were recorded simultaneously in conscious SAD and SO rats. During moving and grooming states, Pa increased in SO rats while it decreased in SAD rats. In SO rats, both RSNA and LSNA increased during moving and grooming states. However, in SAD rats, the magnitude of the increases in RSNA during moving and grooming states were attenuated compared with those in SO rats; LSNA tended to decrease during moving and grooming states in SO rats. During REM sleep, Pa increased in SO rats but it decreased in SAD rats. RSNA decreased while LSNA increased during REM sleep in SO rats; but, in SAD rats, RSNA decreased while LSNA tended to decrease during REM sleep. These results suggest that arterial baroreceptor may involve in differential control of RSNA and LSNA, which may play a critical role in resulting in state dependent changes in Pa during normal daily activity in conscious rats.

P366 (1P1-108)

Chemical stimulation of dorsal raphe nucleus produces an increase in frontal blood flow in rat

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It has been demonstrated that patients with depression show decreased prefrontal blood flow and less serotonin (5-HT) level in the brain. In the present study we observed a change in frontal blood flow (fBF) when 5-HT neurons in the dorsal raphe nucleus (DRN) were chemically stimulated in anesthetized rats. Microinjection of L-glutamate into DRN induced an increase in fBF, accompanied by respiratory activation and decreased blood pressure. Since those respiratory and circulatory changes were observed during DRN stimulation in our previous study, we suggest that activation of 5-HT neurons in DRN may cause the increase in frontal blood flow.

P367 (1P1-109)

Development of the inhibitory synaptic transmission to the superior salivatory neurons in the rat

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We showed the excitatory (glutamate) and inhibitory (GABA and glycine) synaptic inputs to superior salivatory (SS) neurons in neonatal rat brainstem slices. There is a possibility that the synaptic transmission to SS neurons develop postnatally with the development of oral function. In the present study, we studied the development of the inhibitory synaptic transmission to SS neurons electrophysiologically. We used Wistar rats at postnatal day 2 (P2) to P14. The SS neurons innervating the submandibular salivary glands and tongue were labeled by retrograde axonal transport of a fluorescent dye. Gramicidin perforated patch-clamp recordings from the labeled cell were performed. To examine the reversal potential (V_{rev}), the inhibitory postsynaptic currents (IPSCs) were evoked by electrical stimulation near the recording cell at various potentials. Additionally, to examine the effect of $GABA_A$ receptor activation on the excitability of the cells, GABA (1 mM, 100 ms) was applied via pressure ejection near the recording cell at its resting potential. The V_{rev} was shifted with age, and that at P2-P4, P5-P7 and P8-P14 were -52.6 ± 5.1 mV (n = 9), -62.3 ± 1.6 mV (n = 12) and -71.4 ± 1.5 mV (n = 12), respectively. Exogenous GABA application produced depolarization in 40% cells at P2-P4, and produced hyperpolarization after P8. Such functional change of synaptic transmission might be involved in the transition from sucking to mastication.

P368 (1P1-110)

Submandibular salivary secretion during feeding of various foods in rats.

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It is well known that salivary secretion is mainly induced during feeding behavior. In the present study we examined flow rate of saliva induced by feeding of various texture of food. For this examination male Wistar rats were placed on a food deprivation schedule and allowed access to various foods for 3 hours per day for one week. The foods were food pellets, powder diet, and 3 types of mashed diet (powder diet : water = 1:1.5, 1:1, and 1:0.5). After the training schedule, the animals were anesthetized and the duct of the submandibular gland was cannulated with polyethylene tubing for recording salivary flow rates, and a pair of stainless electrodes was inserted into the masseter muscle for recording EMG activities. After recovery from anesthesia, the recording experiment was performed. The following results were obtained: 1) The average salivary flow rates for 3 min were in the order powder diet > hard mashed diet > medium mashed diet > food pellet > soft mashed diet; 2) Dry weights of food consumption were in the order soft mashed diet > medium mashed diet > hard mashed diet > powder diet and food pellet; 3) Total EMG activities for 3 min were similar in various foods; 4) Bruxism usually yielded biggest EMG activities during recording session, but did not induced salivary secretion. These findings suggest that the movement of the jaw, sensory inputs from the periodontal membrane, or the amount of food consumption is not correlate with the flow rate of saliva. And the dry powder diet is the most sialagogue food.

P369 (1P1-111)

Intravenous Mg^{2+} infusion inhibits adrenal catecholamine release by acting on both pre- and post-ganglionic sites

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Using microdialysis technique, we investigated the effects of intravenous Mg^{2+} infusion on the releases of pre-ganglionic ACh and post-ganglionic catecholamine in the left adrenal medulla of anesthetized rats. **Protocol 1:** The dialysis probe was perfused with Ringer solution containing neostigmine and the left splanchnic nerves were electrically stimulated at 4 Hz for 2 min before and after Mg^{2+} infusion. $MgSO_4$ 25 μmol/kg/min for 30 min (n=7): Immediately after infusion, plasma Mg^{2+} levels increased from 0.9 ± 0.04 to 2.5 ± 0.1 mM, but the nerve stimulation-induced releases of ACh, norepinephrine (NE), and epinephrine (Epi) were not affected. 60 min after the stop of infusion, plasma Mg^{2+} levels recovered to 1.5 ± 0.1 mM, but the nerve stimulation-induced releases of ACh, NE, and Epi were suppressed. $MgSO_4$ 50 μmol/kg/min for 30 min (n=7): Immediately after infusion, plasma Mg^{2+} levels increased 0.9 ± 0.03 to 3.8 ± 0.2 mM and the nerve stimulation-induced releases of ACh, NE, and Epi were suppressed. **Protocol 2:** The dialysis probes were perfused with Ringer solution and ACh (1 mM) was locally administered for 1 min through the dialysis probe before and after Mg^{2+} infusion (n=5). Immediately after infusion ($MgSO_4$ 50 μmol/kg/min for 30 min), the exogenous ACh-induced releases of NE and Epi were suppressed. These results suggest that intravenous Mg^{2+} infusion inhibits catecholamine release from adrenal medulla by acting on both pre- and post-ganglionic sites and the pre-ganglionic effect is dose- and time-dependent.

P370 (1P1-112)

Effects of extracellular ATP on the membrane potential and the spontaneous action potential firing in rat area postrema neurons.

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Recently, histochemical studies have reported the presence of P2X purinoceptors in the area postrema (AP). To examine the effect of extracellular ATP on the AP neuronal excitability, we performed the patch-clamp recording from brain slices (150-200 μm thick) obtained from SD rats (8-21 days postnatal). Voltage-clamp recordings revealed the pre- and post-and/or extrasynaptic modulation by ATP. To clarify how the ATP-induced currents affect the membrane potential and the action potential firing, we also performed the current-clamp recordings. Thirteen of twenty cells tested showed a marked depolarization of the resting membrane potential during the bath application of ATP (up to 1 mM). Two of these cells also showed marked increases in the frequency of spontaneous action potential firings. Two cells showed a marked hyperpolarization of the resting membrane potential with decreases in the frequency of spontaneous action potential firings. Each cell was also classified into different groups according to whether it displays the hyperpolarization-activated cation current (I_h) or not. ATP-induced depolarization was seen in either groups, but the hyperpolarization of membrane potentials or outward currents elicited by ATP was seen in cells not displaying I_h. These results suggest that the functional separation of purinoceptors among the AP neurons.

P371 (1P1-113)

Morphological properties and chemosensitivities of neurons in the nucleus gelatinosus solitarius in rat brain slices

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The nucleus gelatinosus solitarius (SolG) is a structure just adjacent to the area postrema (AP). The AP is one of the circumventricular organs that lack a blood-brain barrier. The SolG is considered to be involved in the vomiting center in the medulla. Electrophysiological and morphological data from SolG neurons are virtually absent. To examine the morphological and electrophysiological properties, and chemosensitivities of SolG neurons, whole-cell patch-clamp recordings with a neurobiotin tracer were performed in the rat brain slices. In the morphological study, we found dendrites of SolG neurons were found to be extended into the AP, indicating the functional relevance between the AP and the SolG. Neurons in the both regions showed sensitivities to 5-HT, nicotine and ATP. 5-HT_3 receptors, nicotinic ACh receptors and P2X purinoceptors were found to be located both pre- and post- and/or extrasynaptically in the SolG. Further, we found a cell that showed excitatory responses to the activation of every one of these receptors. This study suggests that SolG neurons could access to circulating substances as well as AP neurons and the information of circulating substances might be integrated by SolG neurons.

P372 (1P1-114)

Effect of 3 days sodium loading on renal sympathetic nerve activity and urinary sodium excretion

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It has been unclear whether changes in renal sympathetic nerve activity (RSNA) influence chronic regulation of sodium excretion and arterial pressure because there is little information on the chronic effect of changes in RSNA on sodium excretion and arterial pressure. In the present study, a month recording of RSNA in freely moving rats was carried out to study the long-term regulation of RSNA and sodium excretion. Wistar rats were instrumented chronically with electrodes for the measurements of RSNA and electrocardiogram. At least 7 days after the implantation of the electrodes, the animals were housed in individual metabolic cage and then RSNA, heart rate and sodium balance were measured over three weeks. Animals were allowed to drink four different concentration of sodium chloride solutions; that was 0, 50, 154, 308 meq/l NaCl. The experiments were consisted of 3 days control (50 meq/l NaCl) period, 3 days unloading (0 meq/l NaCl)/loading (154, 308 meq/l NaCl) period, and 3 days recovery (50 meq/l NaCl) period. The sodium loading with 308 meq/l NaCl suppressed RSNA significantly over the 3 days loading period and then it recovered above the control level while either 0 meq/l NaCl and 154 meq/l NaCl loading had no effects on RSNA. Sodium excretion changed significantly in proportion to the sodium loading levels during 0, 154 and 308 meq/l NaCl. These results demonstrated that changes in RSNA were not always correlated with the changes in sodium excretion during 3 days sodium unloading/loading period in rats.

P373 (1P1-115)

Differential control of renal and lumbar sympathetic nerve activity during freezing behaviour in rats

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Stressful sensory input results in an immediate decrease in motor activity under certain conditions in rats. Although this acute response has long been recognized as "freezing behaviour", sympathetic response during this behaviour remains unknown. In the present study, we measured renal (RSNA) and lumbar (LSNA) sympathetic nerve activity simultaneously during loud noise exposure to study whether sympathetic outflows may be controlled differently during freezing behaviour in conscious rats. Wistar male rats were instrumented chronically with electrodes for the measurements of RSNA, LSNA, electroencephalogram, electrocardiogram and electromyogram, and catheters for the measurements of systemic arterial and central venous pressures. At least three days after the surgery, rats were exposed to white noise with 90 decibel (dB) over 10 min following 10 min control period. RSNA increased immediately after onset of the noise exposure and remained high level throughout the 10 min exposing period. By contrast to the RSNA response, LSNA did not change significantly throughout the experimental period. Heart rate decreased immediately after onset of the noise exposure, then it stated to recover gradually but did not reach the control level. Systemic arterial pressure showed a transient and small increase at the onset of the noise exposure but it returned to the control level thereafter. The present study demonstrated that RSNA and LSNA were differently regulated during freezing behaviour in rats.

P374 (1P1-116)

Effect of electro-acupuncture stimulation on plasma glucose response to insulin in streptozotocin-induced diabetic rats

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Effects of electro-acupuncture (EA) stimulation mimics those of exercise in part. Since exercise increases glucose uptake in diabetic patients, the present study aimed to investigate whether EA stimulation can increase glucose uptake in streptozotocin (STZ)-induced diabetic rats. Rats were treated with STZ 2-3 weeks prior to the experiments. Experiments were performed under urethane-chloralose anesthetized, artificially ventilated condition. For EA stimulation two stainless steel needles were inserted perpendicularly about 5 mm into the right tibialis anterior muscle. The EA stimulation was delivered for 10 min at 10 mA, 20 Hz. Although EA stimulation had no effect on plasma concentration of glucose in the STZ rats, it changed plasma glucose response to insulin. Intravenous administration of 0.2 unit/kg insulin had no significant effect on plasma concentration of glucose before EA stimulation, whereas intravenous administration of the same dosage insulin significantly decreased plasma concentration of glucose after EA stimulation. These results suggest that EA stimulation can facilitate glucose uptake in response to insulin in STZ rats.

P375 (1P1-117)

Effects of neuropeptide W on single unit activity of paraventricular nucleus neurons in conscious rat

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Neuropeptide W(NPW) is a novel hypothalamic peptide that activates the orphan G protein-coupled receptors, GPR7 and GPR8. We have recently demonstrated that intracerebroventricular (i.c.v.) administration of NPW30 increases the blood pressure, heart rate, and plasma norepinephrine and epinephrine concentrations in conscious rats. This observation indicates that central NPW regulates sympathetic nervous outflow and affects cardiovascular function. NPW was recently reported to be an important stress mediator in the central nervous system that modulates the hypothalamus-pituitary axis. To examine the effects of NPW on the neural activity of the hypothalamic paraventricular nucleus (PVN), which is an integrative center of the autonomic and endocrine functions, we simultaneously recorded the single unit in the PVN neurons, arterial blood pressure, and heart rate in conscious freely-moving rats. Single unit activities were examined for their spontaneous firing patterns and responses to stressors, including disturbance in arterial blood pressure and systemic administration of cholecystokinin-8 (CCK). Non-phasic-type neurons showed excitation to i.c.v. administration of NPW; furthermore, these neurons showed sensitivity to CCK, but did not respond to phenylephrine(PE) and nitroprusside(SNP). Our data suggest that central NPW, at least in part, activates PVN neurons, which are affected by CCK, probably via the vagus nerves, independently of blood pressure changes.

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P376 (2P3-110)

Impairment of rats' spatial memory induced by foot shock stress in Morris water maze

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It has thought that the central information processing of spatial memory using Morris water maze in rodent is hippocampal-dependent paradigm. Recent studies have shown that various types of stressors influence on the rat cognitive function. Especially, a kind of adrenocortical steroid hormone glucocorticoid secreted by activation of hypothalamo-pitituitary-adrenal (HPA) axis has been studied on learning and memory. Animals given immobilization stress prior to training trial had no effects on subsequent learning acquisition and retention test, whereas there was significant effect on impairment of rats· memory retrieval if they were subjected to the stress before retention test. Here we investigated whether foot shock stress (eighty times, 0.5mA for 5s with a 10s intershock interval) would also impair rats· spatial memory retrieval. Furthermore, we measured plasma corticosterone concentration in different timing of following the stress and assessed the relevance between the circulating corticosterone levels and the spatial memory retrieval. The foot shock stress immediately before the retention test significantly impaired the memory retrieval compared to other groups in which given the stress at some different timings. Plasma corticosterone concentration was elevated 20min after the stress in this experiment. These data suggest that memory retrieval, but not learning acquisition, deficit has relevance to the stress intensity and timing and circulating corticosterone levels.

P377 (2P3-111)

Effects of coffee drinking on reduction of mental stress in young women

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Coffee is among the most common luxury beverages for Japanese consumers. We investigated the relationships between ingestion of coffee and reduction of mental stress in 256 young Japanese women. We administered a questionnaire to the subjects about preference for coffee and associated feelings of relaxation. An organoleptic test also was performed. Finnaly, effects of coffee were investigated after subjects were palced under mental stress (New Stroop test for 4 min, followed by Uchida-Kraepelin test for 20 min). The degree of mental stress was evaluated by chromogranin A, which is secreted with catecholamines in saliva.
 Among the 68% of subjects whose questionnaire responses indicated that they liked coffee, 68% felt relaxed when they drank it. In an organoleptic test, coffee extracted at 80°C exhibited greater relaxation effect than coffee extracted at 65 or 95°C. This effect showed a significant negative relationship to sourness, suggesting that relative lack of sourness enhances the relaxed feeling. Coffee drinking also decreased experimentally induced mental stress evaluated by salivary chromogranin A concentration.

P378 (2P3-112)

Behavioral analysis of HPC-1/syntaxin1A knock-out mouse

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HPC-1/syntaxin 1A is believed to regulate the exocytosis of synaptic vesicles. In order to examine the physiological function in vivo, we have produced HPC-1/syntaxin1A knock-out mice. Surprisingly, the null mutant mice revealed normal development and basal synaptic transmission in cultured hippocampal neurons appeared to be normal. In this study, we have examined the learning and behavioral phenotypes of mutant mice using behavioral approach. It was appeared that consolidation of conditioned fear memory was impaired in homozygous mutant mice but not in heterozygote. The mice exhibited impairment of cued fear memory extinction. We have also examined latent inhibition of cued fear memory (LI) to access behavioral property. Interestingly, LI was impaired both in heterozygous and homozygous mutant mice unlike the case of conditioned fear memory test. Similar results were observed in pre-pulse inhibition with acoustic startle reflex. Implication of these behavioral abnormalities in HPC-1/syntaxin1A knock-out mouse will be discussed.

P379 (2P3-113)

A difference of pain-related behaviors between young and adult in rat mono-iodoacetate injection model of osteoarthritis

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Osteoarthritis (OA) is an age-related involutional degenerative joint disease. OA attacks the knee joint particularly, and is associated with chronic pain. Intra-articular injection of mono-iodoacetate (MIA) has been used for the development of experimental pain-related OA model of osteoarthritis. Usually young rats (less than 7 weeks) were used in this model as MIA acts as metabolic inhibitor of the chondrocytes, and no report of the MIA model in the adult and aged rats was found expect no insert age in the papers until now.The objective of this study was to investigate the difference of pain related behaviors (lifting hindpaw) between the young (7w) and adult (over 24 month) rats in the MIA (60mg/ml, 50 μl) model of OA. The pain-related behaviors were evoked using von Frey hairs (2-17g) and electrically rotating brush and counted the number of lifting among 10 trials. The surface skin temperature at the knee joint were measured, as an index of joint inflammationIn pain-related behaviors, the young rats showed more frequent lifting of the hind-paw to intense von Frey filament compared with those of the adults 14 days after injection. The baseline temperature of skin surface in the young rat was lower than that of adult, and it clearly increased over 10 days after the MIA injection in the young rats. On the other hand, no clear influence was observed in the adults.These results suggest that the young rat is more suitable for the MIA injection model for studying the OA and its associated pain-related behaviors.

P380 (2P3-114)

Effects of orexin-B on cytosolic calcium in neurons of the nucleus accumbens in rats

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Orexin-B (ORX-B) acts upon orexin 1 (OX_1R) and orexin 2 (OX_2R) receptors, and is involved in the regulation of feeding, sleep-wakefulness and locomotor activity. Orexin neurons project to the shell of the nucleus accumbens (AccSh), which plays an important role in the regulation of reinforced behaviors including feeding, and may participate in the control of feeding behavior through the modulation of AccSh neuron activity. Therefore, the effects of ORX-B on AccSh neurons were investigated using cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) imaging study in rat brain slice preparations. When ORX-B was applied to AccSh neurons in artificial cerebrospinal fluid (ACSF), it increased $[Ca^{2+}]_i$ dose-dependently. The ORX-B-induced increase of $[Ca^{2+}]_i$ was also seen in the presence of tetrodotoxin, suggesting that ORX-B directly affects AccSh neurons, but not via synaptic transmissions. Increase of $[Ca^{2+}]_i$ induced by ORX-B in normal ACSF disappeared completely when tested in N-methyl-D-glucamine (NMDG)-containing ACSF. $[Ca^{2+}]_i$ increase induced by ORX-B in normal ACSF was also attenuated in about 60% of neurons tested in Ni^{2+}-containing ACSF. Results suggest that in many AccSh neurons, orexins increase $[Ca^{2+}]_i$ via non-selective cation channels and Ni^{2+}-sensitive voltage-dependent Ca^{2+} channels, and that orexins may participate in the regulation of feeding behavior through the activation of AccSh neurons.

P381 (2P3-115)

Mesolimbic neuronal activities related to ingestion of taste solutions in freely behaving rats

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The mesolimbic system and its related structures are considered to be important for ingestive behavior. We have revealed that these structures are involved in overconsumption of normally preferred taste stimuli. However, the processing of taste information in palatability-induced intake of food and fluid is still unclear. To obtain further information concerning the role of these structures in ingestion of taste stimuli, we recorded single neuron activities in the nucleus accumbens shell, ventral tegmental area, ventral pallidum, and central and basolateral nuclei of the amygdala during ingestion of taste solutions in freely behaving rats. In the experimental chamber, the rats were previously trained to lick distilled water from each spout of the bottles through a small hole on the chamber's wall. In the recording session, the rats were presented with distilled water and various taste stimuli at each trial in pseudorandom order. After a 2.5 kHz cue tone presentation for 2.5 s, rats were allowed 5 s to access the spout. Thirty to fifty percent of units in each region showed facilitation or inhibition in firing rates during licking behavior. Although in most cases these changes in the firing pattern during licking did not depend on the taste presented at each trial, a small number of units in the ventral tegmental area and amygdala responded to a specific taste. These results suggest that the mesolimbic system is implicated in taste-guided licking behavior.

P382 (2P3-116)

Estrogen facilitate the running behavior induced by oxytocin injection into the rat ventromedial hypothalamus

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Our previous study has demonstrated that excitation of neurons in the rat ventromedial hypothalamus (VMH) induced running behavior. In this study we examine the effect of neuropeptide oxytocin injection into the VMH on the running behavior. Microinjection of oxytocin into the VMH induced a dose-dependent increase in the running behavior in male rats. On the other hand, simultaneous injection of oxytocin receptor antagonist, d(CH2)5-Tyr(Me)2-Orn8-Vasotocin, with oxytocin inhibited the running behavior. Oxytocin administration into the VMH of ovariectomized (OVX) female rats also induced running behavior. In the estrogen-treated OVX rats oxytocin injection into the VMH further increased the running behavior than OVX rats. In the histological study, effective cites of oxytocin injection on inducing running behavior is located in the ventro-lateral part of the VMH. These findings suggest that oxytocin receptor in the VMH is involved in the induction of the running behavior, and in female rats estrogen facilitate the effect of oxytocin on running behavior.

P383 (2P3-117)

Masticatory muscle EMG activity during wakefulness and sleep.

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Sleep-related masticatory muscle activity has been reported to occur in association with physiological changes of sleep process (e.g., arousal) in humans. This study aimed to assess masticatory muscle activities during wakefulness and sleep in the guinea pig. Animals were prepared for chronic experiments to monitor electroencephalogram, electro-occulogram and electromyograms from dorsal neck, bilateral masseter and digastric muscles in freely moving states. After the animals recovered from surgery, these signals were recorded for 6 to 8 hours during light-phase. Sleep stages were scored for every 10-second epoch. Muscle activities were integrated for each epoch and compared between ingestion and three quiet states (quiet wakefulness, non-REM and REM sleep). All muscles showed the highest activity during ingestion. Neck muscle activity decreased clearly from quiet wakefulness to non-REM sleep and further to REM sleep. Masticatory muscle activity was 5 to 8 times lower in quiet states than during ingestion. However, the difference between the quiet states was less evident in the masticatory muscles. These results suggest that, when epoch-based analysis was done, masticatory muscle activity in the quiet states is usually very low and the state-dependent muscle activity may be different between masticatory and neck muscles.

P384 (2P3-118)

Responsiveness for acute stress correlates with menstrual distresses in healthy women.

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It is widely recognized that fluctuations of steroid hormones during women's menstrual cycle induce various physiological and psychological changes and sometimes cause undesirable condition such as premenstrual syndrome (PMS). In the present study we take advantage of menstrual distress questionnaire (MDQ) to screen subjects having normal menstrual cycle but being aware of distresses especially on luteal phase. Healthy women subjects (N=16, aged22±1) are divided into two groups of those who marked high scores on this questionnaire (MDQH) and others who scored relatively low (MDQL). Their steroidal hormones (cortisol, estradiol, progesterone and testosterone) concentrations in saliva are analyzed with ELISA and restoring time of skin temperature after cold stimulation (RT) is measured which indicates the autonomic nervous activity of cutaneous peripheral artery. Then the salivary hormonal levels of MDQH subjects are rather higher than of MDQL while they showed less fluctuation and RT is significantly longer throughout the cycle. The acute stress-loading test is also demonstrated on these two groups (N=22, aged24±3) by means of Stroop's color and word confliction task. And subjects of MDQH are found to be less responsive for acute stress and show no changes in salivary cortisol level and RT. Based on these results, we consider that the hyporesponsiveness for stress or stimulation could correlate with women's menstrual distresses out of the therapeutic range and thus it should be taken into account to cope with their complaints.

P385 (2P3-119)

Intracellular transduction of the circadian signal

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In mammals,the circadian peacemaker is located in the suprachiasmatic nucleus(SCN).Many of the SCN neurons exhibit spontaneous firing rhythms with periods specific to each neuron,suggesting that neuronal interaction is critical for the SCN to function as a master peacemaker.Hundreds of gene transcriptions are circadianly regulated,yet,a little is known about the intracellular transduction of the circadian signal.The purpose of our study is to identify the molecules which transmit the circadian signal to the membrane potential.Tetrodotoxin(TTX) inhibits synaptic communication,but a line of evidence supports it does not affect the intracellular circadian oscillation.To exclude the effect of the circadian rhythm of the membrane potential on the intracellular signal transduction,we applied TTX to pull out Na^+ channel independent genes.We cultured the SCN slice of 300μm thick from the adult mouse using culture membranes.We separately monitored *Per1*-luciferase expression rhythms in the SCN to determine the timing of TTX administration.The SCN slices were sampled for 24h in 4 h intervals.Using serial frozen sections,we systematically analyzed circadian expression rhythms of clock and clock controlled genes by *in situ* hybridization.Our results indicated a number of genes showed a robust circadian expression rhythm under the TTX treatment,and some gene expressions were severely dumped by the treatment while others were not.These findings suggest that the rhythmic input is necessary for the former,while the latter involves in the core oscillation and/or rhythmic signal transduction.

P386 (2P3-120)

Non-photic entrainment to scheduled 3-h wheel running activity in the mice circadian rhythm

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The aim of the present study was to examine whether a 3-h spontaneous wheel-running could entrain the mouse circadian rhythm under constant darkness (DD). Four-month-old, male, wild type mouse (n=27, C57BL6J, CLEA Japan, Inc) were used. They were raised in own animal quieter where environmental conditions were kept constant (light from 6:00 to 18:00, light intensity ca 100 lx, room temperature 22±2°). At 4 month old age, they were transferred to individual cage without running-wheel. The animals were exposed to DD for 4 weeks to establish stable free-running rhythm. Then the cage were exchanged with a cage outfitted with a running-wheel (10cm diameter) for 3-h, 3-h from 6:00 to 9:00 of local time. Wheel running treatment was implemented for 80-100 days with a fixed period of 24-h. Afterwards, the spontaneous locomotor activity was measured in DD for 4 weeks. As a result, 15 of 23 mice entrained the scheduled 3-h wheel-running session. The timing of wheel setting during entrainment was related (-0.644, $p<0.008$) to free-running period before treatment under DD, when 3-h wheel running commenced the late subjective night (CT 17-22, CT12 was activity onset of animal). 6 animals showed longer free-running periods than 24-h ranged 24.04-24.44, and their free-running periods were significantly shortened after 3-h wheel treatment (M±SD, 23.93±0.07h). A possible role of 3-h wheel running was demonstrated as the non-photic zeitgeber.

P387 (2P3-121)

Two distinct photoperiodic oscillators in the mouse suprachiasmatic nucleus

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A master circadian clock of mammals is located in the suprachiasmatic nucleus (SCN) which is entrained by the light-dark (LD) cycle. Transcription-translation feedback loops involving several clock genes are the molecular machinery for rhythm generation, where *Per* gene plays a key role. Adaptation to the photoperiod is an essential strategy for organisms living in the high latitudes, and the mechanism is currently explained by two mutually coupled circadian oscillators. However, the localization of these oscillators remains elusidated. Adult mice carrying luciferase reporter genes at downstream of the *mPer1* promoter were used. They were housed in three LD conditions, LD12:12, LD6:18 or LD18:6 for three weeks. The anterior and posterior SCN slices were cultured separately to monitor *Per1* expression for 5days. In addition, spontaneous locomotor activity was monitored by thermal sensors. When transferred from LD12:12 to LD18:6, the activity time was compressed from 12.4h to 7h. On the other hand, in LD6:18 the activity time was lengthened by 1.6h with the activity-onset phase-advanced. In the SCN from LD12:12, the circadian peak in the posterior SCN phase-leaded the peak in the anterior SCN by 2.5h. In LD6:18, the peaks of both rhythms were located at nearly the same time of day, whereas in LD18:6, two peaks were observed in the anterior SCN which were evident in the first several days of culture, but eventually merged into one single peak. The results suggest that the two distinct oscillators reside in the SCN which respond differently to different photoperiods.

P388 (2P3-122)

Effect of daily exercise on the clock gene expression in the central and peripheral tissues of mice

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Circadian rhythms are intrinsic oscillations which control various physiological and behavioral phenomena. In mammals, the master circadian pacemaker (or circadian clock) is located in the suprachiasmatic nuclei (SCN) of the ventral hypothalamus. Although the environmental light/dark cycle (LD) is the most potent synchronizer of the circadian pacemaker in the SCN, it is well known that exercise has effects on various circadian oscillations. Actually, novel wheel-running, which are called non-photic stimuli, induce phase advances in the circadian pacemaker. Recently, it is reported that circadian oscillators can be found in most tissues. Therefore, to explore the effect of voluntary running activity on gene expression under the LD cycle, we examined the daily expression of clock gene such as mPer1, mPer2, and mBmal1 in the central and peripheral tissues of mice. We found that daily exercise induced changes in the expression of clock genes in both central and peripheral tissues. These results suggest that daily exercise regulates the circadian oscillators in both central and peripheral tissues .

P389 (2P3-123)

Effect of Taurine-loading in perinatal period on the conditioning task in the mouse

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The effects of taurine on the behaviour of male mice were investigated using operant conditioning task. This amino acid is found in high concentrations in the central nervous system of mammals and human maternal milk, has been shown to be essential for the development. Male mice (C57BL/6) were used as subjects and divided into four groups, 1) Long-loading: Taurine (400 mg/kg in a day) dissolved in distilled water was provided as drinking water. In the prenatal period, the taurine was given to through subjects' mothers, from the birth to weaning period, through maternal milk and after the weaning, from the drinking water by the end of the experiment. 2) Loading in pre-weaning: subjects were exposed to the taurine by the weaning. 3) Loading in adult: from after the weaning. 4) Control: no loading (drinking distilled water). Subjects were trained for reacting to illuminated key as soon as possible. As indexes of learning, correct reaction ratio and reaction time was subjected for analysis.All subjects acquired this discrimination task in one month period of the training. The learning speed of each experimental group was different depending on the timing but not length of the loading.

P390 (2P3-124)

Photic entrainment of the dorsal SCN

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In mammal, circadian rhythms are driven by a pacemaker located in the suprachiasmatic nucleus (SCN) of the hypothalamus. The pacemaker is entrained to environmental light-dark cycle via the retino-hypothalamic tract, which terminates predominantly in the ventral SCN. The main neurotransmitter of the tract is glutamate. Previous studies show that the ventral part of the SCN responds to light more quickly than the dorsal SCN. However, it is unknown that how the dorsal SCN is entrained to the ventral. The dorsal part of the SCN exhibits an endogenous rhythm of arginine vasopressin (AVP) in vivo and in vitro. Measuring AVP releasing rhythm in the SCN slice culture, we examined phase-shifting effect of N-methyl-D-aspartate (NMDA) on the rhythm of the dorsal SCN. The pulse application of NMDA induced phase delays at early subjective night and phase advances at late subjective night. Even in the presence of bicucullin, a GABA-A antagonist, NMDA also induced phase shift of the rhythm. On the other hand, VIP antagonist inhibited NMDA-induced phase shift. These results suggest that VIP but not GABA would concern the photic entrainment of the dorsal SCN.

P391 (2P3-125)

EFFECTS OF SB334867 ON OREXIN-A MEDIATED FOOD AND WATER INTAKE IN THE BED NUCLEUS OF STRIA TERMINALIS

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Orexins are synthesized by lateral hypothalamic neurons which appear to have extensive projections throughout the neuraxis. Orexins have been shown to influence a variety of homeostatic mechanisms such as feeding and drinking behavior. Orexins act on two receptor subtypes, orexin-1 (OX1R) and orexin-2 (OX2R) receptors. Orexin-A (OXA) binds selectively to OX1R, whereas OX2R binds both OXA and orexin-B (OXB). The presence of OX1R in the bed nucleus of stria terminalis (BST) is verified. OXA microinjections into the BST evoked an increase in liquid food intake (FI) and water intake (WI) in a dose dependent manner. Here, the effect of selective OX1R antagonist SB334867 was examined in the BST in male Wistar rats. 0.26 nmol SB334867 was bilaterally microinjected into the BST alone or 15 min before 0.14 nmol OXA microinjections, whether the increases in liquid FI and WI following OXA applications can be antagonized. OXA enhanced liquid food consumption and increased WI as well. SB334867 pretreated OXA groups did not show any significant differences compared to the control groups neither in liquid food, nor in water ingestion. These findings show that the effects of OXA can be antagonized by SB334867 whereas SB334867 alone did not alter intakes indicating that the effects of OXA are mediated by OX1Rs in the BST.This work was supported by National Research Fund of Hungary (C012, M036687), and by the HAS.

POSTERS
Neurochemistry

P392 (2P3-126)

HOMEOSTATIC RELEVANCE OF ORBITOFRONTAL CORTICAL CYTOKINE-SENSITIVE NEURONS

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In the present experiments homeostatic consequences of orbitofrontal cortical (OBF) interleukin-1β (IL-1β) microinjection were investigated. Food intake (FI), water intake and body temperature (BT) were measured after bilateral OBF microinjection and i.p. injection of IL-1β. Metabolic alterations, namely, blood glucose levels (BGLs), plasma concentrations of insulin, leptin, cholesterol, triglycerides and urate were also determined. Similar to consequences of i.p. administration, short term FI was suppressed, whereas BT was raised remarkably after OBF microinjection of IL-1β. Central application of the cytokine led to a diabetes-like prolonged elevation of BGL, furthermore, plasma levels of insulin and triglycerides were found decreased, whereas that of uric acid increased. In our single unit recording study high proportion of OBF neurons were responsive to the microelectrophoretically applied IL-1β. An overwhelming majority of these neurons were also responsive to microelectrophoretically applied D-glucose, i.e., proved to be the elements of the central glucose-monitoring neural network (GMNN). Our findings, therefore, confirm that neocortically organized IL-1β mediated adaptive reactions through the orbitofrontal cortical GMNN play important roles in the central homeostatic control.Supported by: National Research Fund of Hungary (T 042721, M 036687), the Hungarian Academy of Sciences and Richter Gedeon Pharmaceutical Co., Ltd., Hungary.

P393 (3P3-150)

Effects of rare sugar, D-allose, on rat retinal ischemic model

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The effects of D-allose, one of the rare sugars, against ischemia injury in rat retina were determined.

Retinal ischemia was induced by intraocular pressure to 130mmHg and maintaining that level for 45min. Morphological analysis was performed to study the effects of D-allose on histological changes in the rat retina induced by ischemia. Glutamate release from retina was monitored by using a microdialysis biosensor. Vitreal PO2 was measured oxygen sensitive microelectrode during and after ischemia.

Seven days after ischemia, significant reductions in both number of ganglion cells and thickness of the inner plexiform layer were detected. Pretreatment of D-allose significantly inhibited the ischemic injury of the inner retina. A large amount of glutamate was released during ischemia. After recirculation, glutamate levels were increased again and reached a maximum in 20min. This increase of released glutamate was clearly suppressed by the pretreatment of 200mg/kg D- allose. D-allose also attenuated the increase in vitreal PO2 values during repurfusion. These results suggest that D-allose may protect neurons by decreasing extra-cellular glutamate and attenuating oxidative stress in ischemic injury. injury in rat brain.

P394 (3P3-151)

Eugenol prevents 6-hydroxydopamine-induced dopamine depression and lipid peroxidation in mouse striatum

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As superoxide and hydroxyl radical have been implicated in pathogenesis of Parkinson disease, free radical scavenging and antioxidant have attracted attention as way to prevent progression. We examined effects of eugenol, an essential oil extracted from clove, on 6-hydroxydopamine (6-OHDA)-induced dopamine reduction in mouse striatum. Eugenol administration 3 days before and 7 more days following one intracerebroventricular 6-OHDA injection prevented reductions of striatal DA and its metabolites and increases of thiobarbituric acid-reactive substances, an indicator of lipid peroxidation. Eugenol did not change catalase, glutathione peroxidase, and superoxide dismutase like activities. Eugenol has known to have superoxide and hydroxyl radical scavenging activities in vitro. These results suggest that eugenol prevents 6-OHDA induced DA depression by preventing lipid peroxidation directly. Effects of eugenol treatment in this model suggest possible value in treatment of Parkinson disease.

P395 (3P3-152)

The phosphorylation of Ser^{40} of tyrosine hydroxylase has no effect on the intracellular stability of the enzyme

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It is well established that the phosphorylation of tyrosine hydroxylase (TH) at Ser^{40} is critical in regulating the catalytic activity of the enzyme. However, the influence of the phosphorylation of Ser^{40} on the intracellular stability of TH protein has not been investigated. This study was performed to estimate such a possibility. Although the treatment of rat pheochromocytoma cell line PC-12 cells with forskolin increased the amount of TH phosphorylated at Ser^{40} in the cells, it did not affect the total amount of TH in the cells. Next, human TH type 1 (hTH1) of wild-type and a mutant missing the first 52 amino acid residues were expressed as histidine-tagged forms in PC-12 cells, and then the cells were treated with forskolin. However, the phosphorylation of hTH1 at Ser^{40} did not affect the amount of the wild-type hTH1 protein present in PC-12 cells. Finally, wild-type and a mutant Ser^{40} of which was replaced by Asp (S40D, a mimic of TH phosphorylated at Ser^{40}) were expressed in PC-12 cells as histidine-tagged forms or untagged forms. Neither histidine-tagged nor untagged forms showed any difference in their amounts of wild-type hTH1 and S40D hTH1 present in the cells. Collectively, these results indicate the fact that the phosphorylation of Ser^{40} does not affect the stability of TH protein in PC-12 cells. However, we still do not deny the possibility that the successive phosphorylation of Ser^{19} and Ser^{40} may affect the intracellular stability of TH protein.

P396 (3P3-153)

Corticotropin-releasing factor receptor antagonist attenuates LPS-induced increase of GTP cyclohydrolase I expression at murine locus coeruleus

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It has been reported that corticotropin-releasing factor (CRF) is involved in the regulation of norepinephrine neuron responses to stress such as an immobilized stress. Furthermore, systemic lipopolysaccharide (LPS) injection upregulates the transcription of the genes encoding *CRF* and *CRF type 1 receptor* in the paraventricular nucleus of the hypothalamus. We have already reported that an increase in norepinephrine turnover within the murine locus coeruleus is accompanied by septic shock triggered by LPS intraperitoneal injection. We also elucidated that the expression levels of the enzymes involved in the catecholamine biosynthesis were altered by peripheral LPS injection. Collectively, the effects of CRF on the expression levels of the enzymes at murine locus coeruleus were investigated by peripherally injecting CP-154,526 (CP-154), a CRF receptor type 1 antagonist. Pretreatment with CP-154 attenuated the increase in expression levels of GTP cyclohydrolase I mRNA due to intraperitoneal LPS injection at 4 h after the injection. However, no effects on the expression level of tyrosine hydroxylase mRNA at the site were observed. Taken together with the fact that LPS injection enhances tetrahydrobiopterin biosynthesis at locus coeruleus, CP-154 may attenuate the increase of NE turnover by way of suppressing the enhanced GCH expression level at the site caused by peripheral LPS injection.

P397 (3P3-154)

Functional investigation of neural stem cells through cell surface N-glycan

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Asparagine-linked oligosaccharide (N-glycans) on the cell surface undergo dynamic changes during embryogenesis; i.e., in the peripheral tissues, mannose type N-glycan is changed to complex type N-glycan. On the contrary, in the brain, mannose type N-glycan appears to be retained in adulthood. In the last meeting, we have investigated the carbohydrate diversity in embryonic mouse brain and showed the expression of complex type N-glycan on the cell surface of the subventricular zone cells by immunohistochemical analysis. In this study, we performed quantitative analysis of embryonic brain cells by using Fluorescence-activated cell sorting (FACS) system, and revealed that a half of the nestin positive cells in the embryonic mouse brain boar complex type N-glycan. We, then, tried to purificate the neural stem cells (NSC) from mouse brain through complex type N-glycan on surface. As a result, complex type N-glycan positive cells enable to form neurosphere at greater extent (36.7 fold) compared to N-glycan negative cells, and enable to differentiate to neurons and glial cells. These results indicate that unique expression of complex N-glycans are valuable substances for the prospective purification of living NSC.

P398 (3P3-155)

Recovery of hypoxic-ishchemic damage by low dose of erythropietin in P3 rats

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Selective vulnerability of late oligodendrocyte progenitors (preOLs: NG2-positive and O4-positive cells) to hypoxic-ischemia (H-I) was reported, explaining the etiology of periventricular leukomalacia (PVL). In rats, preOLs is detected in white matter at postnatal day 2 (P2)-P4. To investigate whether H-I targeting to preOLs caused pathological changes more similar to PVL and whether erythropoietin (EPO) has effect to reduce brain damage by H-I, right common carotid artery occlusion (RCAO) was carried out in P2-P4 rats followed by hypoxic condition (6% 0_2) for various time (0-90 min). RCAO with 6% O_2 for 60 min resulted in high proportion of death: 64% in P2, 50% in P3, and 89% in P4. Histological examination 2 days after H-I revealed that no obvious change was shown in P2 rats. However, typical histological changes of PVL were found in most of surviving P3 rats, suggesting that RCAO followed by 6% 0_2 for 60 min in P3 pups induced histological changes more similar to human PVL in P3 pups. Various dose of EPO (1-30,000 U/kg, i.p.) was treated to animals just before H-I, and the mortality and histological alterations were assessed. With lower concentration of EPO (50-100 U/kg), death rate became to 27-38%, and the damage in cortical and callosal white matter was decreased, indicating that low dose of EPO is protective to cerebral white matter damage in P3 rats.

P399 (3P3-156)

Low oxygen and cytokine mixture increased number of dopaminergic neurons from ES-derived nestin-positive cells

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Dopamine(DA)ergic differentiation from ES cells was induced by 5 steps in McKay·s method: stage 1, maintenance of ES cells; stage 2, formation of EBs; stage 3, selection of nestin-positive cells; stage 4, expansion of nestin (+) cells; stage 5, induction to DAergic neurons. To investigate whether physiological low oxygen found in development and cytokines expressed in the DA-depleted striatum increase the production of DA neurons from ES-derived neural progenitor cells (NPCs), NPCs were treated with cytokine mixtures (100 pg/ml IL-1β, 1 ng/ml IL-11, 1 ng/ml LIF, 1 ng/ml GDNF) or lowered O_2 (3.5%) on stage 4 and stage 5, followed by tyrosine hydroxylase (TH) immunostaining. Low oxygen from stage 4 increased total number of TH(+) cells (1.78-fold) and number of TH(+) cells per sphere (1.58-fold of control) as compared to normal O_2. Cytokine mixture significantly increased TH(+) cells (2.13-fold) compared to non-treated control. IL-1β during stage 4 exhibited major contribution in the effect of cytokine mixtures. Data suggest that physiologically relevant low oxygen in development and cytokines and trophic factors that were enhanced in injured brain cause in increase of DAergic neurons from ES-derived NSCs.

P400 (3P3-157)

Tumor necrosis factor-α induced by peripheral LPS injection plays an important role in the process of programmed cell death in murine olfactory bulb.

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Persistent inflammation and successive innate immune reaction have been considered to cause Parkinson's disease (PD). Recently, we have found that peripherally injected lipopolysaccharide (LPS) induced tumor necrosis factor (TNF)-α production in astrocytes in the olfactory bulb (OB) and enhanced the number of apoptotic cells in the OB. This finding could be one of the explanations for the olfactory dysfunction observed frequently and early in the patients suffering from PD.

Herein, we attempted to clarify the relative roles of TNF-α playing in the induction of programmed cell death after peripheral LPS injection, in which TNF receptor-deficient mice ($TNFR^{-/-}$) were used. The intraperitoneal injection of 50 μg LPS into wild-type mice induced the successive increases in mRNA expression levels in the OB of the genes encoding TNF-α and caspase-8. The number of TUNEL-positive cells in the OB increased to a significant level in LPS-injected wild-type mice at 24 h after the LPS injection. On the contrary, LPS (50 μg)-injected $TNFR^{-/-}$ mice did not reveal any increase in caspase-8 mRNA expression level and the number of apoptotic cells in the OB, despite the significant increase in the TNF-α mRNA expression level at the site. These findings suggest the fact that a crucial role should be assigned to TNF-α in the induction of programmed cell death triggered by peripheral LPS injection.

P401 (3P3-158)

The differences between the amount of Ca^{2+} release from the Ca^{2+} store and the amount of Ca^{2+} increase in the cytoplasm.

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Caffeine causes the periodical Ca^{2+} release from the Ca^{2+} store in cultured bullfrog sympathetic ganglion cells. We have already reported that the time course of cytoplasmic Ca^{2+} increment does not reflect faithfully that of Ca^{2+} decrease in the Ca^{2+} store. To understand how this difference arise, the dependency of the amount of Ca^{2+} release from the Ca^{2+} store on caffeine concentration applied was investigated. Dependency of Ca^{2+} increase in cytoplasm on caffeine concentration was different from that of Ca^{2+} decrease in the Ca^{2+} store. At the application of 5mM caffeine, the amount of Ca^{2+} released from the store was half of that at the application of 10mM caffeine, and the amount of cytoplasmic Ca^{2+} increase was about 20%, suggesting that all amount of Ca^{2+} released from the Ca^{2+} store did not cause the cytoplasmic Ca^{2+} increment.

P402 (3P3-159)

Rat spinal cord injury model produced by asphyxia.

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Regeneration of spinal cord injury is important thema in rehabilitation science as well as basic on. This experience of aim is to recognize the process after spinal cord injury by asphyxia. To establish an animal model of spinal cord injury produced by asphyxia, we used adult rats with aorta occulusion . Fifteen minutes after occulusion electrical reflex activity of apinal cord disappeared. After thirty minutes occulusion , irreversible function changes appeared, long term depression of reflex activities and disorder of motor functions. We traced time course of electrical and functions change after thity minutes occulusion for twelve hours.

P403 (3P3-160)

Changes in the chloride homeostasis-related genes in trigeminal nuclei of the adult rats with nerve injury

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Changes in the chloride homeostasis-related genes in trigeminal nuclei of the adult rats with nerve injuryBing Wei, Atsuo FukudaDepartment of Physiology, Hamamatsu University School of Medicine, Hamamtsu, Shizuoka 431-3192, JapanAbstractAccording the modern pain control theory predicts that a loss of inhibition in the dorsal horn of the spinal cord is a crucial substrate for chronic pain syndromes. It involves a transsynaptic reduction in the expression of the potassium-chloride exporter KCC2. we have previously shown KCC2 mRNA downregulation in injured facial motoneurons, that impairs Cl^- homeostasis and makes GABA act depolarizing. We report here changes in the expression level of the KCC2 and NKCC1 mRNAs in the trigeminal nuclei of an experimental rat model of trigeminal neuropathic pain produced by a chronic constriction injury to the infraorbital nerve. By means of in situ hybridization histochemistry, our data indicates that 1 to 3 weeks after the operation the KCC2 mRNA was downregulated in the spinal nucleus. The result suggests that the KCC2 downregulation may play pivotal roles in the trigeminal neuralgia by rendering GABA action depolarizing.

P404 (3P3-161)

Responses of extracellular dopamine release in the nucleus accumbens to somatic afferent stimulation in anesthetized rats

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In anesthetized rats, responses of extracellular dopamine release in the nucleus accumbens to innocuous and noxious mechanical cutaneous stimulation of the back were investigated. Coaxial microdialyis probe was stereotaxically implanted in the nucleus accumbens and dialysed with artificial cerebrospinal fluid at a speed of 2 µl/min. Dialysate output from the probe was directly injected into the high liquid chromatograph and the amount of dopamine released from the nucleus accumbens for 5 min was consecutively measured with electro-chemical detector. Innocuous stroking stimulation for 5 min applied to the bilateral back significantly increased dopamine release for 10 min after the onset of stimulation. The increase in dopamine release was observed by stroking of the contralateral, but not iplilateral back. Bilateral noxious pinching stimulation had no effect on dopamine release in the nucleus accumbens. These results indicate that excitation of the innocuous mechanical afferents can stimulate dopamine release without emotional influence.

P405 (3P3-162)

High-field mouse MRI probe for stereotaxic analysis

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Our new project is to detect minimal brain injury in the shaken-baby syndrome. We will take a rat model that was established by Ueda et al (Neuroscience Letters, **385** 82-86 2005). A high-spatial resolution image and a diffusion analysis can be useful to detect and follow pathological changes in the brain. It is reasonable to take a higher field to get a better image of small brains of neonatal, infantile, and juvenile rats that are similar size to mouse. In order to establish a basic system in vertical high-field MR micro-imaging system, we had made a probe with the stereotaxic coordinates for mice inside a gradient system. A set of the conventional fixation devices (a bite bar and a pair of ear bars) was installed in an acrylic tube. The position of the bite bar can be adjusted to the level position. 1% enflurane in a gas mixture of 36% O_2-2%CO2-62% NO delivered through the tracheal canula by artificial ventilator. The body temperature was kept in adequate range by a hot water circuit, and the temperature was monitored by fluorescence thermometer. ECG was also monitored by PowerLab. Using this probe, we can get reasonable quality of gradient-echo image, spin-echo diffusion image, and multi-slice multi-spin-echo image in 100 µm pixel resolution with a slice thickness of 0.5 mm. Vital conditions of mice kept constant for 4 hours and longer. We are now trying to detect a minimal hemorrhage in the infant rat brain that was shaken by a shaking machine.

P406 (3P3-163)

Analysis of molecular mechanisms involved in microglia activation

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Microglia are resident macrophages in the central nervous system. In response to injuries or inflammation, microglia are stimulated to exhibit drastic changes in morphology and functions, and called as "activated microglia". Activated microglia proliferate vigorously, migrate and accumulate to the site of the inflammation, and phagocytose pathogens and cellular debris. Further, activated microglia produce various bioactive molecules to assist repair and regeneration of the nervous system. In this report, to elucidate molecular mechanisms of microglia activation, we focused on signaling molecules including small G protein Rac and microglia-specific calcium binding protein Iba1. Activated microglia exhibit extremely active motility and phagocytosis, suggesting the existence of microglia-specific mechanisms regulating the actin cytoskeleton. We previously reported that expression of Iba1 is upregulated during microglia activation, and that Iba1 is involved in motility and phagocytosis of microglia. Furthermore, Iba1 was shown to be cooperating with Rac in regulation of the actin cytoskeleton. Herein, we demonstrated intracellular behavior of these signaling molecules in microglia activation.

P407 (3P3-164)

Does endogenous vasopressin induced by central salt loading increase vasopressin and oxytocin mRNA in the paraventricular and supraoptic nucleui of the hypothalamus in rats?

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We have previously showed that endogenous vasopressin increases the vasopressin (AVP) and oxytocin (OT) mRNA production in the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus after central salt loading in conscious rats. We reached this conclusion by exposing hybridization signals to x-ray films. However, we are also asked to confirm this results by showing the specific signals on the brain region using double labeling procedure (Fos-like immunoreactivity: FLI and mRNA). For this purpose, all hybridized sections were dipped in emulsion (Art emulsion, Fuji film) and then stored at 4°C for 14 days and 28 days for AVP and OT assays after completion of immunostaining of FLI. The distribution of the hybridization signal on the sections were similar to that of FLI in the SON. From these results, we confirmed that central vasopressin affects its own synthesis and OT synthesis in the SON.

P408 (3P3-165)

The carbohydrate structures of the oligo-sialylated sugar chains increased by aging in rat peripheral nerve glycoproteins.

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We investigated the structural changes in asparagine-linked sugar chains of rat sciatic nerve glycoproteins during aging and reported previously that high-mannose-type sugar chains were abundant in younger animals, whereas two sialylated/sulfated oligosaccharides, OIBA1 and OIBA2, increased during maturation. We investigated further on the sugar chains increased by aging and found that there were several oligo-sialylated sugar chains (referred to as OIBA-3. OIBA-4, and OIBA-5). The common structure of these sugar chains was determined to be a bi-antennary complex-type oligosaccharide; Galβ1-4GlcNAcβ1-2-Manα1-6(Galβ1-4GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc. A sulfate residue linked to the 6-O-GlcNAc on the C3 antenna. The difference among them was revealed to be sialic acid contents. We analysed the structures of these sugar chains by exo-glycosidase digestion, HPLC analysis, and MALDI-TOF-MS. The oligosaccharide structures of the OIBA familiy were determined as follows; $(Sia)_{0\text{-}3}$-Galβ1-4GlcNAcβ1-2-Manα1-6($(Sia)_{0\text{-}1}$-Galβ1-4(SO_4-6)GlcNAcβ1-2Manα1-3)Manβ1-4GlcNAcβ1-4(Fucα1-6)GlcNAc. These data indicated that highly sialylated oligosaccharides increased in peripheral nerve glycoproteins of aged rats.

P409 (3P3-166)

The role of guidance receptor system in cell dynamics and mouse behavior

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During neural development, guidance molecules such as semaphorins and netrin play crucial roles in neuronal network formation by controlling axonal pathfinding. Semaphorin4A (Sema4A), a member of class 4 semaphorin induced growth cone collapse of hippocampal neurons. The binding of Sema4A to the growth cone indicated the presence of a receptor transmitting signals to intracellular effectors to induce growth cone collapse of hippocampal neurons (Yukawa K et al. Int J Mol Med 16: 115-118, 2005). Transfection experiments of receptor candidate genes into Cos7 cells demonstrated that Sema4A could bind to guidance receptors including Plexin-B1, Plexin-B2 and Plexin-B3. To identify functional Sema4A receptor and the signal transduction machinery, we performed Cos7 cell contraction assay where the completion of intracellular signal transmission induced contraction of Cos7 cells. Expression vectors for receptor candidate genes and Rho family GTPase (Rnd1, Rnd2 or Rnd3) were transfected into Cos7 cells. As a result, we confirmed that Plexin-B1 could transmit intracellular signal of Sema4A through Rnd1. Our data also suggested that Plexin-B2 and B3 could transmit intracellular signal of Sema4A through Rho family GTPase different from Rnd1. Concomitantly, our mouse behavioral analyses now screen knockout mice of semaphorins and guidance receptors to get a clue whether the guidance receptor system controls mouse behavior. The screening disclosed a hyperactive behavior of knockout mice of Sema4D, another class 4 semaphorin.

POSTERS
Endocrine glands & hormones

P410 (1P2-118)

Leptin activates α2 AMPK in C2C12 muscle cells through Atm and PI-3kinase/Nur77/ CaMKKβ-dependent pathways.

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Leptin promotes fatty acid oxidation in skeletal muscle by activating α2 AMP-kinase (AMPK), directly at the muscle level and via the hypothalamic-sympathetic nerve. We examined the role of AMPK upstream kinase such as LKB1, Atm, and Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ) in leptin-induced AMPK activation in C2C12 muscle cell line that expresses leptin receptor Ob-Rb. Leptin produced a biphasic activation of α2 but not α1 AMPK in C2C12 cells with an early peak by 1 hr and a late peak by 12-24 hr. Antisense RNA of Atm abolished the 1st peak, while RNAi of CaMKKβ suppressed the late peak. RNAi of LKB1 decreased neither the 1st nor 2nd peak. Consistently, leptin induced expression of CaMKKβ and transcription factor Nur77 from 6 to 24 hr. Nur77 increased CaMKKβ expression via unique NGF1-B (Nur77)-response element. Leptin stimulated Nur77 expression by PI3-kinase (PI3K)/PKCζ pathway, promoting JAK2/ IRS1/PI3K complex. Furthermore, leptin increased intracellular Ca^{2+} from 6 to 24 hr through PI3K/PLCγ- and Ca^{2+}-channel-activation. Nur77 and CaMKKβ expression decreased in skeletal muscle of leptin-deficient mice (ob/ob). In sum, leptin activates α2 AMPK in C2C12 muscle cells through two distinct mechanisms: the early activation is mediated by Atm and the late activation is due to PI3K/Nur77/ CaMKKβ. Significant decrease of Nur77 and CaMKKβ expression in ob/ob mice suggests that the signaling pathway as well as Atm is important for leptin-induced AMPK activation in skeletal muscle.

P411 (1P2-119)

Gender differences in hypothalamus-pituitary-adrenocortical (HPA) activity during acute psychological stress

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Two primary systems are particularly involved in setting on the physiological stress response, HPA and sympathetic-adrenomedullary (SAM) systems. The present study compared the responses of both the HPA and SAM activities to the acute psychological stress between genders. Subjects were selected according to their score in Spielbergers Trait Anxiety Inventory (STAI) to assess the predisposition to personal anxiety, and high (score >55) and low (score <45) anxiety groups were included. The video of corneal surgery was served as the stressor for 15 min. Salivary cortisol and amylase were assayed as indexes of the HPA and SAM activities, respectively. Salivary β-endorphin was also assayed as a possible index of HPA activity. There were no differences of all the resting salivary parameters levels among the groups. As expected, during the stressful video viewing, all the salivary parameters were significantly increased in all groups. There were no differences in amylase levels between high and low anxiety of both genders. However, cortisol and β-endorphin levels of high anxious females were significantly lower than those of high anxious males. Thus, in contrast to the traditional view, high anxious females exhibited lower levels of HPA hormones than high anxious males during stressful video viewing. Our findings suggest that high trait anxiety in females may be associated with an inability to respond to sufficient activation of HPA to acute psychological stress.

P412 (1P2-120)

RORα mutation induces changes of mRNA levels in neurotrophins and their receptors of developing cerebellum

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Abnormal cerebellar development of *staggerer* (*sg*) mouse has been reported due to the mutation of retinoic acid receptor-related orphan nuclear receptor (ROR)α, a member of the steroid, thyroid, and retinoid receptor superfamily. However, the role of RORα on cerebellar development has not yet been clarified. The appropriate mRNA levels of neurotrophins and their receptors are necessary for development of cerebellum. However, the changes in expressions of these genes in *sg* mouse cerebellum have not yet been studied. To understand whether neurotrophins and their receptors are involved in cerebellar impairments of *sg* mouse, we investigated these gene expression profiles during postnatal development by RT-PCR and *in situ* hybridization. The decreased mRNA levels of BDNF, NT-3 and NT-4 at P15 or P21 and increased mRNA levels of NGF, TrkA and TrkB were shown in homozygous *sg* mouse compared with those in wild type. Interestingly, the expression patterns of these mRNA in *sg* mouse are similar to those in hypothyroid animals, suggesting a possible cross-talk between RORα and thyroid hormone receptors. The hybridization signals for BDNF, NT-3 and TrkB mRNAs were mostly located in the granule cells, suggesting the important roles of neurotrophins in granule cells during *sg* postnatal development. These results indicate that the mutation of RORα alters the expression of neurotrophins and their receptors, which may be partly responsible for abnormal cerebellar development of *sg* mouse.

P413 (1P2-121)

The effect of PCBs in TR-mediated transcription on native-TRE

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Polychlolinated biphenyls(PCBs) are known as environmental contaminants that cause various effects in many organs including the central nerves system.We have previously reported that PCB5005 (hydroxylated PCB) suppressed Thyroid hormone(TH) receptor (TR)-mediated transcription on several artificial TH-response elements (TREs).In this study, we examined TR-mediated transcription on native TRE. We used malic enzyme (ME) response element contains three TRE half sites. Transcriptional activity of ME was measured by using transient transfection based repotor gene assay in fibroblast-derived clonal cells (CV-1) and neuroblastoma-derived clonal cells (TE671).TR-mediated transcription was suppressed by PCB5005.However PCB6002 did not affect on the transcription.On the other hand, PCB187 induced the TR-mediated transcription congeners.Next, to study the effects of these congeners on endogenous ME gene expression, we applied the reverse-transcriptional polymerase chain reaction (RT-PCR). PCB5005 exposure suppressed the expression of ME mRNA. These results indicate that exposure of PCBs affects TR-mediated transcription on native promoter.

P414 (1P2-122)

Secretin facilitates oxytocin release from the neurohypophysis

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It has been known that oxytocin receptors are implicated in autism. Autistic subjects have been reported to have lower serum concentrations of oxytocin. Genetic linkage studies and single nucleotide polymorphism analyses for autistic disorder also show positive association of the oxytocin receptor with autism. Furthermore, mice lacking the oxytocin receptor gene show autistic phenotypes, such as socio-behavioral deficits. On the other hand, secretin, a gastrointestinal hormone, has been considered as a possible treatment for autism. However, the relationship between oxytocin and secretin remains to be determined. In the present study, we investigated whether secretin activates oxytocin neurons. We measured plasma oxytocin concentrations after an intracerebroventricular (icv) injection of secretin. The icv injection of secretin caused a dose-dependent increase in plasma oxytocin concentrations. The results indicated that secretin induces the activation of oxytocin neurons, consistent with a hypothesis that the effect of secretin on autism is mediated by oxytocin/oxytocin receptor system.

P415 (1P2-123)

Effect of SXR on progesterone receptor (PR)-mediated transcription

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The regulation of transcription of target genes by nuclear hormone receptors, such as estrogen receptor (ER) and progesterone receptor (PR), is related to the growth and development of many organs and tumors such as breast cancer. Recently, it has been reported that steroid and xenobiotic receptor (SXR) is expressed in a series of breast cancer cells. It is known that more than 60% of prescribed drugs bind to SXR to induce the transcriptional activities of target genes such as cytochrome P450 (CYP) 3A4. In the present study, to investigate the action of SXR on PR-mediated transcription, we performed transient cotransfection-based reporter assays using African green monkey kidney fibroblast-derived CV-1 cells. SXR activated the PR-mediated transcription through progesterone response element (PRE) in the presence of PR agonist up to 3-fold in a receptor-concentration dependent manner . On the other hand, without PR, SXR did not activate the transcription through PRE. These results indicate that SXR may directly or indirectly interact with liganded PR, but not with PRE in the presence of agonist. These results indicate that SXR may alter the effect of progesterone in breast cancer cells. In conclusion, together with the effect of SXR on ER-mediated transcription, SXR may deteriorate the breast cancer, and may be a useful clinical marker to classify breast cancers.

P416 (1P2-124)

Paracrine role of GABA in adrenal medullary cells

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GABA is known to produce a depolarization or secretion in adrenal medullary (AM) cells of various species. However, whether the GABAergic system is intrinsic or extrinsic in the adrenal medulla is ambiguous. Thus, we addressed this issue using immunological techniques. The immunoblotting and immunohistochemistry revealed that GAD, a GABA synthesizing enzyme, was present in rat AM cells, but not adrenal cortical cells. VGAT, a vesicular GABA transporter, was also found in the rat adrenal medulla. The fractionation study with bovine adrenal medulla showed that GAD and VGAT were recovered in a crude membrane fraction, but not in a chromaffin granule fraction, suggesting GABA is stored in synaptic-like microvesicles, but not chromaffin granules. Perfusion of a GABA-containing solution in the rat AM cells loaded with Fura-2 resulted in an increase in Ca^{2+} signal in some, but not all, of AM cells that responded to electrical stimulation. The maximum response of Ca^{2+} signal evoked by both electrical stimulation and GABA did not differ from that elicited by electrical stimulation alone. This result suggests that GABA did not produce an inhibition of membrane excitability through a shunt effect. The immunoblot showed that GABA receptors in rat AM cells consisted of at least α 1, α 3, and γ 2 subunits. The results suggest that GABA functions as a paracrine or autocrine in rat AM cells

P417 (1P2-125)

Estrogen responsiveness of neuronal system-related genes in the hypothalamus of adult female rats

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In adult female rats, estrogen has crucial effects on the estrus cycle, reproduction and sexual behavior. The hypothalamus is the brain area most obviously related to estrogen actions on these physiological events. Electrophysiologically, it was demonstrated that estrogen exerts different actions between the anterior part containing the preoptic area and the posterior part containing ventromedial hypothalamus. Total RNA was prepared from the anterior and the posterior parts of the hypothalamus of 7 week-old ovariectomized rats after the treatment with estrogen or without the treatment. To evaluate the profile of estrogen responsiveness of genes, total RNA was subjected to cDNA microarray analysis. Based on the results of microarray and quantitative real-time PCR analyses, we selected four neuronal system-related genes, acetylcholine receptor α 4, GABA-A receptor δ , serotonin receptor 6 and GABA transporter 2. In the previous meeting, we presented the estrogen-mediated transcriptional regulation of the four selected genes. The examination of the regulation of these genes at the translational level is now in process by Western-blotting and immunohistochemistry. Recently, we obtained results that the expression of the acetylcholine receptor α 4 protein differed depending on the area of the hypothalamus and the time of exposure to estrogen. We present here the recent results and discuss the molecular bases for the site- and time-specific regulation of neuronal system-related genes in the hypothalamus by estrogen.

P418 (1P2-126)

Expression profiling of estrogen responsive genes for the sexual differentiation of the brain

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In the hypothalamus, there are two sexually dimorphic nuclei, the anteroventral periventricular nucleus (AVPV) and the sexually dimorphic nucleus of the preoptic area (SDN-POA), which have been associated with sex-specific regulation of reproductive neuroendocrinology and behavior. The AVPV is sexually dimorphic with over three times as many dopaminergic neurons in the female rat compared with male. On the other hand, the volume of the SDN-POA is several times smaller in female than in male. Estrogen, derived from circulating testosterone from pup testis, masculinizes the developing AVPV and SDN. However, molecular mechanisms of estrogen signaling in the sexual differentiation of the brain have not been clarified hitherto. In this report, using a customized DNA microarray with selected estrogen-responsive genes (172 genes), we attempted to show the gene cascades to establish the sexual dimorphism in the AVPV and SDN of rat brain. Several genes of the postnatal day 5 (PD5; the day of birth: PD1) female brain were up- or down- regulated, significantly, by masculinization with estrogen treatment on PD1. Six of these genes showed the same expression pattern between the AVPV and SDN. We are currently investigating estrogen-responsive gene expressions in the PD1 - PD4 female brains to profile estrogen function. The data would contribute to clarify the molecular mechanisms of estrogen signaling in the sexual differentiation of the brain.

P419 (1P2-127)

Estrogen augments BK Currents in GnRH Neuronal Cell Line GT1-7.

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Gonadotropin-releasing hormone (GnRH) neurons in the hypothalamus constitute the final common path for the neuroendocrine control of reproduction. The activity of GnRH neurons is modulated by cyclic fluctuations in circulating concentration of estrogen. However, the nature of estrogen action upon these cells has not been clarified. We report here a direct action of estrogen on the regulation of potassium current, which is closely related to the neuronal excitability, in GnRH neuronal cell line, GT1-7. Delayed rectifier potassium current (I_K) and calcium-activated voltage-gated potassium (BK) current were recorded by perforated patch clamp configuration in GT1-7 cells cultured in DMEM with 10% fetal bovine serum for 3 days. BK current was increased by addition of 17β-estradiol (E2) in culture medium in a concentration-dependent manner. This action of E2 was completely blocked by ICI-182,780, a potent estrogen receptor antagonist. Acute application of E2 had no effect on the BK current. We further examined the calcium currents whether the effect by E2 on BK currents was mediated through augmentation of calcium currents. E2 had no effect on the calcium currents in GT1-7 cells. E2 exerted no effect on I_K. These results indicate that E2 increases the BK current by activating estrogen receptor without affecting calcium currents.

P420 (1P2-128)

Genome-wide analysis of the genes differentially expressed in proliferation-inhibited lactotroph by the anti-mitogenic action of estrogen

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Estrogen stimulates cell proliferation in anterior pituitary gland, which is one of the typical estrogen-responsive tissues. Recently, we have found that 17-β estradiol (E2) had estrogen receptor-mediated mitogenic action as well as anti-mitogenic (in presence of IGF-1 or insulin) action on rat lactotroph primary cell culture. However, at present, the cellular and molecular mechanisms underlying the IGF-1- or insulin-dependent anti-mitogenic action of estrogen upon lactotroph remain unknown. In this study, primary cell culture of the anterior pituitary cells was treated for 4 hours with IGF-1 (50 ng/ml) alone or with IGF-1 and E2 (1 nM) in combination. 1 nM E2 was chosen because anti-mitogenic action is typically demonstrated by such concentration of E2. After the treatment, total RNA was isolated from the sample and used for genome-wide gene expression analysis with CodeLink™ Rat Whole Genome Bioarrays (33,849 gene probes, Amersham Biosciences). Our data analysis revealed that 0.49% (165/33,849) of the gene probes were down-regulated and 0.38% (129/33,849) were up-regulated by E2 in presence of IGF-1. These affected genes turned out to be cell cycle-related genes, growth factors, and signal transducers. Expression analysis by quantitative RT-PCR validated the results obtained by CodeLink Bioarray analysis, and thus our data strongly implied some involvement of the E2-affected genes in the IGF-1-dependent anti-mitogenic action of estrogen on the lactotroph primary cell culture.

P421 (1P2-129)

Estrogen enhances the stress response via amygdala pathway in female rats

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It is well known that the limbic system and the hypothalamo-pituitary-adrenocortical (HPA) axis is activated in response to stressful stimuli in rats. In addition, estrogen enhances the stress responses in the female rat, but it remains unknown how estrogen affects stress responses. In the present study, we examined the possible pathway of estrogen affecting stress reponses. Adult female rats of Wistar-imamichi strain were ovariectomized 2 weeks prior to the experiment. The stainless steel tube containing cholesterol (Chol) or 17β-estradiol (E_2) (Chol : E_2 = 100 : 1 by weight) was stereotaxically implanted in the paraventricular nucleus (PVN) or the amygdala. Three days after the implantation, blood samples (300 μl) were taken every 1 h through an indwelling cardiac catheter from 1100 h to 1600 h to monitor the serum corticosterone (CS) concentration as a marker of HPA axis activity, and restraint stress was applied from 1200 h to 1300 h. The serum CS concentration was assayed by a radioimmunoassay. The serum CS concentration increased at the onset of restraint, and gradually decreased after the release from the restraint in all experimental groups. E_2 implantation in the amygdala caused a significant enhancement of the CS release response during the restaint, comparing Chol. On the other hand, no significant difference was observed between the two groups implanted with E_2 or Chol in the PVN. The implantation did not affect the vaginal smear and the serum E_2 concentration. These results suggest that amygdala is the possible pathway of E_2 enhancing stress responses.

P422 (1P2-130)

Involvement of central prolactin-releasing peptide on stress-induced activation of hypothalamo-pituitary-adrenal axis in rats

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To confirm a role of prolactin-releasing peptide (PrRP) on stress response, we examined the effects of restraint stress (RTS), nociceptive stimulus and acute inflammatory stress in rats on the expression of the PrRP gene in the hypothalamus and medulla oblongata using *in situ* hybridization histochemistry. Moreover, we examined the effects of pretreatment with indomethacin on acute inflammation-induced PrRP gene expression and pretreatment with an anti-PrRP antibody on nociceptive stimulus-induced c-*fos* gene expression in the hypothalamic paraventricular nucleus (PVN). RTS, nociceptive stimulus and acute inflammatory stress upregulated the PrRP gene expression in the medulla oblongata. Acute inflammation-induced increase in the PrRP gene expression was significantly attenuated almost completely by prereatment with indomethacin. Pretreatment with anti-PrRP antibody attenuated significantly nociceptive stimulus-induced c-*fos* gene expression in the PVN. RTS, nociceptive stimulus and acute inflammatory stress activate PrRP neurons. Especially, activation of PrRP neurons by acute inflammation was mediated mainly by prostaglandins. Pretreatment with anti-PrRP antibody attenuated stress response via neurons in the PVN. These results suggested that PrRP is a potent and important mediator of stress response in the hypothalamic PVN in rats.

P423 (1P2-131)

Adenovirus-mediated conditional luciferase reporter assay system for detection of the transcriptional activation of estrogen response element (ERE) in pituitary lactotroph cells

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Activated estrogen receptor binds to specific genomic DNA termed estrogen response element (ERE) and enhances the transcription of target genes. There are numerous studies to examine ERE transcriptional activation in cell lines or tumor cells but little is known about primary culture cells especially in the pituitary. To determine the direct transcriptional activity of ERE in primary culture cells, we established the adenovirus-mediated reporter assay system for ERE transcriptional activation in lactotroph cells. 2×ERE, which was originally constructed and the most responsive for estrogenic induction among 1× to 4×ERE, was used for reporter DNA construct. Because the pituitary cell population consists of several cell types including estrogen-responsive gonadotroph cells, Cre-loxP system was used to localize the luciferase gene expression in lactotroph cells. Cre recombinase gene with nuclear localization signal was expressed under the control of prolactin promoter and the luciferase reporter gene was flanked by loxP sites. Double-fluorescence immunocytochemistry revealed that both Cre recombinase and luciferase proteins were expressed specifically in lactotroph cells when pituitary cells in primary culture were infected with two adenovirus vectors carrying these proteins. Treatment with 1 nM estradiol increased the ERE activity about 9-fold in the presence of dextran/charcoal-treated serum. This system will be useful to detect the ERE transcriptional activity in primary lactotroph cells.

P424 (1P2-132)

Novel mechanism of SXR action on ER-mediated transcription in breast cancer cells.

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Estrogen receptor (ER) is a key regulator of proliferation and differentiation in breast cancer cells. On the other hand, steroid and xenobiotic receptor (SXR) regulates the expression of the cytochrome P-450 3A (CYP3A) gene family. It has been reported that SXR is expressed in breast cancer cells. To study whether SXR affects on estrogen action, we performed transient transfection-based reporter assays. SXR potentiated ER-mediated transcription in the presence of estradiol in MCF-7 breast cancer cells. To study the mechanism of SXR potentiation on ER-mediated transcription, we performed GST pull down, mammalian two hybrid, and electropholetic mobility shift assays. We showed that (i) SXR did not bind with ER, (ii) SXR did not bind to ERE. Thus, we focused on the effect of SXR on the binding between ER and corepressors. In reporter assays, silencing mediator for retinoid and thyroid receptors (SMRT) reversed the potentiation of ER activity by SXR. The SMRT was dissociated from ER by SXR in a receptor concentration-dependent manner. These results suggest that SXR induced ER-mediated transcription by squelching limiting amounts of SMRT. In conclusion, our results indicated that SXR induces ER signaling, which may play crucial role for cell growth, cell differentiation in breast cancer cells.

P425 (1P2-133)

Effects of PCBs on nuclear receptors-mediated transcription in breast cancer cells

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Polychlorinated biphenyls (PCBs) are environmental chemicals that may cause a series of abnormal effects. Due to its stability and lipophilicity, they are accumulated in liver and/or adipose tissues. It has been speculated that PCB may associate with the generation and development of breast cancer. Thus, we focused on the effect of PCBs on estrogen rceptor (ER)- or progesterone receptor (PR)-mediated transcription using transient transfection-based reporter assays. In CV-1 cells, hydroxylated (OH)-PCB activated the estradiol (E2)-induced ER-mediated transcription in a dose dependent manner at concentration from 10^{-12} to 10^{-9} M. However, the transcriptional activity is suppressed at higher concentrations. The magnitude of induction was about 2.5 fold compare to the level without PCB. On the other hand, no significant difference was observed when PR was cotransfected in the presence of OH-PCB. Since steroid and xenobiotic receptor (SXR) potentiated the ER-mediated transcription (Rokutanda et al, personal communication), we also investigated the effect of PCBs on SXR-induced ER-mediated transcription. OH-PCB further activated the transcription in both CV-1 and MCF-7 breast cancer derived cells. These results indicate that PCBs potentiated the ER-mediated transcription in breast cancer cells. In conclusion, PCB may associate with the production and development of breast cancer.

P426 (2P3-127)

Alteration in external Na^+ concentration affects the movement of mouse sperm flagella

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The present work was carried out to examine the "Escape from High Na^+ Hypothesis" presented by S. Yoshida for mitochondria, i.e., α-probacteria (the ancestor of mitochondrion with flagella) were so susceptible to high Na^+ concentration that they escaped into eukaryotic cells and transformed into mitochondria (symbiosis). The movement of sperm, comparable to α-probacteria, was estimated using a phase-contrast microscope and a high-speed video camera designed by T. Etoh; the beat frequency was monitored at 250 - 500 frames/s. Bathing media always contained glucose, since it is essential for sperm motility. When $[Na^+]_o$ was raised from normal (140 mM) to 190 mM or to 240 mM, beat frequency of the sperm gradually decreased with time. This effect cannot be ascribed to elevated osmolarity, because adding mannitol to the medium did not introduce any significant changes to sperm motility. In addition, a Na^+ ionophore (monensin) suppressed sperm movement when it was added to the normal 140 mM Na^+ solution. In order to observe changes in $[Na^+]_i$ in response to $[Na^+]_o$ alteration, Na^+ dynamics was monitored with a Na^+ indicator, SBFI, using ARGUS-50 (Hamamatsu Photonics). The fluorescence image analysis showed that $[Na^+]_i$ increased in the midpiece but not in the head of the sperm when $[Na^+]_o$ was raised or when monensin was added to the normal solution. In summary, an increase in $[Na^+]_o$ generates $[Na^+]_i$ elevation in the mouse sperm, especially in the flagellar portion of the mouse sperm, and it causes a slowdown in sperm activity.

POSTERS
Reproductive physiology

P427 (2P3-128)

CENTRAL ACTION OF PROLACTIN ON THE INDUCTION OF REPRODUCTIVE BEHAVIOR IN THE MALE NEWT

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In breeding season, the sexually mature male newt, *Cynops pyrrhogaster*, vibrates the tail in front of the female at an early stage of courtship. The effects of intracerebroventricular (ICV) and intraperitoneal (IP) injections of ovine prolactin (PRL), antiserum against newt PRL, and antibody against the newt PRL receptor on the expression of the tail-vibrating behavior of male newts were studied to see whether PRL acts centrally or peripherally to induce the behavior. Both of ICV and IP injections of PRL to gonadotoropin-primed males enhanced the expression of the behavior dose-dependently. The minimum effective dose of PRL was 0.1 μg for ICV injection, whereas it was 100 μg for IP injection. The minimum effective amount of the antiserum was 0.05 μl for ICV injection, whereas it was 20 μl for IP injection. Neither ICV nor IP injection of preimmune rabbit serum affected the expression of the behavior. Furthermore, ICV, but not IP, administration of 0.3 μg of anti-newt PRL receptor antibody blocked the spontaneously occurring courtship behavior in sexually developed males. Neither ICV nor IP injection of the same amount of normal rabbit IgG affected the expression of the behavior. These results strongly suggest that endogenous PRL enhances the behavior by acting centrally through the PRL receptors localized in the brain area.

P428 (2P3-129)

Sex difference in the activity of melanin-concentrating hormone neurons in the lateral hypothalamic area off rats under free feeding condition

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We have shown that there are sex differences in the response of melanin-concentrating hormone (MCH) neurons to glucose using phosphorylated cyclic AMP response element-binding protein (pCREB) as a marker of neural activity. That is, glucose injection in 48 h-fasted rats decreased the number of MCH neurons expressing pCREB more promptly in females than in males. We therefore suggest that MCH neurons play a role in sex differences in feeding behavior. In the present study, we examined changes in the activity of MCH neurons under normal (free) feeding condition. Male and female rats were killed at various time point and preparations were subjected to immunohistochemical processing for the double staining of MCH and pCREB. Approximately 10% of MCH neurons expressed pCREB between meals irrespective of sex. Next, we found that approximately 40% of MCH neurons expressed pCREB 10 sec after meal initiation irrespective of sex. Five min after meal termination, on the other hand, the number of MCH neurons expressing pCREB was significantly decreased only in females but not in males. No changes in the activity of orexin, cocaine and amphetamine-related transcript, and neuropeptide Y neurons were observed. The results further suggest MCH neurons play an important role not only in sex differences in feeding behavior but also in controlling feeding behavior per se.

POSTERS
Development, growth & aging

P429 (2P3-130)

Cloning and Characterization of *Xenopus* p26, an S100-like Calcium-binding protein in *Xenopus* Egg

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The early egg fertilization process requires a change in the intracellular calcium concentration. To understand the calcium-dependent molecular mechanisms of fertilization of eggs, we isolated a 26 kDa Ca^{2+}-binding protein from *Xenopus* eggs, a homologue of *Rana Catesbeiana* p26 (renamed from p26olf) isolated from the olfactory epithelium. The primary structure of *Xenopus* p26 shows comparatively high amino acid identity (61%) to *Rana* p26, and consists of two S100-like regions aligned in tandem as seen in *Rana* p26. By ^{45}Ca blot analysis and flow dialysis experiments using ^{45}Ca, p26 was found to bind ~4 Ca^{2+} with an apparent Kd value of ~9.5 μM. Genomic Southern analysis implicated that *Xenopus* p26 is a unique orthologue of *Rana*. Northern blot analysis showed that *Xenopus* p26 is expressed in *Xenopus* eggs and also in other tissues. Immunohistochemical study revealed that *Xenopus* p26 is localized prominently in the cytoplasm of the cortex of both the animal and the vegetal hemisphere of *Xenopus* eggs. Blot overlay analysis showed that several egg proteins bind to *Xenopus* p26 in a Ca^{2+}-dependent manner, indicating the presence of target proteins of *Xenopus* p26. These results indicated that *Xenopus* p26 is a novel S100-like protein in eggs and could be involved in the early Ca^{2+}-dependent event during (or after) fertilization.

P430 (2P3-131)

Differences in the appetite-stimulating effect of orexin, NPY, ghrelin among young, adult, and old rats.

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Aging is associated with a progressive decrease in appetite and food intake. Orexin-A, NPY (neuropeptide Y), and ghrelin, which are appetite-stimulating peptides, are known to play a critical role in food intake. In this study, the stimulatory effect of intracerebroventricular administration of these peptides on food intake was compared among young (4-month-old), adult (11-month-old) and old (24-27-month-old) male Wistar rats. A stainless steel cannula was implanted stereotactically into the left lateral ventricle. After a 7-day recovery period, different doses of orexin-A (0 to 3 nmol), NPY and ghrelin (0 to 1 nmol) were injected into the left lateral ventricle without anesthesia. Food consumption was measured at 1, 2, and 4 hr after injection. We also examined the plasma levels of acylated and desacyl ghrelin in young and old rats by ELISA. Intracerebroventricular administration of orexin-A and NPY stimulated food intake in young and adult rats, but, no effects were observed at any dose in old rats. Ghrelin increased food intake in a dose-dependent manner in all groups and the effect of ghrelin was reduced with advancing age. Either acylated or desacyl plasma ghrelin level did not differ between young and old rats significantly. In conclusion, the result that the orexigenic effect of these peptides, orexin-A, NPY and ghrelin were diminished in old rats could be responsible for the age-related decrease in food intake.

P431 (2P3-132)

Age-related changes in the coeruleospinal antinociception system in rats

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The nucleus locus coeruleus (LC) and the nucleus subcoeruleus (SC) have been confirmed to be involved in descending modulation of nociceptive transmission at the spinal cord dorsal horn. We examined the age-related difference in modulating nociceptive processing at the spinal dorsal horn. Four age groups (8, 20, 50 and 70 months old) of male SD rats were used. Each group of rats was divided into the following two subgroups: LC/SC-lesioned (DC, 1 mA, 20 s) and LC/SC-intact rats. In behavioral experiments, following hindpaw inflammation (carrageenan, 2 mg, 0.1 ml), there was a significant difference in the development of hyperalgesia between the LC/SC-lesioned and the LC/SC-intact rats in young age (8 and 20 months old) but not in old age groups (50 and 70 months old). In dorsal horn neuronal activity, following hindpaw inflammation, a significant difference in both spontaneous activity and heat-evoked response was observed between the LC/SC-lesioned and the LC/SC-intact rats in young age, but not in old age. Electrical stimulation of the LC/SC significantly inhibited heat-evoked responses of dorsal horn neurons in young age, while the inhibitory effect of LC/SC stimulation was not observed when applied in old age rats. The microinjection of S-glutamate (50 nmol, 0.5 μl) into the LC/SC produced an inhibition of heat-evoked responses of dorsal horn neurons in young age but not in old age rats. These results suggest that there is an age-related difference in the function of the coeruleospinal antinociception system.

P432 (2P3-133)

The Drosophila DC0 Mutation suppresses age-related memory impairment without affecting lifespan

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Age-related memory impairment (AMI) is one of the most significant phenotype of brain aging and experienced by elderly people even without showing symptoms of age-related neuronal disease such as Alzheimer disease (AD). Identification of genes underlying AMI could light on the molecular mechanisms of AMI and gives clues for therapeutic strategy. However, behavioral genetic for AMI has not been much carried out because of the long lifespan of animal models. Previously, we reported that memory mutant amnesiac (amn) does not show further memory decay upon aging. Since amn encodes neural peptide supposed to control adenylyl cyclase (AC) in mushroom bodies (MBs), neuronal center for memory, we screened mutants of the genes suspected or reported to express in MBs to isolate AMI mutants. Here, we found that heterozygous DC0-PKA mutants (DC0/+) sustain robust young normal memory into old age with no effect on lifespan and its expression level upon aging. Moreover, we found that long-lived mutant does show normal AMI. These findings suggest that DC0-PKA specifically regulate AMI, and extension of longevity is neither essential nor sufficient for suppression of AMI.

P433 (2P3-134)

Changes of Alzheimers disease- and neural stem cell growth-related genes by aging

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Alzheimers disease (AD), the most common form of dementia in the elderly population, is characterized by an insidious onset with memory impairment and an inexorable progression of cognitive decline. Amyloid β peptide (Aβ) is a product of sequential cleaving of amyloid precursor protein (APP) by two proteolytic enzymes, β- and γ-secretases, which cleave the APP at the N and C terminus of Aβ, respectively. The age-related activities of these secretase may induce changes in turnover of Aβ and elevate the risk of AD. From a therapeutic point of view, there is increasing interest in using β- or γ-secretase inhibitor to treat AD. In addition, neurotrophin signaling is a critical mechanism involved in synaptic plasticity, learning and memory and neuronal health. Neurotrophins can also regulate proliferation and differentiation of neural stem cells. Thus, changes in neurotrophin signaling may also be involved in age-related memory impairment and induction of AD. We examined age-related changes of mRNA expressions in these proteins. The hippocampus and cerebral cortex were dissected from young (4 weeks old), adult (50 weeks old) and aged (95 weeks old) rats and the expressions of APP, β-site APP-cleaving enzyme (BACE), presenilin 1 (γ-secretase) and neprilysin mRNA for AD-related genes and neurotrophin receptor mRNA for neural stem cell growth-related genes in these tissues were measured by Real-Time PCR.

P434 (2P3-135)

Proteins involved in survival and growth of human neuroblastoma cells, GOTO

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Neuroblastoma is one of the most common pediatric solid tumors, and is quite conspicuous. Therefore, the therapy of neuroblastma is a critical problem for health for children. Generally, cancer cells essentially require more nutrients including serum to growth as compared to normal cell. To investigate what kind of molecular event occurs in neuroblastoma cells in response to low serum stimulation could be very useful for understanding the cancer cell and the therapy. Therefore, we have performed proteomic analysis of neuroblastoma cells, GOTO in response to from 10% to 1% serum. The cells were analyzed for differential expression by fluorescence differential two-dimensional electrophoresis (DIGE) and liquid chromatography-Tandem Mass Spectrometry (LC-MS/MS). MTT assay and trypan blue exclusion assay were also performed to check growth and viability of the cells. As a result, six proteins were identified as cell signaling regulating proteins, apoptosis-related proteins or cellular stress-related proteins. The cells simulated showed decreased viability and growth at the day 1 after stimulation, but recovery of them at second day and later. Taken together, we here describe novel six proteins, which may be involved in survival and growth in neuroblastoma cells, GOTO.

P435 (2P3-136)

X-ray Diffraction and Biochemical Analyses of Developing Rat Lens

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Objective: The short-range order of crystallins has been reported to be necessary for lens transparency. Developmentally, lens fiber cells continue differentiating and the rate of protein synthesis changes dramatically. Therefore, we investigated the time course of X-ray diffraction patterns and protein concentrations in developing rat lens to reach a better understanding of the required conditions to keep transparency even with high protein concentration. Methods: We obtained X-ray diffraction patterns from excised whole rat lens and measured protein concentrations at postnatal day 5 (P5), P10, and P15. The X-ray experiments were performed at 37°C. The X-ray beam (wave length: 0.1 nm) passed lens-center in a direction parallel to optic axis. Protein concentrations of lenses were measured by Bicinchonianate method, using bovine serum albumin as standard. Lens volume was obtained from weight and specific gravity. Results and Discussion: Although scattering patterns of X-ray were observed at P5 and P10, the diffraction pattern of broad ring with a characteristic spacing of around 15 nm appeared at P15. Total protein concentration of the lens fiber cell cytoplasm significantly increased especially from P10 to P15 (274.4 ± 39.6, 347.4 ± 41.6, and 569.0 ± 60.7 [mg/ml lens volume] at P5, P10, and P15, respectively, mean ± SD, each group: n = 5). Conclusion: Short-range order of crystalline developed with a marked increase of protein concentration of the lens fiber cell cytoplasm in short time.

P436 (2P3-137)

A transcriptional repressor, RP58, is essential for migration and differentiation of cortical neurons

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Neuronal positioning is thought to be crucial for cerebral development, but little is known about the underlying regulatory mechanisms. We generated mice lacking the RP58 gene encoding a transcriptional repressor. In vivo labeling with BrdU revealed that presumptive subplate neurons are diffusely distributed in all cortical layers and that neuronal migration represents a reeler-like outside-in pattern in the mutant mice. In this study, we performed molecular marke analysis using Tbr-1, ER81 and mSorLA probes. In the RP58 deficient mice, ER81 positive layer V neurons, which were normally laminated above Tbr-1 positive layer VI neurons, were found beneath Tbr-1 positive cells. These results confirm the outside-in pattern of the mutant cortex. The mSorLA positive layer II-III neurons were not detected in the mutant mice. The result suggests that RP58 is essential for the differentiation of layer II-III neurons.

P437 (2P3-138)

Completion of neuronal migration regulated by loss of Ca^{2+} transients

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The migration of immature neurons constitutes one of the major processes by which the central nervous system takes shape. Completing the migration at the final destination requires the loss of cell body motility, but little is known about the signaling mechanisms underlying this process. Here we show that a loss of transient Ca^{2+} elevations triggers the completion of cerebellar granule cell migration. Simultaneous observation of the intracellular Ca^{2+} levels and cell movement in cerebellar slices of the early postnatal mice revealed that granule cells exhibit distinct frequencies of the transient Ca^{2+} elevations as they migrate in different cortical layers, and complete the migration only after the loss of Ca^{2+} elevations. The reduction of the Ca^{2+} elevation frequency by decreasing Ca^{2+} influx, or by inhibiting the activity of PLC, PKC, or Ca^{2+}/calmodulin, halted the granule cell movement prematurely. In contrast, increasing the Ca^{2+} elevation frequency by increasing Ca^{2+} release from internal stores, or by elevating intracellular cAMP levels, significantly delayed the completion of granule cell migration. The timing of the loss of Ca^{2+} elevations was intrinsically set in the granule cells and influenced by external cues. These results suggest that Ca^{2+} signaling, dictated by multiple signaling systems, functions as a mediator for completing the migration of immature neurons.

P438 (2P3-139)

Cell Prolifration After Domoic Acid Induced Neuronal Cell Death

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Domoic acid (DA) is the structural analogs of Kainic acid (KA) belongs to a group of amino acid analogues called excitotoxins. DA is shown to be about 10 times more toxic than KA. In rodents, DA produces extensive neuronal damage in the hippocampal pyramidal neurons and behavioral effects ranging from inactivity to seizures and distinguishable by its unique ability to elicit a scratching response. In the case of intraperitoneal DA administration, neuronal damage was observed in a wide range. In this study we examined the neuronal damage after intraperitoneal DA administration and cell proliferation in the adult rat whole brain. The most extensive neuronal cell damage was observed in CA3 subfield as evaluated by HE staining. While many TUNEL positive cells were observed in the granular cell layer of cerebellum. The process of neuronal cell death was made up mingled necrosis and apoptosis. TUNEL histochemistry revealed a time dependent sequential apoptotic cell death after DA administration. During the first 2 days postinjection, apoptosis in the cerebellum was only evident in the brain. At 3 days, the entire hippocampus and cortex become apoptotic. The distribution of the BrdU positive cells were most abundant in the dentate gyrus and not correlated to the degree of the neuronal damage.

P439 (2P3-140)

Influence of extracellular GABA and taurine to $GABA_A$ receptor-mediated actions in radially migrating cortical plate cells.

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It is known that role of $GABA_A$-R mediated actions is important for early CNS development. The radially migrating cells derived from the ventricular zone (VZ) may affected by the actions. GABA content in the brain of GAD67-GFP knock-in mouse decrease compared with the wild type mice. Therefore, in this study, we investigate the influence of the circumferential GABA concentration to radially migrating cells by using GAD67-GFP knock-in mice. Furthermore, as it was known that $GABA_A$-R is affected by taurine, the influence of taurine to radially migrating cells was also investigated.The radially migrating cells were labeled by means of in utero electroporation at E14. Three days after the electroporation, labeled cells located in CP, intermediate zone, and VZ were counted. There was no significant difference in distribution among genotypes of GAD67-GFP knock-in mice. $GABA_A$-R mediated currents were recorded by whole-cell recording from labeled cells. Evoked GABA current had dose-dependent manner and had no differences among genotypes. These results suggest that the cells generated in the VZ possess rather equivalent $GABA_A$-Rs and migrate radially independent of circumferential GABA concentration. Therefore, we have examined the influence of circumferential taurine to $GABA_A$-R mediate actions *in vivo* by using taurine metabolism blocker, D-cystein sulfinate.

P440 (2P3-141)

The role of galectin-1 in the fish retina during optic nerve regeneration

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Galectins constitute a family of lectins that bind to β-galactoside and are present in a wide range of animal species. Although galectin-1 has been recently shown to promote axonal elongation in the PNS, it is unclear whether galectin-1 implicates in the CNS regeneration. Unlike the mammals, the fish optic nerve can regenerate their axons after transection, so we studied effects of galectin-1 on axonal regeneration after fish optic nerve transection. We isolated a cDNA fragment for goldfish galectin-1 with probes of PCR products amplified by a primer constructed from zebrafish sequence. We got a band of 396bp from goldfish retina. Since nucleotide sequence of this band was 98% homology to that of zebrafish galectin-1, we detected the transcripts of galectin-1 in the goldfish retina. Using this cDNA probe, *in situ* hybridization was performed in the goldfish retina following optic nerve injury. The level of the mRNA increased at 3days, and peaked at 10-20days, and then returned to the control level by 30days after nerve injury. The increase of the mRNA was located strongly in the ganglion cell layer, and weakly in the inner nuclear layer and outer nuclear layer. The levels of galectin-1 protein showed a similar expression pattern to that of the mRNA. The period of increased levels of galectin-1 mRNA and protein correlated well to the period when ganglion cells start to regrow their axons after lesion. So, we are now in progress to evaluate the effect of galectin-1 on neurite outgrowth in retinal explant culture.

P441 (2P3-142)

A comparative study of zebrafish visual system during development and regeneration

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Fish optic nerve can regenerate after nerve transection. In this study, to evaluate the regeneration of visual function after nerve injury, we quantified the optokinetic and chasing behaviors of moving zebrafish with optic nerve transection by a computer image processing system. In an optokinetic movement, zebrafish can follow the rolling drum with black-and-white vertical stripes. In a chasing movement, one fish can chase the other. The optic nerve injury induced a complete loss of their movements in zebrafish. The recovery of optokinetic behavior in adult zebrafish was 25 days after nerve injury, whereas the recovery of chasing behavior in adult two zebrafish was 80-100 days after nerve injury. In the development, young zebrafish can gain the optokinetic and chasing behavior by 15 days and 60 days after hatching, respectively. Furthermore, we cloned some interesting genes upregulated in the retina during optic nerve regeneration. Purpurin and GAP-43 were very different in their expression pattern of the upregulated period. The expression of purpurin was very transient for less than 10 days, whereas that of GAP-43 was sustained for more than 100 days after optic nerve injury. Now, we compare their expression pattern in the developing zebrafish retina. Such a comparative study of molecular and behavioral phenotypes during development and regeneration might be useful for understanding molecular structure of zebrafish visual system.

POSTERS
Nutrition, energy metabolism & body temperature

P442 (1P2-134)

Behavioral study of sodium preference in zinc deficient rats

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Our previous study demonstrated that zinc deficient (ZnX) rats fed zinc deficient diet during 4 weeks enhanced sodium preference (Futani *et al*., 2005). In the present study, we conducted behavioral experiment to investigate whether feeding period of zinc deficient diet affect this sodium preference or not. As the zinc deficient animals, male Wistar rats fed the zinc deficient diet during 1 week after the weaning were used. Results were as follows; (1) In the long-term (48 h) two-bottle preference test, the preference percents for 0.1 and 0.3M NaCl in some ZnX rats were higher than those in the control rats. (2) In the short-term (10 min) test, there was no significant difference in the preference percents between ZnX and control rats. These results suggest that 1 week is enough period for some rats to occur high sodium preference and taste may not play a role for enhancement of this sodium preference.

P443 (1P2-135)

Dietary zinc deficiency increases the number of peripheral granulocytes in rats

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Zinc is known to be an essential trace element for all organisms. In human subjects body growth and development is reported to be strictly dependent on zinc. The nervous, reproductive and immune systems are also particularly influenced by zinc deficiency. However, the effects of zinc deficiency on the number of immuno-responsible cells have not yet been fully elucidated. Therefore, we studied the effects of zinc deficiency on the number of white blood cells in rats. Four weeks old male Sprague Dawley rats were used, and randomly divided into the control diet group (CON: Zn=53.5mg/kg diet) and zinc deficient diet group (ZDD: Zn=1.9mg/kg diet) . Both group rats were fed in each diet for 26 days. During the experimental period, the numbers of white blood cells, neutrophils, eosinophils, basophils, lymphocytes and monocytes were analyzed with a flow-cytometer. The number of neutrophils in the ZDD in 18th to 26th days was significantly higher than those of the CON group. The numbers of eosinophils and basophils of the ZDD in 14th day were 2.0 and 5.2 times significantly higher, respectively, than those of the CON. The numbers of lymphocytes in the ZDD in 18th to 26th days were significantly lower than those of the CON. However, The numbers of total white blood cells and monocytes were not changed in both groups. These results suggest that zinc deficiency increases the number of natural immunity cells and decreases the number of acquired immunity cells in rats.

P444 (1P2-136)

Zinc deficiency induced changes of the numbers, volume and distribution width of red blood cells in rats

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Zinc has been shown to regulate the growth, acid-base equilibrium and oxygen transport functions. Zinc is also known to play an important role in red blood cell formation, which is related to the physiochemical properties of red blood cells (RBC), hematopoietic differentiation factors and hematopoietic hormones. However, the effects of zinc deficiency on the number and volume of RBC and hemoglobin levels are still unknown. Therefore, we examined zinc deficiency-induced changes of the number and volume of RBC and hemoglobin concentration in rats. Four weeks old male Sprague Dawley rats were divided into zinc deficient diet group (ZDD: zinc=1.9mg/kg diet) and the control group (CON: zinc=53.5mg/kg diet), and both group rats were fed for 26 days. During the experimental period, the number and volume of RBC and hemoglobin concentration in rats were analyzed by a flow-cytometer. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) in the ZDD were significantly lower than those in the CON. The number of RBC and mean corpuscular hemoglobin concentration (MCHC) in the ZDD were significantly higher than those in the CON. The peak of distribution of RBC in the ZDD was shifted to small size and the distribution width was significantly reduced, as compared with those in the CON. These results suggest that zinc deficiency in rats clearly induces the increase of RBC numbers, and the reduction and homogeneity in the volume of RBC.

P445 (1P2-137)

Zinc deficiency induced change of rat liver cytosolic alcohol dehydrogenase activity and the recovery effect

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Zinc is an essential cofactor for many enzymes, including those that regulate the metabolism of vitamin A and ethanol in the liver. Alcohol dehydrogenase (ADH) is also a metalloenzyme in which zinc acts as a prosthetic group and a stabilizer of the quarternary protein structure. However, the effects of zinc deficiency diet (ZDD) on liver cytosolic ADH activity and the recovery effects from ZDD have not yet been fully elucidated. Therefore, we studied the effect of ZDD and the recovery effects on the liver ADH activity in rats. Four weeks old male Sprague Dawley rats (n=32) were divided into four groups : (a) ZDD (zinc=1.9mg/kg diet) for 26 days, (b) the control group (zinc=53.5mg/kg diet) for 26 days, (c) ZDD diet (zinc=1.9mg/kg diet) for 26 days and then replaced on the zinc supplemented control (zinc=53.5mg/kg diet) for 19 days, and (d) the control group for (c). Liver cytosolic ADH activities were assayed spectrophotometrically at 38°C, and liver cytosolic protein assays were performed by the method of Lowry *et al*. Liver cytosolic specific ADH activities were significantly reduced to about 1/4, by ZDD for 26 days. However, a significant reduction in the activity of liver cytosolic ADH was recovered to the control levels when zinc intake (zinc=53.5mg/kg diet) of the normal levels was supplemented for consequtive 19 days to the ZDD in rats. These results suggest that liver cytosolic ADH activity in rats regulated by the magnitude of zinc intake.

P446 (1P2-138)

Humoral factors involved in strong salt appetite in zinc deficient rats.

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Previous studies have demonstrated that zinc deficient rats drink 0.3M NaCl, avoided by normal animals, in preference to water. We investigated the participation of humoral factors in such salt intakes in zinc deficient rats by analyzing serum concentrations of aldosterone, angiotensin II and sodium. Animals were fed a zinc deficient diet (group 1) or the normal control diet (groups 2 and 3) ad libitum. Group 3 was pair fed with group 1. Serum constituents and preference for NaCl solutions (48-hr two-bottle choice test) were determined at one and four weeks after the onset of feeding. The intake of 0.3M NaCl solution in preference to water occurs in group 1, but not in groups 2 and 3, from one week after the onset of feeding. There was no significant difference in serum concentrations of sodium among all groups throughout experiments. However, estimates of group 1 tended to be lower than those of other two groups four weeks after the onset of feeding. Angiotensin II concentrations decreased significantly in group 1 compared with in two other groups, whereas aldosterone concentrations in group 1 were approximately two times as high as those of two other groups. These results suggest that in zinc deficient animals with low serum concentrations of sodium, aldosterone is upregulated through synthetic pathways different from rennin-angiotensin system. Increased central action of aldosterone may lead to increased sodium appetite.

P447 (1P2-139)

The difference in behavioral responses of neonatal hypoxic-ischemic encephalopathy rat model between left and right carotid artery ligations assessed by a simple swimming direction test

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The neonatal hypoxic-ischemic encephalopathy (HIE) rat model, in which one of carotid arteries ligation followed by hypoxic insult, has been already established. However, differences of effects in the left and right carotid arteries ligations have not been yet clarified by behavioral study. In this study, we examined differences in the effects of left and right carotid arteries ligation on this HIE model by behavioral study. We ligated the left (L-group, n=12) and the right (R-group, n=8) carotid arteries of 7-day-old rats under inspired isoflurane anesthesia, then exposed the rats to 8% oxygen for 15 min at 37 degree, then returned the animals to their mothers. A sham operation group (S-group, n=7) was also prepared. Swimming direction test was performed between 3 and 6 weeks later. Each rat was put in the center of a circular pool (diameter, 150cm; water depth, 15cm). We assessed the rat's swimming direction (clockwise or counterclockwise) when the rat arrived at the pool wall on ten trials per day on different days. The L-group and R-group swam clockwise and counterclockwise respectively, significantly more frequent compared with the S-group. These findings imply that the effects of HIE insult may be easily assessed by a simple swimming direction test.

P448 (1P2-140)

Salivary free radical-scavenging activity is affected by physical and mental activities

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Free radicals/reactive oxygen species (ROS) are related to various disorders including inflammation, aging, and cancer. However, living systems have essential antioxidant mechanisms by which these harmful radicals can be scavenged, i.e., free radical-scavenging activity (FRSA). In the present study, we examined how salivary FRSA is affected by physical and mental activities, which included 1) ingestion of green tea or coffee, 2) a swimming or dancing lesson, 3) watching a comic video or stimulation by lavender or isovaleric acid odors, and 4) smoking. The FRSA was determined by using the DPPH (1,1'-diphenyl-diphenyl-2-picrylhydrazyl) method. In the study on the individual activities, beverage ingestion increased FRSA, whereas exercise decreased it. Watching an amusing video program or stimulation by a pleasant aroma increased FRSA. In contrast, an unpleasant odor had no effect on FRSA. The FRSA decreased immediately after smoking, but thereafter increased after. Thus salivary FRSA was affected by not only physical activities, but also mental activities. The FRSA in saliva may be a parameter for reflecting the health status of individuals.

P449 (1P2-141)

Does atrial natriuretic peptide inhibit LPS-induced activation of microglia ?

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We recently found that arial natriuretic peptide (ANP) inside and outside the blood-brain barrier inhibits the lipopolysaccharide (LPS)-induced fever in rats in vivo. Since ANP inhibits LPS-induced activation of transcription factors and subsequent production of pyrogenic cytokines in macrophages in vitro, it is possible that brain ANP inhibits the activities of the cytokine-producing brain macrophages, microglias, as well. In the present study, we used the primary culture of microglia to investigate whether ANP inhibit LPS-induced microglial activations through its effect on the activation of proinflammatory transcription factors, NF-κB and AP-1. We examined the production of interleukin-1β and nitric oxide and morphological changes of microglia for its activation. We have gotten interesting results, which we will report in the forthcoming "Meeting of the Physiological Society of Japan".

P450 (1P2-142)

Differential Inhibitory Action of Propolis-derived Substances, Cafeic Acid Phenyl Ethyl Ester and Quercetin, on C6 Cell Growth.

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Propolis, one of the oldest medicines, attracts much attention from the medical community, because of its antibiotic and anti-tumor activities. There are accumulating evidences indicating that propolis ingredients such as caffeic acid phenyl ethyl ester (CAPE) and quercetin (QU) have a variety of novel action including neuroprotective action in addition to well-known anti-tumor activities. It is of significance for the propolis study to elucidate molecular mechanisms underlying these novel actions of propolis. Propolis itself, however, cannot be used in in-vitro experiments that utilize cultured tissues because of its poor solubility in water. To circumvent this problem, we have carried out an in-vitro study of anti-tumor action of Brazilian propolis using water-dispersible form of propolis (WDP). Using rat C6 glioma cells, we have demonstrated a dose-dependent anti-tumor action of WDP. The effects of WDP are compared with those of active ingredients of propolis. Both QU and CAPE were found to inhibit cell-growth of C6 cells in serum-supplemented DMEM. However, QU failed to induce C6 cell death after serum-deprivation, while both WDP and CAPE killed C6 cells. Thus, the inhibition of C6 growth induced by WDP could be accounted on the basis of cytotoxic action of CAPE. QU does not kill C6 cells, but inhibits cell proliferation.

P451 (1P2-143)

Effects of estrogen on insulin sensitivity in ovariectomized rats

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Metabolic syndrome is more prevalent in men than in women. However, postmenopausal women became to have its higher incidence. We investigated whether insulin sensitivity is deteriorated in ovariectomized rats, and is improved by chronic estrogen replacement. Female Wistar rats aged 9 weeks were ovariectomized. After 4 weeks, the rats were assigned either to a placebo-treated (P) group (n=8) or a group treated with 17β-estradiol (E2) (n=8), subcutaneously implanted with either placebo- or 17β-estradiol (1.5 mg / 60-day release) pellets. After 4 weeks of estrogen or placebo treatment, the body weight, percent body fat and wet weights of visceral fat were increased in the P compared with the E2 group, while cumulative food intake per body weight was enhanced in the E2 group. During a 1 g/kg intravenous glucose tolerance test, the glucose and insulin responses (incremental areas under the curve) were greater in the P group than the E2 group. Plasma concentration of free fatty acid was marginally lower, and triglyceride was significantly lower in the P group than the E2 group. Plasma levels of leptin and TNF-α were not different between the two groups, but adiponectin was higher in the P group than the E2 group. These results suggest estrogen deficiency increases visceral fat mass, and deteriorates insulin sensitivity.

P452 (1P2-144)

The effect of 5-HT2A/C receptor agonist on food intake, body weight and blood glucose levels in Zucker fatty rats.

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It has been suggested that central serotonin is anorexigenic and this action is mediated, at least in part, by 5-HT2A/C receptors and activation of POMC neurons. This study examined the systemic effects of 5-HT2A/C receptor agonist on food intake, body weight and blood glucose levels in Zucker fatty rats in which leptin receptor is impaired. Fatty rats were intraperitoneally (i.p.) administered with a 5-HT2A/C agonist DOI at 1.0 mg/kg (n=4) or saline (n=4) from 7 to 10 weeks of age, and food intake, body weight and blood glucose levels were measured. Control Lean rats received the same treatment. In long term measurements, daily food intake and weight gain in Fatty rats were significantly decreased in DOI group as compared to control group. In Lean rats, neither food intake nor weight gain was significantly different between DOI and control groups. There were no difference in blood glucose levels between DOI and control groups both in Fatty and Lean rats. In Fatty rats, upon termination of i.p. DOI food intake was temporarily increased, however weight did not change significantly, indicative of increased energy consumption. In short term measurements, food intake was reduced at 1-2.5 hrs after i.p. DOI in Fatty but not Lean rats, and blood glucose level was lowered at 0.5-3 hrs both in Fatty and Lean rats. These results indicate that activation of 5-HT2A/C restricts feeding and ameliorates obesity in Fatty rats and that some of these effects may be produced by restoring the downstream signaling pathway of the leptin receptor impaired in Fatty rats.

P453 (1P2-145)

Expression of exercise induced hsp70 in runner leukocytes

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Heat shock proteins (HSP) are synthesized by cells of all organism resistance to internal and external cellular stresses including physical stress, metabolic stress, and disease. Exercise is a sufficient stimulus to induce and enhance the synthesis of HSP70 and prolonged endurance exercise also causes an inflammatory reaction. Exercise induced a transient elevation in circulating leukocytes, driven largely by a granulocytosis but also influenced by an increase monocytes and lymphocytes. Present study was designed to investigate the expression of HSP70 in human peripheral blood leukocytes after acute moderate intensity exercise in trained runners and untrained subjects. Ten male long distance runners (TR, n=10, 21.3 yrs) and untrained control subjects (UT, n=10, 22.4 yrs) participated in this study. Subjects ran on a treadmill for 1 hr at 70% of heart rate reserve (HRR). Blood were taken immediately before and immediately after exercise, and at 30 minutes after exercise. HSP protein was evaluated by immunoblotting. Baseline HSP70 protein levels in TR was significantly lower than that in UT. Although expression rate of exercise induced HSP70 in both groups were similar, but UT showed significant higher HSP70 levels versus TR after exercise and at 30 minutes after exercise. We conclude that 1 hr treadmill running at 70% HRR intensity (moderate to heavy) is a sufficient stimulus of leukocytosis, neutrocytosis, lymphocytosis, and HSP70 expression in leukocytes. Adaptation to training are observed in TR subjects

P454 (1P2-146)

Effect of fluid ingestion on thermal and cardiovascular responses during recovery after exercise

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We investigated effect of fluid intake on thermal and cardiovascular responses during 1-h recovery after exercise. Six subjects (five untrained males and one female) cycled (at 60%VO_{2max}) for 60 min in hot-humidity conditions to produce dehydration of 1-2.5% body weight. During the recovery at thermoneutral(at 28°C with rh 40%), the subjects underwent two trials of no fluid (C) and ingested water (F) of 500 ml (containing electrolytes and carbohydrates) after the exercise.We continuously measured weight loss as index of insensible perspiration, tympanic (T_{ty}), rectal (T_{re}), and skin (T_{sk}) temperatures, skin blood flow (SkBF), heart rate (HR, included R-R interval), systolic (SBP) and diastolic (DBP) blood pressures, palm sweat rate (SR). Mean arterial pressure (MAP) was calculated as (SBP+2×DBP)/3. Rate-pressure product (RPP) and cutaneous vascular conductance (CVC) calculated HR×SBP and SkBF divided by MAP, respectively. CVC, MAP, SkBF, T_{re} and mean T_{sk} were not significantly different between the tests. Insensible perspiration and R-R interval were significantly increased by fluid intake at the early stage. Palm SR and RPP in the F were significantly lowered at the early stage, and stable trend during the recovery, as compared with the C. T_{ty} also decreased in comparison with the C at the initiation of the recovery. The present results suggest that fluid ingestion is reduce thermal and cardiovascular strains after exercise-induced thermal dehydration, and may be to alleviate the autonomic regulatory responses during rehydration in humans.

P455 (1P2-147)

A new simple apparatus for the study of rodent behavioral thermoregulation using a principle of alternative selection of ambient temperatures

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Recent instruments for the study of thermoregulation in rodents are usually large and often require troublesome trainings of animals. In this study, we designed, made and tested a new simple instrument for investigating rodents' behavioral thermoregulation. The apparatus was composed of two stainless-steel hollow plates (plate A and plate B) with a length of 20cm and width of 5cm. Each plate had an inlet and an outlet that were connected to a separate constant-temperature bath (bath 1 and bath 2). The water temperatures of the baths were controlled at one designated temperature within 10 and 45 degrees and pumped into the plates. A change switch for the water supply was inserted between both the plates and the baths. In the normal switch position, bath 1 supplied water plate A, and bath 2 supplied water plate 2, and in the reverse position, vice versa. Plates A and B were arranged and covered with a surrounding transparent fence 20cm high in a climatic chamber. A rodent stayed inside the fence and moved on plate A or plate B. The position of the rodent was observed by a video camera. We tested thermoregulatory behavior of eight male mice using this instrument. By shuffling the plate temperatures between 10 and 45 degree, the mice moved to the plates with a temperature close to the 35 degree. The findings implied our instrument might be useful as an apparatus for the study of behavioral thermoregulation.

P456 (1P2-148)

Effects of therapeutic hypothermia after hypoxic-ischemic insults in neonatal rats assessed by motor function using rotor rod test

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Effects of therapeutic hypothermia soon after hypoxic-ischemic (HI) insults in infants is now receiving attention in perinatology. Although the neonatal HI rat model is established in the experimental study, effects of therapeutic hypothermia in rats' actual motor activity has rarely been tested. In this study, we investigated the effects of therapeutic hypothermia in motor function of HI neonatal rat model using rotor rod test. We ligated the left common carotid artery in ten seven-day-old rats under inspired isoflurane anesthesia, then exposed the rats to 8% oxygen at 40 degrees for 15 min. Therapeutic hypothermia was induced in 5 of the rats (hypothermia group) immediately after the insult by maintaining the rats' body temperature at 30 degrees for 12 hrs. In the other the rats' body temperature was maintained around 36 degrees (normothermia group). Rotor rod test was performed two months after the HIE insults. On the 1st day of experiments, all rats fell from the rod rotating at 5 rpm within 60 sec. On the 2nd day, all rats in the hypothermia group stayed on the rotating rod for 60 sec, although all rats in the normothermia group again fell within 60 sec. On the 3rd day, all the rats stayed on the rod at 7 rpm for 60 sec. Hypothermia soon after HI insult may improve motor function compared to normothermia.

P457 (1P2-149)

Natriuretic peptide: an another endogenous antipyretrics?

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Natriuretic peptide (NP) such as atrial NP (ANP), brain NP (BNP) or C-type NP (CNP) is a bioactive hormone well known to induce a decrease in blood-pressure, and natriuresis. By contrast, angiotensin II (ANG II), an another bioactive peptide, has the opposite effects such as an increase in blood pressure and a retention of sodium within the body. In other words, NP and ANG II participate in the blood pressure and body fluid regulation through the physiological mechanisms conflicting with each other. Recently, we have found that ANG II and its type 1 receptor are involved in the bacterial endotoxin (lipopolysaccharide; LPS)-induced fever. Since NP reportedly contributes to the inhibition of the inflammation, it is likely that NP has an opposite (inhibitory) effect on the fever to that of ANG II. We examined this possibility and have gotten interesting results, which we will report in the forthcoming "Meeting of the Physiological Society of Japan".

P458 (1P2-150)

Calcium-independent phospholipase A_2 involves in prostaglandin E_2 production in brain endothelial cells during fever

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Prostaglandin E_2 (PGE_2), the brain mediator of fever, is produced in brain endothelial cells through the actions of PGE_2-synthesizing enzymes including cyclooxygenase-2 (COX-2) and microsomal-type PGE synthase (mPGES). However, the type of phospholipase A_2 (PLA_2) working in the upstream of COX-2 is unknown. We have previously shown a possible involvement of calcium-independent PLA_2 ($iPLA_2$) in brain PGE_2 production during lipopolysaccharide (LPS)-induced fever, since systemic pretreatment of rats with an inhibitor of $iPLA_2$, BEL, suppressed fever and brain PGE_2 production. Here, we examined whether BEL directly acts on brain endothelial cells to suppress PGE_2 production using an ex vivo preparation of brain blood vessels. PGE_2 release from subarachnoidal blood vessels, which were isolated from LPS-injected rats and incubated ex vivo, was suppressed by BEL (10μM) added to the incubation medium. Unexpectedly, BEL also suppressed COX-2 expression in endothelial cells of isolated blood vessels. These results indicate that BEL directly acts on brain endothelial cells to suppress PGE_2 production, and suggest that arachidonic acid produced through the BEL-sensitive pathway might not only be a substrate for COX-2 but also be involved in the induction of COX-2. Our findings support the idea that $iPLA_2$ is working in the upstream of COX-2 in brain endothelial cells to produce PGE_2 during LPS-induced fever.

P459 (1P2-151)

Effect of α-lipoic acid on body temperature and fever in pigeons

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The effect of thiol-reductant α-lipoic acid on diurnal temperature (Tc) changes and fever was assessed in pigeons (*Columba livia*) in ambient temperature of 26 ± 1°C, with lights on at 09:00 and off at 21:00. Intravenous (iv) injection of 10 μg/kg *E. coli* lipopolysaccharide (LPS) at 13:00 evoked after latency of 30 min first a variable decrease of Tc, followed by an increase starting 90 min later towards values which were 0.78 ± 0.04°C higher than control between 18:00 and 20:00. Tc decreased in the dark phase parallel to the decline of Tc of afebrile pigeons but with a flatter slope. Iv injections of 12.5, 25.0 and 37.5 mg/kg α-lipoic acid at 16:00 lowered Tc dose-dependently by 0.75 ± 0.09°C, 1.47 ± 0.14°C, and 1.90 ± 0.18°C, respectively, the hypothermic effects lasting 50, 90 or 140 min, respectively. Treatment with the non-competitive glutamate *N*-methyl-D-aspartate (NMDA) receptor antagonist dizocilpine maleate (MK801) blocked the α-lipoic acid-induced hypothermia. Injection of 25 mg/kg α-lipoic acid 3 h after LPS caused a decrease of Tc by 1.05 ± 0.20°C, lasting about 60 min. Tc then returned to level not significantly different from the afebrile state between 18:00 and 20:00. The hypothermic action of α-lipoic acid is assumed to be induced by reduction of vicinal thiol groups of the NMDA receptor, because the hypothermic effect is blocked by NMDA receptor antagonist MK801. The data support the hypothesis that the NMDA receptor is involved in thermoregulation of birds and that augmented oxidation of vicinal thiol groups attached to its ion channel leads to hyperthermia or causes fever.

P460 (1P2-152)

Acidic fibroblast growth factor induces fever via the afferent hepatic and gastric vagus nerves in rats.

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Exogenous aFGF given via the icv route activates the sympathetic outflow innervating interscapular brawn adipose tissue and adrenal medulla. We investigated the effect of exogenous aFGF given via the iv route on body temperature in rats (Wistar strain) under urethane-chloralose anesthesia using thermistor thermometer or in free moving animals using telemetry system. When animals were given aFGF (100 ng/kg) 30 min before the onset of dark period, nocturnal core temperature (Tc) elevated for 9 h after the aFGF challenge. Peak in Tc induced by aFGF was about 1.5 °C (39.6±0.2) higher than that (38.5±0.1) in animals given saline. Records of tail temperature in rats given aFGF showed three phasic thermal responses (two peaks followed by a trough). The aFGF-induced hyperthermia significantly reduced by a resection of the vagal hepatic branch and abolished completely by the combined hepatic vagotomy with bilateral gastric vagotomy. Furthermore, pretreatment with 30 mg/kg methylpredonisone, artificial glucocorticoid, given intraperitoneally significantly attenuated the aFGF-induced hyperthermia. These data suggest 1) that exogenous aFGF given via the iv route induces fever, and 2) that afferent signals from the hepatic and gastric vagus nerves play an important role in the aFGF -induced fever, 3) that aFGF-induced fever might be triggered by glococorticoid-sensitive inflammation processes within gastrointestinal regions.

P461 (1P2-153)

Effects of alcohol on thermoregulation in humans

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We investigated the effects of alcohol on thermoregulatory responses and thermal sensations during cold exposure in humans. Eight healthy men participated in this study. Experiments were conducted twice for each subject at a room temperature of 18 °C. After a 30-min resting period, the subject drank either 15% alcohol (alcohol session) at a dose of 0.36 g/kg body weight or an equal volume of water (control session). Deep body temperature gradually decreased throughout 90-min measurement both in the alcohol and control sessions (from 36.9 ± 0.1 °C to 36.6 ± 0.1 °C) without any statistically significant differences. Metabolic rate in the control session started to increase 30 min after the onset of measurement. On the other hand, in the alcohol session, metabolic rate remained unchanged in spite of a decrease in body core temperature. Whole body cold sensation became strong in the control session during cold exposure, whereas it changed to "not cold at all" after alcohol drinking, which would inhibit the behavioral regulation, if available. In the previous study we have already shown that both autonomic and behavioral thermoregulation is also modulated to decrease body temperature in hot environment (Yoda et al., 2005). Thus, alcohol influences all thermoregulatory mechanisms including behavior so as to decrease body core temperature. These results suggest that alcohol affects some elements common to all the effector mechanisms, most presumably thermosensitive neurons in the brain.

P462 (1P2-154)

Effect of intra-PO/AH or intraperitoneal administration of antipyretic drugs on stress-induced hyperthermia and motor activity in rats.

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It is known that stress-induced hyperthermia is attenuated by intraperitoneal administration of antipyretic drugs. To investigate the site of action of antipyretic drugs on stress-induced hyperthermia and motor activity, adult male rats were subjected to cage switch stress or 30-min immobilization and administrated intra-preoptic/anterior hypothalamic (PO/AH) or intraperitoneal antipyretic drugs. The intraperitoneal administration of sodium salicylate significantly attenuated the hyperthermia induced by cage switch compared with saline group, but the intra-PO/AH administration of sodium salicylate did not attenuate the hyperthermia induced by cage switch compared with saline group. The intraperitoneal administration of acetaminophen slightly attenuated the hyperthermia induced by cage switch compared with saline group, but there was no significant difference. When rats were subjected to 30-min immobilization, stress-induced hyperthermia was significantly attenuated by intraperitoneal administration of sodium salicylate compared with saline group. Furthermore, the intraperitoneal administration of sodium salicylate induced hypothermia during several hours. These data suggest that stress-induced hyperthermia is not mediated by central nervous system and the stronger stressor may induce the production of prostaglandin during several hours.

P463 (1P2-155)

Contribution of accumulated muscle metabolites following moderate exercise to the enhanced sweating during mechanical stimulation

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Previously we and others reported enhanced sweat rate (SR) during passive cycling (PC) after moderate exercise. This response may have occurred due to a combination of accumulated metabolites coupled with mechanoreceptor stimulation. To test whether accumulated metabolites associated with dynamic exercise contribute to this response, 6 healthy males performed 15-minute bouts of supine right leg exercise on a tandem cycle ergometer (1.5 kpm at 50 rpm). Following this exercise bout, subjects performed one of the following conditions for 5 minutes per condition; 1) stopped exercise (NC), 2) PC of the exercised leg (PR), and 3) PC of the non-exercised leg (PL). These protocols were randomly assigned with at least 30 min rest between bouts. PC was accomplished via a second person cycling the tandem ergometer, which allows for mechanical stimulation of the muscle with minimal activation of central command. One-leg exercise increased heart rate, mean arterial pressure (MAP), esophageal temperature and SR. At the end of exercise there were no significant differences in these parameters among the exercise bouts. SR was greater during both PR and PL bouts relative to NC bout, although no significant difference was observed between PR and PL conditions. These results suggested that accumulated metabolites in the active muscle are unlikely to contribute to the enhanced sweating during mechanical stimulation following moderate exercise.

P464 (1P2-156)

Central TGF-beta induces fever by producing prostaglandin E2 in rat brain

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We have demonstrated that the TGF-beta in the cerebrospinal fluid (CSF) was activated by poly I:C injection (i.p.) and the neutralization of TGF-beta with antibody partially suppressed the poly I:C-induced fever. We will now need to examine whether central TGF-beta itself induces fever. 2 hours after injection of TGF-beta into the cisterna magna of rat, significant elevation of core body temperature was observed. To elucidate the mechanism of TGF-beta-induced fever in detail, we examined the concentration of prostaglandin E2 (PGE2), the mediator of fever, in the CSF and the expression of cyclooxygenase-2 (COX-2), the enzyme producing PGE2, in the rat brain 5 hours after TGF-beta injection. The significant increase of PGE2 in the CSF and the induction of COX-2 in the endothelial cells in the rat brain were observed. Moreover, pretreatment with Nimesulide, a selective inhibitor of COX-2, significantly suppressed the elevation of core temperature induced by TGF-beta administration. These results suggest that central TGF-beta activated by poly I:C injection elevates core temperature through the COX-2 - PGE2 pathway in brain.

P465 (1P2-157)

Role of TGF-beta in the brain in induction of central fatigue and fever by Poly I:C

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Previously, we have demonstrated that transforming growth factor-beta (TGF-beta) was activated in the brain and related to the central fatigue and energy metabolism during exercise. Infection of virus or bacteria elevates various kind of cytokine in the brain, and it has been known that some cytokines mediate sickness behavior including decreased food intake, lowered spontaneous motor activity, and fever. Similar symptoms are often observed after exercise. Then, we postulated that TGF-beta might also be activated in the brain and be associated with various physiological changes during infection. In the present study, we examined the relation between TGF-beta and physiological changes caused by viral infection. Intraperitoneal administration of double stranded synthetic RNA (Polyinosinic: polysytidylic acid: Poly I:C) was used as an experimental model of viral infection. When spontaneous motor activity began to decrease by Poly I:C, activated form of TGF-beta increased in cerebrospinal fluid. We next examined the role of TGF-beta activated by Poly I:C administration. Poly I:C-induced fever was partially inhibited by neutralization of TGF-beta using anti-TGF-beta neutralizing antibody. However anti TGF-beta antibody was not able to inhibit the decrease in spontaneous motor activity. These results indicated that in the brain TGF-beta, known to anti-inflammatory cytokine, might act as pro-inflammatory cytokine during infection.

P466 (1P2-158)

Changes in Vascular Functions in Heat-Acclimated Rats

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We have shown that when rats and humans were subjected to daily heat exposure limited to several hours at a fixed time of day and then transferred to a constant thermoneutral ambient temperature, the pattern of nycthemeral variations in their core temperature (T_{cor}) was altered so that T_{cor} fell for 3 - 4 hours during the period when they had previously been exposed to heat. In addition, heat acclimation-induced changes in thermoregulatory functions, e.g. enhancements of thermoregulatory responses to acute heat load, were clearly seen during the period of the previous heat exposure time. It has been well known that cutaneous blood flow has a crucial role in maintaining heat loss especially in hot environment. In this study, therefore, we examined effects of heat acclimation on function of the aorta and tail arteries and also investigated how repeated timed daily heat exposures affect the daily cycle of their function in rats. Wister rats were subjected to heat (33°C) only during the second half of the dark period for 10 consecutive days. After the heat exposure schedule, the aorta and tail artery were sampled at three points of a day, and in them NE-induced smooth muscle contraction, NE-induced release of adenyl nucleotides and adenosine, and expressions of mRNAs of several factors related to vasomotion were measured.

P467 (1P2-159)

Effect of estrogen replacement on the circadian rhythms of feeding, activity and body temperature in ovariectomized rats

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We examined the effect of estrogen replacement on the circadian rhythms of feeding, activity and body temperature in ovariectomized rats. Seven-week-old female rats were ovariectomized and were assigned into estradiol- (E2-T) and cholesterol-treated (C-T) groups. Animals were implanted subcutaneously in the intrascapular space with Silastic tubing containing estradiol or cholesterol. A miniature temperature data logger was also implanted in the abdominal cavity. After the one-week recovery period, food intake, activity and intraperitoneal temperature (Tabd) were measured under 12:12h light-dark condition over a week. Daily food intake was significantly higher and daily total activity was lower in C-T rats than E2-T rats. Tabd during the late light phase and early dark phase was higher in C-T rats than E2-T rats, but was not different during the late dark phase and early light phase between the groups. The diurnal pattern of activity level was similar between E2-T and C-T groups, but activity was persistently higher in E2-T rats than C-T rats throughout the day. Food intake was also higher in the C-T rats than E2-T rats throughout the day, but the difference was more significant during the late light period and early dark period. Thus, E2 modifies the diurnal patterns of feeding and body temperature change differently from the activity pattern in ovarietomized rats.

P468 (1P2-160)

Cold-sensitive phenotype in mice lacking oxytocin receptor gene.

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We studied the physiological functions of oxytocin receptor (OXTR) in energy homeostasis using OXTR deficient (*oxtr-/-*) mice, that we recently generated by genetic engineering.Male *oxtr-/-* mice developed obesity by 13-week-old. Histrogical analysis of adipose tissue in male *oxtr-/-* mice showed lipid accumulation in gonadal white adipose tissue (WAT), and most of cells in brown adipose tissue (BAT) were filled with large lipid droplets, suggesting a typical feature in dysfunction of thermogenesis.When *oxtr-/-* mice were exposed to cold, their rectal temperature rapidly dropped.In *oxtr-/-* mice, UCP1 expressed in BAT was increased normaly by cold exposure, but the ratio of expressions of $\alpha 2$ to $\beta 3$ adrenergic receptors (ARs) was altered in comparison with that in wildtype animals. Since these ARs were known to have opposite effects on thermoregulation, the inbalance of ARs might cause this dysfunction.We predicted that OXT/OXTR systems act thermoregulation via central nervous or hormonal systems because both OXT and OXTR were not expressed in mature brown adipocyte, and analysed OXT mRNA expressions in thermogenic ceter of hypothalamus, but no alterations were observed after cold exposure.We further study about the thermoregulatory system controlled by OXT and OXTR.

P469 (1P2-161)

Central control of thermoregulatory vasomotor response to hypertonic stimulation in rats

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Heat loss responses are suppressed during dehydration, and hyperosmolality in the extracellular fluid is thought to be involved in this mechanism. In an *in-vitro* brain slice, warm-sensitive neurons in the medial preoptic area (MPA) are deactivated in a hyperosmotic medium. In contrast, neural activities in the median preoptic nucleus (MnPO) increase during both heat and osmotic stimuli. In the present study, we hypothesized that tail vasodilatation induced by local warming of the MPA in urethane-anesthetized rats would be suppressed by a selective infusion of hypertonic saline to the brain. In addition, rats with the MnPO lesion would lack this suppression. The MPA warming at ~40 °C increased skin temperature at the tail. Hypertonic saline (1500 mM) infusion into the left internal carotid artery suppressed this response when the MPA was warmed in its dorsolateral area. In MnPO-lesioned rats, there was no effect of hypertonic-saline infusion on the tail skin temperature during the MPA warming. These results indicate that the tail vasodilatation elicited by MPA warming is suppressed during osmotic stimulus. Moreover, the dorsolateral area in the MPA is a crucial site for such a response. The MnPO is also involved in this mechanism.

POSTERS
Exercise physiology

P470 (1P2-162)

Zebrafish model to analyze behavioral thermoregulation

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The neural mechanism of behavioral thermoregulation is poorly understood. In this study, we aimed to establish a new model to analyze behavioral thermoregulation using zebrafish. Zebrafish is a tropical fish living in fresh water. The body length of newborn fish is about 3 mm, and that of adult fish is about 2 cm. We can detect whole-body behavior of newborn fish under microscopic observation. Because the body of newborn fish is transparent, we can directly observe neurons in the brain. There are large numbers of transgenic lines. In addition, it is easy to inhibit the gene expression by injecting antisense morpholino oligonucleotide into embryo within a chorion.

We investigated whether newborn fish (3~5 past fertilization) perform thermoregulatory behavior against heating. Breeding water temperature (control) was 28.5^{o}C. When water temperature was raised to 32.5~40 ^{o}C, locomotor activity (swimming) much increased. This suggests that fish performs thermoregulatory escape behavior.

In conclusion, we established an effective new model to analyze behavioral thermoregulation.

P471 (1P3-163)

Assessment of cardiac contractility during a cold pressor test

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In preceding studies, we have shown that the ratio of the first derivative (d*P*/d*t*) of carotid artery pulse (CAP) to the pressure (*P*), CAP (d*P*/d*t*)/*P*, is an easily measurable, noninvasive index of cardiac contractility even in moderate exercise (Ifuku et al. 1994). Using this cardiac index, we reported the regulatory mechanism of cardiac function during a cold pressor test in athletes last meeting. In the present study, we compared the time course of changes in this cardiac index with previous studies performed using muscle sympathetic nerve activity (MSNA) (Victor et al. 1987; Yamamoto et al. 1992). Fifteen healthy subjects were subjected to the cold pressor test which required them to immerse the right hand in chilly water (4 °C) for 2 min. Mean blood pressure and heart rate changed by almost following the time course as found with MSNA, whereas changes in the cardiac index showed a different time course from that of the latter. Cold stress maximally increased the mean blood pressure and MSNA during the second minute, however, it maximally increased the heart rate and the cardiac index during the 30-60 s period. The time course of change in the cardiac index also differed from that of the heart rate during the recovery period; the cardiac index returned toward the control value but the heart rate was significantly lower than the control value. The findings suggest that the cardiac contractility did not reflect the level of MSNA during a cold pressor test in terms of time course.

P472 (1P3-164)

Alteration of myonuclear number after skeletal muscle atrophy

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Skeletal muscle atrophy occurs in response to unloading condition and is counteracted by mechanical stress to muscle during unloading condition. This study was designed to investigate whether myonuclear number regulates changes in muscle mass induced by altered loading conditions. Adult F344 female rats were assigned to either weight-bearing control or hindlimb-unloaded (HU) group. HU-rats had their hindlimbs suspended for 3 weeks with or without isometric resistance exercise (IRE). IRE (stationary support on a cylindrical wire mesh with 60 or 80 degree incline) was performed three times daily. An additional weight of 50-70% body mass was hung from the rat's tail during IRE. Each training bout lasted 10 min, for a total of 30 min/day. Myonuclear number and fiber size were measured in the medial gastrocnemius muscle using histochemical and immunohistochemical techniques. HU decreased muscle mass and fiber size of all types identified with myofibrillar ATPase staining. IRE ameliorated decrease of muscle mass by 49%. The decreases in fiber size were counteracted by IRE within the range from 33% to 86%. The IRE effectively prevented decrease in fiber size of type IIa (86%) and type IIx (74%) at type I predominant region. Myonuclear number decreased in all types of fibers with HU, but these decreases ameliorated by IRE. These results demonstrate that changes in myonuclear number are associated with changes in myofiber size induced by unloading or intermittent resistance-reloading.

P473 (1P3-165)

Effects of daily regular exercise during infant and adolescent periods on the frequency of obese-diabetes in middle-age

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The present study was conducted to examine the effects of regular exercise in childhood and adolescent years on the body weight (BW),and indices of glucose-fatty metabolism in middle-age. Methods: Thirty seven rats were randomly assigned to a childhood exercise group (CEG), which exercised from 5 to 20 weeks of age, an adolescent exercise group (AEG), which exercised from 20 to 35 weeks of age, and a sedentary control group (SCG). The rats exercised voluntarily every day using a rotatory wheel.Result and discussion: Reduction of BW in the AEG was remarkable, and showed the largest reduction of BW 5 weeks following the start of exercise. After the exercise session, food intake in the CEG had a sharp decrease similar to that of the SCG, however a significantly heavier weight of food intake in the AEG continued. Recovery of BW after the ceasing of the exercise session was delayed in the CEG, but those in the AEG quickly recovered. Quick rebounding of BW after the midpoint of the exercise session, which was observed in the AEG, could be explained by both an increase in food intake and a decreased amount of voluntary exercise. Serum leptin concentration in the exercise groups were significantly lower compared to that of sedentary rats, might contribute to the acceleration of food intake. The current result indicated that regular exercise in childhood might be more highly recommended than in the adolescent period, to prevent the incidence of obese-diabetes in middle-age.

P474 (1P3-166)

Inalterable early recovery in oxygen uptake after sub-maximal exercise with or without artificially increased dead space

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We have reported that the net oxygen uptake during outdoor running was about 0.2 lO_2/kg.km independent of running speed in adult students. The purpose of this study was to investigate the effect of artificially increased dead space on the net oxygen uptake during running and recovery on a treadmill. Twelve healthy students performed the graded sub-maximal exercise twice with or without an additional hose. The volume of the hose which was connected to the respiratory mask was 820 ml. The graded exercise tests consisted of running for 10 minutes and recovery for 2 minutes. The values of the net oxygen uptake during treadmill running were almost constant; between 0.167 - 0.172 lO_2/kg.km, at running speeds of 7.9 - 11.9 km/h. However, in the results using dead space, the values of net oxygen uptake decreased with an increase in running speed from 0.170 lO_2/kg.km at 9.0 km/h to 0.105 lO_2/kg.km at 12 km/min. These individual net oxygen uptakes at a running speed of 12 km/h were correlated with the percentage values of the maximal oxygen uptake at heart rate 160 beats/min using dead space. The early recovery in oxygen uptake after exercise was not affected by the artificially increased dead space. The results of the present study indicate that the decrease in net oxygen uptake with dead space during running at a faster speed is caused by the decreased capacity in individual respiratory function. Also the effect of the artificially added dead space on oxygen uptake disappears during the early recovery period after exercise.

P475 (1P3-167)

Effect of captopril and exercise training on ANP action in DOCA-salt Hypertensive Rats.

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It is known that exercise training improves high blood pressure in humans. The exercise therapy is recommended to the mild hypertensive patients in the drug treatment. However, the effect of execise is not known in details. Therefore we focused on the action of antihypertensive peptide, atrial natriuretic peptide (ANP). We designed protocol to evaluate the effect of combination of drug treatment and exercise training. Captopril was used as drug, and swimming was used as exercise training.

4 week-old male Wistar rats were purchased for the experiment. They were randomly divided into control group (C), hypertension group (H), captopril administration group (D), swim training group (T) and captopril administration group with swim training (DT). To develop DOCA (deoxycorticosteroneacetate) -salt hypertension, DOCA was administered i.m. to all the groups except C at a dose of 20 mg every week for 2 weeks. Thereafter captopril was orally administered to D and DT at a dose of 50 mg/kg twice a week. T and DT swam for 15 min/day three times a week. The swimming time was increased gradually over the 1-week period from 15 min/day to 90 min/day. They continued swim training for 9 weeks. DOCA-salt hypertensive rats were supplied with 1% saline for drinking water for 11 weeks. After 11 weeks, rats were anesthetized with pentbarbital. And tissue samples were excised.

The ANP concentrations in the left ventricule (LV) tended to be higher in T and DT. The heart-to-body weight ratio (HW/BW) of LV was significantly higher in T and DT. These results seems to represent effect of exercise.

P476 (1P3-168)

Sex difference and menstrual cycle effect on the muscle blood flow regulation to the active muscle under reduced perfusion pressure.

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It is known that estrogen affects endothelial function, and that the vasodilatory response to reactive hyperemia is enhanced by estrogen. It is still unknown whether the female reproductive hormones have an influence in the regulation of muscle blood flow to the exercising muscle. We hypothesize that estrogen improves muscle blood flow to the exercising muscle under reduced perfusion pressure via improved endothelial function. In the present study, we examined the muscle blood flow response to static handgrip exercise (20% MVC) under reduced perfusion pressure in female and male subjects. In female subjects, we conducted experiments three times; ovulatory (O), luteal (L), and menstrual (M) phases. Perfusion pressure during exercise was reduced by inflating upper arm cuff at 20 or 40 mmHg. We also measured blood flow response following forearm ischemia (upper arm cuff at 200 mmHg for 5 min) in order to evaluate endothelial function. The reduction of blood flow to the exercising muscle induced by the reduction of perfusion pressure was relatively small in O and L than in M and male subjects. The blood flow response following forearm ischemia was higher in O than other conditions. These results suggest that female reproductive hormones might play a role in the regulation of muscle blood flow to the exercising muscle.

P477 (1P3-169)

Decline of Attention by Mental Fatigue and Physical Fatigue: Detection by Event-related Potentials

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The purpose of this study is to detecte the defference between a decline of the attention by the mental fatigue and one by the physical fatigue by event-related potentials (ERPs). Ten healthy college students (mean age: 20 years old) performed a multi steps exercise loading test using a treadmill (physical task) and a continuous additional task using Uchida-Kreperin test paper (mental task). In physical task, the loading strength was increased stepwisely until the heart rate of the subjects reached expected maximum. In the mental task, the subjects were loaded with the continuous additional task for 2 hrs. In this study, audito EPRs and the concentrations of lactic acid in blood, MHPG (3-methoxy-4-hydroxyphenylglycol), and interleukin 6 (IL-6) were adopted as parameters of the fatigue. Audito EPRs were led by source derivation (SD) method. Parameters mentioned above were obtained just before and after each task, and then, 1hr and 2hrs after. The amplitudes of P3a decreased in both of tasks. Lactic acid increased only just after the physical task but MHPG was kept high after the mental task. However, IL-6 did not change in both tasks. These results suggest that a decline of attention in a transient physical fatigue is caused by laktazidose and a decline of attention in transient mental fatigue is caused by the excess-consumption of monoamine in brain.

P478 (1P3-170)

Effects of Tai Chi exercise on human EEG and regional cerebral blood flow (CBF): Contribution of brain serotonergic system.

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Effects of Tai Chi exercise on brain activity have not been fully evaluated. In the present study, we investigated changes in EEG and regional CBF during and after Tai Chi exercise (24 forms, two times). CBF was assessed as a sum of oxygenated and deoxygenated hemoglobin using near-infrared spectroscopy (NIRS). As a result, we found the alpha power augmentation on EEG during and after Tai Chi exercise. Tai Chi exercise evoked a gradual CBF increase in the prefrontal cortex (PFC) and a gradual CBF decrease in the parietal cortex, indicating that PFC was activated by Tai Chi exercise. In addition, the urinary and blood serotonin (5-HT) levels increased after Tai Chi exercise. Since PFC has efferent projections to 5-HT neurons in the brain stem, we suggest that PFC activation causes augmentation of brain 5-HT system, which in turn, induces increase in alpha power on EEG.

POSTERS
Environmental physiology

P480 (1P3-172)

The effect of elastic socks on travelers' thrombosis.

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[Purpose] It is well known that of clamped seating for long duration causes deep vein thrombosis and pulmonary thromboembolism (so-called Travelers' Thrombosis; TT). Exercise, hydration and the use of elastic socks are well known as preventive measures for TT, but there have been few scientific reports about it. We examined the effect of elastic socks (SSL Healthcare Japan Ltd., Flight Socks) on leg edema caused by 6 hours seating with 8 healthy volunteers.**[Method]** Lower leg girth and lower leg fluid volume were measured using impedance method. Blood pressure and heart rate and self reporting questionnaire concerning edema were also checked every one hour. Body weight, muscle volume and fat volume were measured before and after seating using Multi-frequency Body composition analyzer (TANITA Corp. MC-190).**[Results]** Lower leg edema and fluid volume were gradually increased in the group without elastic socks, but those were markedly prevented by elastic socks especially among women. There were negative correlation between lower leg muscle volume and fluid volume change (r=-0.7007) and positive correlation between fat volume and fluid volume change (r=0.7474) without elastic socks. When wearing elastic socks, there was positive correlation between muscle volume and fluid volume change (r=0.5535), but no correlation between fat volume and fluid volume change was observed. **[Conclusions]** Elastic socks markedly prevented the lower leg edema and fluid retention. It might be a part of evidence that elastic socks prevent the expression of TT, but further experiments (i.e. blood flow etc.) are required.

P479 (1P3-171)

Seasonal changes of sweat function in young men and women

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Purpose We investigated whether sweating function changes between summer and winter in young persons, and, if so, attempted to examine underlying mechanisms. Method Subjects were six male and nine female students. The female subjects had no custom of exercise whereas the male subjects had occasional slight exercise. The sweat test was done in summer and winter, in which room temperature was raised stepwise from 30 to 42°C by 3°C every 30 minutes (40% in relative humidity) and tympanic temperature, local sweat rates (SR), skin temperatures and the rate of sweat expulsion (Fsw) were measured. VO_2max was also measured. Result In the female subjects, the regression line relating SR to body temperature was more steepened in summer than in winter ($p<0.05$), whereas in the male subjects, the regression line shifted in summer to the left of winter ($p<0.05$). The regression line relating Fsw to body temperature shifted to the left in summer both in the female and male subjects ($p<0.05$). The regression line relating Fsw to SR was more steepened in summer in the female subjects, whereas it shifted to the left in summer in the male subjects ($p<0.05$). Conclusion The results indicate that sweat capacity was increased in summer by central and sweat gland mechanisms both in the female and males subjects. It appears that young persons attain heat acclimation during summer season, even when they spend the most time of summer day under air-conditioned environments.

P481 (1P3-173)

EFFECTS OF LUMBAR-BACK WARMING ON EEG, BODY TEMPERATURE AND SWEATING

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Local heat application of skin may elicit various physiological responses and/or beneficial clinical effects. We examined the effect of lumbar-back warming on thermoregulatory responses as well as EEG in 8 healthy young subjects using a steaming pad (16cm x 30cm) newly developed to generate warm steam (<45°C) by the chemical reaction of iron with water and environmental oxygen. After outfit of thermocouples on the skin (10 points) and EEG electrodes (10-20 system), each subject wearing a T-shirt and short pants lay on supine position for 15 min followed by attachment of two steaming pads on both upper and lower back and maintained same position for another 15 min in a neutral environment of 23°C and 35%RH. Mean skin temperature rose by approximately 1 degree, while the palm temperature increased by 2 degree and foot temperature and oral temperature was unchanged. Weight loss due to sweating was 52±27 g (mean±SD) and mean% alpha oscillation was significantly increased from 14% to 20% during lumbar-back warming. There was no effect on blood pressure and heart rate. Subjects reported feeling of relaxation and great comfort during warming. These results suggest that the relaxation effect of lumbar-back warming with the steaming pad is comparable to that of bathing in a warm bath, but it has much less effects on the circulatory system.

P482 (1P3-174)

Arithmetic stress facilitates spontaneous platelets aggregation in healthy youth

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Introduction: Recently, platelet hyperaggregability is suggested to be implicated in the pathogenesis of acute coronary syndromes (ACS), cerebral infarction (CVD) etc. It is widely suggested that these diseases are related to stress. In the present study, the relation between stress and platelet aggregation was studied in healthy youth. Material and Method: Twelve healthy young volunteers were loaded with arithmetic stress. We took peripheral blood samples from the volunteers before and after arithmetic stress loading. We measured platelets aggregation without the addition of any aggregating agents (spontaneous platelet aggregation; SPA) using the novel aggregometer which can detect aggregates as small as two platelets by laser light scattering (LS). Thus, we explored whether SPA is accelerated by arithmetic stress. We also measured the concentration of stress-related hormones and investigated the relation between SPA and stress-hormones. Result: After arithmetic stress loading, the concentration of norepinephrine (NE) was significantly increased among the measured stress-related hormones. SPA was also accelerated by arithmetic stress, which was correlated with the increase in NE concentration. Discussion: Stress releases NE from sympathetic nerve terminals and the binding of NE to $\alpha_2$adrenoreceptor of platelet triggers platelet aggregation. Thus, stress is a possible factor of ACS and CVD.

P483 (1P3-175)

Sympathetic responses to G stress-induced hypotension following after lower body positive pressure in rats

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G protection by lower body positive pressure (LBPP, anti-G suit) is an important life support system for fighter pilots. The purpose of this study is to evaluate sympathetic responsiveness to G stress-induced hypotension in rats with an LBPP equipment. Anesthetized rats were exposed to acceleration (3 and 5G) from head to tail by a centrifuge (arm length: 115 cm) with LBPP. Arterial pressure at a level of the brainstem (APLB), central venous pressure (CVP), electrocardiogram, heart rate, and renal sympathetic nerve activity (RSNA) were measured. APLB and CVP were kept to a control level during LBPP at any G load. Hypotension was, however, observed before the start of LBPP (Hypotension-1, 47.6±6.7 mmHg at 3G or 49.2±7.5 mmHg at 5G) and after the cessation of LBPP (Hypotension-2, 42.9±4.6 mmHg at 3G or 29.1±3.3 mmHg at 5G), because the G valve does not function under 2G. Hypotension-2 was significantly deeper than Hyptension-1 at 5G. RSNA significantly increased to 135.3±9.0% at Hypotension-1, and to 173.4±12.8% at Hypotension-2 at 5G. No significant difference was found in RSNA responsiveness to hypotension between Hypotension-1 and Hypotension-2. These results indicate that the sympathetic system can similarly response to Hypotension-1 and Hypotension-2, suggesting that dysfunction in the sympathetic system may not cause the greater hypotension observed after the cessation of LBPP.

P484 (1P3-176)

Mechanical pain threshold and mechanical sensitivity of C-fiber receptors in the muscle of aged rats

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Musculoskeletal pain is the most common symptom in elderly. To know possible mechanism for this musculoskeletal pain, we examined muscle pain threshold and mechanical sensitivity of muscle C-fiber afferents in rats at different ages. Rats were grown with restricted food supply and the body weight was almost the same when they were used for experiments (mean around 380 g). Mechanical pain threshold of the muscle measured with Randall-Selitto method tended to be lower at 130 week old (1001 ± 74.9 mN) than those at 80 week old (1167mN ± 79.8mN) and at 13 week old (1098 ± 33.6 mN). There was, however, no statisctically significant difference among these threshold values. Some what more clear difference was found in the mechanical sensitivity of C-fiber receptors recorded in the extensor digitorum longus muscle (EDL muscle)-common peroneal muscle preparation in vitro: The mechanical threshold was significantly lower (55.0 ± 5.3 mN) in 130 week old rats than that of 13 week old rats (85.2 ± 8.2 mN), and the magnitude of response to 198 mN/10s stimulation tended to be higher in aged rats (41.5 ± 5.5 impulses) than that of 13 week old rats (29.9 ± 4.5 impulses). To solve the discrepancy between pain threshold and mechanical sensitivity of C-fiber afferents, changes in mechanical properties of the skin, subcutaneous tissue and fascia, in expression of ion channels and transducers, and in spinal mechanism at different ages must be studied.

P485 (1P3-177)

Mechanism of enhanced lipopolysaccharide (LPS)-induced fever by exposure to stressful ambient temperatures in rats

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Our previous studies revealed that levels of plasma corticosterone in rats exposed to alternatively changing ambient temperatures (repeated temperature changes between 4 degrees C and 27 degrees C, each lasting for 1h) were significantly higher than those exposed to constant low ambient temperature (4 degrees C). Frequently changing, but not constant, ambient temperatures may have induced a stronger stress. Furthermore, intraperitoneal LPS-induced fever in these two groups were larger than those in the control group, exposed to a constant ambient temperature at 25 degrees C. Enhancement of LPS-induced fever was larger when stress in terms of corticosterone was stronger. In the present study, two additional experimental groups, exposed to alternatively changing ambient temperatures (repeated temperature changes between 18 degrees C -1h and 34 degrees C -1h) and constant high ambient temperature (34 degrees C), exhibited significantly higher levels of plasma corticosterone than the control group (25 degrees C) only day one post-exposure. But there was no difference between these two experimental groups. The aim of the present experiments was to elucidate underlying mechanisms of enhanced LPS-induced fever in animals exposed to stressful ambient temperatures. For this purpose, changes of hematic constituent and endogenous plasma endotoxin(LPS) were monitored.

P486 (1P3-178)

Effects of estrogen on vascular nitric oxide and oxidative stress ovariectomized rats during psychological stress

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We reported that chronic estrogen replacement bunted pressor response to cage-switch (CS) stress in ovariectomized (OVX) rats and oral administration of a nitric oxide synthase (NOS) inhibitor diminished the difference. In this study, we examined whether estrogen attenuates stress-induced pressor response by mediating nitric oxide (NO) and reactive oxygen species (ROS) productions. Adult OVX rats were assigned placebo-treated (P) or estrogen-treated (E) group implanted with either placebo- or 17β-estradiol pellets. After 4 weeks, these rats underwent to CS stress. We measured plasma concentrations of NO metabolites (NOx) and 4-hydroxy-2-nonenal (HNE), a lipid peroxide. Furthermore, endothelial NOS (eNOS) and the phosphorylation at Ser1177 and neural NOS expression were examined in mesentery. The basal level of NOx was not different between the groups, but HNE was significantly higher in the P group. CS stress increased plasma NOx and eNOS phosphorylation in the P group, but not in the E group. These results imply that pressor response to CS stress enhances eNOS activation and NO release in OVX rats. However, excessive ROS might decrease NO bioavailability and induce sustained hypertension during psychological stress in OVX rats. Further study is required to address as to whether the effect of estrogen is mediated through NO/ROS imbalance in peripheral vessels.

P487 (1P3-179)

Heart rate variability of rat under sinusoidal rotation

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The space adaptation syndrome may partly be caused by lack of input to the otlith system as well as cardiovascular modification due to a fluid shift. We reported sinusoidal linear acceleration induced heart rate (HR) modification synchronized to the acceleration. However a potential contribution from the vestibular system could not well evaluated. We examined the contribution of the vestibular system applying off-centered sinusoidal rotation (OCSR) to rat with and without vestibular function. Vestibular disfunction was achieved by repeated injections of streptomycin sulfate. The OCSR was applied in two different postures relative to the rotation vector, radial and tangential, as well as a posture, the head on the rotational axis. The rotational stimuli were applied keeping a constant maximum radial acceleration and varying the tangential acceleration. Power spectra of HR were calculated by FFT. In case of OCSR stimulation fixing body in both positions, a synchrony was observed in the HR power spectrum of the individual acceleration frequency. When the head was on the rotational axis, the synchrony was not observed in neither of two directions. The synchrony was diminished with vestibular disfunctioned rat in all OCSR postures. It strongly implied that the input from vestibular system may play an important role in the heart rate variability.

P488 (1P3-180)

Change of delayed onset muscle soreness (DOMS) in aged rat

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DOMS is commonly observed after unaccustomed, strenuous exercise. It usually reaches a peak 24 to 48 hours after exercise. The mechanism for DOMS is not clear yet. In our previous experiment, we demonstrated the existence of tenderness by behavioral pain tests and by c-Fos protein expression in the spinal dorsal horn after eccentric contraction (ECC), which is known to induce DOMS more effectively, in young rats (8 w). To know if there is any change in DOMS in aged rats, we examined mechanical threshold of extensor digitorum longus muscle (EDL) and c-Fos expression of dorsal horn in the aged rats (130-139 w) after ECC. The mechanical threshold started to significantly decrease 1 day after ECC and remained decreased up to 5 days after ECC. It finally returned to the pre-ECC value 7 days after ECC. It became clear that DOMS continued for a longer period than the young (8 week-old) rats, in which DOMS lasted between 1 and 3 days after ECC. In the aged rats, the compression (160 g, 60 times) was applied to the EDL 3 days after ECC. Two hours later animals were perfused and fixed, and c-Fos expression in the dorsal horn was examined. c-Fos-immunoreactive neurons in the superficial dorsal horn at L4 increased significantly in not only at L4 but also in L5 spinal segment. This distribution is wider than that in the young rats (in L4 only). This result suggested that pain information from the muscle in aged rats was transmitted to the wider level of spinal cord than young rats.

P489 (1P3-181)

Effects of lowering barometric pressure on sympathetic nerve activity in anesthetized rats

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Our lab reported that lowering barometric pressure (LP) by 27 hPa aggravated pain-related behaviors in chronic pain rats, and this effect was blocked by surgical lumbar sympathectomy. LP also increased systemic blood pressure (BP) and heart rate (HR) in unanesthetized neuropathic and normal rats. Therefore, we hypothesize that sympathetic nerve activity (SNA) plays an important role in pain-aggravation by LP. In the present study, I directly recorded SNA in anesthetized rats and examined its change by LP. Normal and complete Freund's adjuvant- monoarthritic rats (male SD rats) were used. Rats were anesthetized with a urethane and α-chloralose mixture, some with isoflurene. SNA was recorded from the left sympathetic nerve trunk between L3 and L5. LP in several magnitudes and speeds was done in the decompression chamber (Daikin). LP up to 45 hPa did not significantly change SNA in normal rats, although noxious pinch to the skin or lowering body temperature increased SNA. Neither BP nor HR was significantly changed by LP. Because aggravation of pain behavior was observed only in animals with persistent pain, we also exposed monoarthritic rats to LP, but without any effect. Isoflurene anesthesia did not change the results. Previous study and these results indicate that the difference from previous observation in BP and HR and absence of change in SNA might be caused by anesthesia. To examine this possibility, recording these parameters in decerebrated rats after removal of anesthesia and recording sympathetic nerve activity in conscious rats are to be done.

P490 (1P3-182)

Effects of bilateral vestibular lesions on arterial pressure control during head-down tilt in anesthetized rabbits

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Head-down tilt (HDT) has been used to simulate microgravity environment. HDT causes a headward fluid shift as real microgravity does, but HDT also induces a change in vestibular input which is absent during real microgravity. Some studies have shown that the vestibulo-sympathetic reflex (VSR) has an additive influence to cardiovascular reflexes (i.e., baro reflexes) on sympathetic nerve activity. Thus HDT, using animals without VSR, may provide a better model for microgravity. Little information is, however, available for relationship between the otolith inputs and cardiovascular responses during HDT. In the present experiments, we examined changes in mean arterial pressure (MAP) and renal sympathetic nerve activity (RSNA) during 45°HDT using anesthetized rabbits. Chemical labyrinthectomy was performed bilaterally under anesthesia 2 days before experiments in order to make vestibular lesions (VL group). The animal's head was mounted on a tilting table during experiments. In control rabbits, RSNA decreased markedly during HDT, which was followed by a MAP decrease. After 10 seconds from the onset of HDT, the RSNA went up to the pre-HDT baseline activity, and then MAP recovered to the pre-HDT baseline level. In VL group, RSNA did not change significantly and the MAP was very variable and tended to increase during HDT. These results suggest that vestibular apparatus is involved in arterial pressure control during HDT, which is possibly mediated by the sympathetic nerve outflow.

P491 (1P3-183)

Gravity and Cardiovascular Regulation of Fish

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As reported previously, aquatic animals , fish or amphibian, lack synchrony between heart rate variability (HRV) and respiration unlikely mammals and avian living on the ground directly exposed to gravity. We then speculate the HRV may be originated after moving in on the ground adapting cardiopulmonary functions against the gravity induced fluid movement. In this report, we tested a gravitational tolerance of cardiovascular system of fish (carp and goldfish) using reverse water immersion method changing the posture under air. The fish was restrained and kept maintaining respiration by circulating water at upright posture in water and then rapidly drained surrounded water. ECG and respiratory activities were monitored with implanted silver wire electrodes (0.1mm) in thoracic cavity and gill muscles respectively. The ECG analysis indicated that upright posture in air significantly elevated the heart rate indicating an ischemia while the respiration rate remained constant. At a horizontal posture after 10 min. exposure at upright position in air, the heart rate largely decreased beyond the normal level indicated a bradycardia. In the same time, the HRV drastically increased for more than 15 min. showing a strong arrhythmia. The results strongly suggested that a baroreflex did exist in fish cardiac system, but the response could not compensate the large blood shift induced by gravity. It was also suggested that the reflex may not be responsible for vasoconstriction like mammals. We consider the gravity exposure may play an essential role in evolutional developments of cardiovascular antigravity reflex.

P492 (1P3-184)

Does the aortic baroreflex in adult rat fail during and after the 16 days spaceflight?

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We previously reported that the 16 days spaceflight (FLT) reduced the sensitivity of the aortic baroreflex in rat neonates and showed fewer unmyelinated fibers in its afferent (UMFa) as compared to the ground controls, which might be related with the lower baroreflex response, and that the dams housed for nursing the neonates also showed fewer UMFa. In the post-flight examination, these adult rats were principally served for the histological analysis, however, we tried the baroreflex test (phenylephrine 50 g/kg bolus injection) under anesthesia (urethane 1.0-1.8 g/kg) in two dams of the FLT groups. The indices of the decrease in heart rate in response to the maximal increase in mean arterial pressure (delta-HR bpm/delta-MAP mmHg) were -1.40 and -2.65 in the two FLT animals, and -3.20 and -4.69 in the two ground animals. The results suggest the possibility of a failing of baroreflex sensitivity during and after the spaceflight even in adult rats, relating the fewer UMFa.

P493 (1P3-185)

Eye movements of flatfish evoked by body tilting

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Eye movement serves to hold the gaze steady or to shift the gaze to an object of interest. On Earth, signals from otolith organs can be interpreted either as linear motion or as tilt with respect to gravity. In microgravity, static tilt will no longer give rise to changes in otolith activity. However, linear acceleration as well as angular acceleration stimulates the otolith organ. Therefore, during adaptation to microgravity, otolith-mediated response such as eye movements would alter. Flatfish provide a natural model for the study of adaptive changes in the vestibulo-ocular reflex. During metamorphosis, vestibular and oculomotor coordinate of flatfish displaced 90 degrees about the longitudinal body axis. Therefore, it is expected that microgravity induce the sensory mismatch in adult flatfish. In this study, we analyzed the eye movements of flatfish for body tilting. The eye movements for body tilting along the different body axis were video-recorded. The vertical and torsional eye rotations were analyzed frame by frame. In normal flatfish, vertical eye movement of left eye to leftward tilting was larger than that to rightward tilting. For head up or head down tilting, clear vertical eye movements were observed. These results suggested that sacculus and lagena were important for otolith-ocular eye movements.

P494 (1P3-186)

Immunohistochemical analysis on the kidney in neonatal rats which reared for 30 days after spaceflight.

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Microgravity shifts body fluid to upper body during spaceflight. And the survival ratio of rat newborns after spaceflight is low, however, why newborns died in space is still unclear. They imply that the postnatal development is the key to realize generation transition in space. The aim of this study is to examine the structural development in kidney macroscopically.Eight- and 14-day old rats were launched at these ages in the Space Shuttle Colombia for 16 days (STS-90; Neurolab). Some of 8-day rats were reared on the ground for 30 more days after landing as the re-adaptation group. Six of 11 flight rats of the 8-day group showed ambilateral hydronephrosis. Despite the hydronephrosis and tubule expansion existed, it was thought that these kidneys were potentially functional in filtration process. This hydronephrosis was remained in the re-adaptation group, but it wasn't observed in the 14-day group.

POSTERS
Pathophysiology

P495 (1P3-187)

Antioxidative effect of water treated by granular ceramics

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It has been reported that treatment of water by granular kaolinite ceramics modified the physical and biological properties of water. It was shown that the treated-water was positively charged without any absorption-deposition phenomena to substances contained in water. The positive charge of the treated-water was indirectly affirmed by thermally stimulated depolarized current measurements and Nuclear Magnetic Resonance spectrometry analysis. In the present study, we investigated an antioxidative effect of treated-water employing fibroblast cell culture system and Vitamin C preservation test. After cells were cultured in ordinary culture medium until sub-confluent state, then the medium was changed to media dissolved in Milli Q water (MEM) or Milli Q water treated by granular ceramics (tMEM), with or without various concentrations of hydrogen peroxide. Treatment of water was done by vigorous agitation of water with ceramic granules in a plastic bottle. After incubation for 24 hrs, the survival rate of the cells cultured in tMEM was significantly high compared to that of the cells cultured in MEM. Dissolved Vitamin C loses its reducing activity in time course. However, the reducing activity of Vitamin C in tMEM was significantly higher than that of Vitamin C in MEM, after prolonged incubation of the solution, suggesting the preventive effect of the treated-water against auto-oxidation of Vitamin C. Although the exact mechanism involved in the cell resistance against oxidative stress is not clear yet, it is strongly suggested that the treatment of water by granular ceramics gave antioxidative property to the water.

P496 (3P3-167)

The effect of moxibustion on collagen-induced arthritis in mice: A role of CD25+ CD4+ regulatory T cells

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It is known that the moxibustion applied to the acu-point of the skin has desirable effects on the self-defense mechanisms. There are many reports that the decrease of the number and/or the dysfunction of natural regulatory T cell ($CD25^+$ $CD4^+$ T cells) induces autoimmune disease such as rheumatoid arthritis. We used collagen-induced arthritis (CIA) mice to examine the effect of moxibustion for autoimmune disease such as rheumatoid arthritis. The method of collagen-induced arthritis (CIA) in mice was as follows. DBA/1J mice were immunized intradermally with type II bovine collagen twice with the 3 weeks interval. CIA mice were divided into three groups. Control group : no treatment, Moxibustion group : Moxibustion treatment with 1mg moxa cone was applied 5 times a day to the MEIMON (GV4) acupoint, 3 times per week for 3 weeks. Prednisolone group : 10mg of prednisolone was administered orally, 3 times per week for 3 weeks. At the initial stage (day28), the number of the white blood cell counts, $CD25^+$ $CD4^+$ T cells and the ratio of the $CD25^+$ T cells in $CD4^+$ T cells were significantly increased in the moxibustion group. On the other hand, in the prednisolone group, the ratio of the $CD25^+$ T cells in $CD4^+$ T cells was significantly increased but the other two indications were not changed. There results suggested that the increase of regulatory T cell induced by moxibustion and prednisolone at the early stage of CIA lowered arthritis severty.

P497 (3P3-168)

suppressive activity of dexamethasone on the production of matrix metalloproteinases and tissue inhibitor of metalloproteinases from human synovial fibroblasts obtained from knee osteoarthritis *in vitro*.

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Glucocorticoids (GCs) are frequently used for the treatment of osteoarthritis (OA) with remarkable success. However, the precise therapeutic mechanisms of the agents are not well understood. The present study was, therefore, designed to explore the mechanisms by which GCs could favorably modify the clinical conditions of OA by using synovial fibroblasts (SF) and dexamethasone (DEX) in vitro. SF was induced from synovial tissues obtained from five patients with osteoarthritis. Cells (1.0×10^5cells / mL) were stimulated with either 0.5μg / mL lipopolysaccharide (LPS) or 1.0μg / mL transforming growth factor β (TGF-β) in the presence of various concentrations of DEX. After 24h, culture supernatants were collected and MMPs levels were examined by ELISA. Addition of DEX at more than 50M into cell cultures could suppress MMP-1, MMP-2, and MMP-3 production from SF induced by LPS, but not TGF-β stimulation. DEX could not suppress the production of TIMPs from SF in response to LPS and TGF-β stimulation, even when DEX at more than 50M were added to cell cultures.These results suggests that DEX could suppress joint tissue remodeling through inhibition of MMP production and favorable modification of clinical condition of OA .

P498 (3P3-169)

Effects of chondrotin sulfate on oxide production from synovial fibroblasts obtained from osteoarthritis in vitro.

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Osteoarthritis(OA) is well known to be one of the most frequent rheumatic diseases observed mainly in elderly people. Although chondroitin sulfate (CS) has been used frequently as a supplement for the treatment of OA, the mechanism by which CS could favorably modify the clinical conditions of OA. The present study was undertaken to examine the influence of CS on the production of prostaglandin E2 (PGE2) and nitric oxide (NO), which are involved in the pathogenesis of OA, from synovial fibroblasts *in vitro*.Fibroblasts were prepared from surgical specimens obtained during arthroplasty for OA. Cells were stimulated with 5.0ng/ml IL-1β in the presence of various concentrations of CS for 24h. PGE2 and NO contents in culture supernatants were analyzed using ELISA test kits and NO2/NO3 assay kits, respectively.Addition of CS at more than 5.0μg/ml into cell culture significantly suppressed the ability of cell to produce NO through the inhibition of iNOS mRNA expression, which was increased by IL-1β stimulation. On the other hand, CS could not suppress PGE-2 production, even when 100.0μg/ml CS was added to cell cultures.These results may suggest that the suppressive effects of CS on NO production may bucontrite, in part, to favorable modification of clinical symptoms of OA.

P499 (3P3-170)

Suppressive activity of chondroitin sulfate on MMP production from synovial fibroblasts obtained from patients with osteoarthritis in vitro

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Osteoarthritis (OA) is well known to be one of the most frequent rheumatic diseases observed mainly in elderly people. It is also recognized that OA is characterized by loss of articular cartilage and secondary bone as well as changes to the synovium, including marginal osteophyte formation and synovitis. Although chontroitin sulfate (CS) has been used frequently as a supplement for the treatment of OA, the mechanisms by which CS could favorably modify the clinical conditions of OA. The present study, therefore, was undertaken to analyze the possible therapeutic mode of action of CS on OA by examining the production of matrix metalloproteinases, which are important molecules in cartilage depletion, from synovial cells in vitro.Fibroblast were obtained from joint specimens of patients who underwent total knee arthroplasty for OA. Cells (1×10^5 cells/ml) were stimulated with 5.0 ng/ml IL-1βin the presence of various concentrations of CS for 24 h. MMP-1, 3 and 13 levels in culture supernatants were evaluated by ELISA. Addition of CS at more than 50.0 μg/ml into cell cultures could suppress the production of MMP-1, 3, and 13. However, the ability of cells to produce TIMP-1 and 2 was not interfered by CS, even when 100 μg/ml CS was added into cell cultures.These results strongly suggest that CS prevents tissue remodeling through the suppression of MMP production and results in favorable modification of the clinical condition of OA.

P500 (3P3-171)

Attenuating effect of bisphosphonate on oxidative responses in rats with adjuvant arthritis

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Reactive oxygen species are well accepted to play important roles in development and maintenance of several types of diseases, including rheumatoid arthritis and osteoporosis as the final effector molecules. Although it is also reported the anti-inflammatory action of bisphosphonates, which is frequently used for the treatment of osteoporosis, the influence of bisphophonate on the development of oxidative responses. The present study, therefore, was designed to examine the influence of bisphosphonate on oxidative responses through the choice of etidronate (ER) and adjuvant arthritic rats *in vivo*. Adjuvant arthritis was induced in male Lewis rats by a single subcutaneous injection of 0.1 ml complete Freund's adjuvant into the right hind paw. Treatment of rats with ER was started on day 7 after adjuvant injection. Oxidative responses were evaluated by measuring hydroperoxide contents in plasma with a newly developed free radical analysis system, FREE. Daily intraperitoneal administration of ER for 2 weeks could suppress hydroperoxide contents in plasma, which was increased by adjuvant injection. The minimum dose of the agent, which causes significant suppression of hydroperoxide levels 3.33 mg/kg, a human recommended therapeutic dose. These results may suggest that ER could suppress the production of reactive oxygen species and results in attenuation of the clinical conditions of disease with bone destruction.

P501 (3P3-172)

The effect of moxibustion on the dynamics of peripheral neutrocytes in the collagen-induced arthritis mice

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To evaluate the effect of moxibustion (MOX) on the immune activity and compare with the changes of immune related cells, we examined the effect of Mox on the symptom severity and the changes of white blood cell (WBC) number, CD11b (adhesion molecule) and CD69 (the early activation antigen) expression of neutrocytes in the collagen-induced arthritis (CIA) mice. DBA/1J mice were immunized with type II collagen. CIA mice were divided into three groups. Control group : no treatment, Mox group : Mox treatment with 1mg moxa cone was applied 5 times a day to the MEIMON acupoint, 3 times per week for 3 weeks. Pred group : 10mg of prednisolone was administered orally, 3 times per week for 3 weeks. Symptom severity was evaluated using arthritis score method. CD11b and CD69 expression of neutrocytes were counted using flow cytometry. Arthritis score was suppressed significantly in the Mox group. WBC number was significantly decreased in Pred group, while not changed in Mox group. CD11b expression was not changed in Mox and control, while significantly increased in Pred group on day 35. CD69 expression in Mox groups was significantly increased on day 31 as compared with Control and Pred groups. The results suggested that Mox might suppress the symptom severity of CIA through the different mechanism from the inhibitiion of inflammatory reaction in vivo.

P502 (3P3-173)

Measurement of rat mast cell surface antigen-1 (MASA-1) mRNA in a rat model of type I allergy

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We have reported that rat mast cell surface antigen-1 (MASA-1) with a molecular weight of about 90 kDa was cloned from a rat mast cell line (RBL-2H3 cells). MASA-1 was specifically expressed in the lesional tissues of human chronic inflammatory diseases associated with fibrosis such as interstitial pneumonia and hypertrophic scar. However, the expression of MASA-1 in type I allergy is not clear. In this study, the expression of MASA-1 protein and mRNA were investigated in a rat model of type I allergy using immunostaining and real time PCR. MASA-1 protein was found to be expressed on day 3 to 25 after antigenic stimulation, coincidentally with the infiltration of inflammatory cells, but not immediately after antigenic stimulation. Increments in MASA-1 mRNA levels were observed from 1 to 25 days after stimulation by real time PCR. MASA-1 mRNA levels at 3 and 5 days after stimulation were significantly higher than that before stimulation. These results suggest that inflammatory cells are involved in the expression of MASA-1 on mast cells. We investigated on the effect of TNF-α, one of the chemical mediators in mast cells, on MASA-1 expression by human mast cell line (HMC-1 cells). TNF-α induced MASA-1 protein expression on HMC-1 cells one day after stimulation by immunoblotting. Thus, MASA-1 might be specifically expressed on mast cells in the late phase of type I allergy, and TNF-α induced the up-regulated expression of MASA-1.

P503 (3P3-174)

Rejection of cytotoxic T lymphocyte-resistant Meth A tumor cells by immunotherapy

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One implication of the varied responses of tumor cells to chemotherapy and other treatment modalities is that the successful eradication of disseminated tumor cells will have to be highly selective and circumvent the problems of biologic heterogeneity of neoplasms. In the past, it has been assumed that cytotoxic T lymphocyte (CTL) is the major effector cells responsible for the rejection. In the present study, we examined the growth of CTL-resistant Meth A tumor cells transplanted intraperitoneally (i.p.) or intradermally (i.d.) into a syngeneic strain (BALB/c) of mice. Intraperitoneally injected Meth A tumor cells continued to grow in BALB/c mice, and the mice died around 2 weeks after injection, as expected. Unexpectedly, however, i.d. injected Meth A tumor cells grew transiently in the same strain of mice with a peak on days 8-12, and were rejected around 3 weeks after injection. When we i.p. injected Meth A tumor cells into i.d. immunized mice, the i.p. injected tumor cells were rejected after a several-days growth of tumor. The peritoneal exudate cells harvested on day 6 exhibited a high cytotoxic activity against Meth A tumor cells. These results suggest that the effector cells responsible for the rejection of i.d. injected tumor cells from BALB/c mice or for the rejection of i.p. injected ones from the i.d. immunized mice may be non-T cells and that the effector mechanism can be memorized.

P504 (3P3-175)

Stage and region dependent expression of a radial glial marker in commissural fibers in kindled mice

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Amygdala kindling is regarded as a model of temporal lobe epilepsy in humans because of many points of similarity. In amygdala kindling, bilateralization of epileptic seizures follows from the accumulation of stimulation and commissural fibers may play a role in this process. However, new progenies of cells following amygdala kindling have not been reported and the precise nature of how bilateralization occurs is not clear. In the present study, we aim to clarify the emergence of radial glia during the progress of amygdala kindling in mouse brain. For this purpose, immunohistochemical staining for 3CB2, which is a specific marker of radial glia, was employed. Immunoreactivity for 3CB2 was observed in the forceps minor, radiation of trunk and forceps major regions at Clonus 3 and more strongly at Clonus 5. In the forceps major, the cingulate gyrus showed immunopositive staining at Clonus 3, but the corpus callosum and alveus hippocampi showed staining only at Clonus 5. In the fimbria hippocampus, the anterior commissure posterior showed staining at Clonus 5. However, the anterior commissure anterior was not stained at the stage progressed to Clonus 5. These findings indicate stage and region dependent expression of progenitor cells in commissural fibers and suggest that these changes may accompany the formation of new circuits in seizure progression during amygdala kindling.

P505 (3P3-176)

The involvement of cyclooxygenase in seizure development in kindling mice

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Cyclooxygenases (COX) are potent mediators of inflammation, blood flow and immunomodulation in the pathologically altered brain. Two COX iso-enzymes have been associated with brain disease, the constitutively expressed COX-1 and the cytokine-inducible COX-2. Here we have analyzed the localization of COX-1 and COX-2 in the mouse kindling model by immunohistochemistry. Bipolar electrodes were implanted in the left side of the mouse amygdala. Mice were stimulated once a day with a biphasic 60 Hz square wave pulse at 80-150 μA. Seizure development was evaluated with Racine's criterion. Mice brains were perfused and fixed at C0 (sham), C3 and C5 stages, respectively. Brains were sectioned and immunostained with several antibodies. COX-1 was predominantly observed in microglia and increased in the hippocampus and areas around third ventricle as seizure development. On the other hand, COX-2 predominantly observed in neurons and increased in the hippocampus. These regions are proposed to play important role in the propagation of limbic seizures. Moreover, both SC-560 (an inhibitor to COX-1) and nimesulide (an inhibitor to COX-2) suppressed the progression of kindling stage, however, did not decrease kindling stage after completion of epileptogenesis. While precise mechanism of COX positive cells such as microglia is not clear, results suggest the involvement of both COX-1 and COX-2 in epileptogenesis and inhibitors to COX might be useful to prevent seizure development.

P506 (3P3-177)

Protective effects of ethyl pyruvate on energy metabolism after ischemic stress

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It is reported that administration of ethyl pyruvate (EP) improves the cellular and organ function in ischemia. In rat brain slices (400 μm-thick) superfused with standard artificial cerebrospinal fluid (ACSF) and bubbled with 95% O_2 and 5% CO_2 gas mixture at 25°C, we investigated effects of EP on high energy phosphate and inorganic phosphate (Pi) after ischemia using ^{31}P-nuclear magnetic resonance (NMR). The brain slices were exposed to hypoxia by stopping the perfusion pump for 1 hour, followed by the reperfusion with normal ACSF (control group) or ACSF with 2mM EP (EP group). Creatine phosphate (PCr) level was significantly lower in the EP group than in the control group one hour after the reperfusion (52±2% vs. 49±3%, $p<0.05$), but there was no difference between them two hours after the reperfusion. During the reperfusion period, PCr/γ-ATP ratio remained relatively unchanged in the EP group compared with the control group, and PCr/Pi ratio was significantly larger in the EP group (0.76±0.12, 0.35±0.05, respectively, $p<0.05$). These results indicate a protective effect of EP on energy metabolism of brain tissue exposed to ischemia. EP is a potent free radical scavenger during the reperfusion and this effect might be protective of the damage due to the reperfusion in the brain.

P507 (3P3-178)

Activation of neurons projecting to hypothalamic paraventricular nucleus by systemic immune challenge

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The aim of the present study was to investigate the distribution of central structures which actived by systemic immune stress also projecting into the hypothalamic paraventricular nucleus (PVN) directly. The double-labelled immunohistochemistric technique to show c-fos expression combining with a neuroanatomical retrograde tracer fluorogold (FG) was used. Intraperitoneal (i.p.) injection of lipopolysaccharide (LPS) 125 ug/kg body weight induced a extensive Fos expression in rat brain including cortex, forebrain, hypothalamus and brainstem. The retrograde tracer labelled cells were found following FG microiontophoresis into unilateral PVN, which scattered in hypothalamus, subfornical organ (SFO), median preoptic nucleus (MnPO), ipsilateral bed nucleus of stria terminalis (BNST), nucleus tractus solitarius (NTS), ventrolateral medulla (VLM) and locus coeruleus (LC). Double-labelled cells were found in forebrain and brainstem sites, especially in BNST and NTS. The results suggested that neurons in BNST and NTS projecting to PVN directly could actived by peripheral administration of LPS which might be important on interactions between immune system and nervous system during systemic immune challenge.

P508 (3P3-179)

Poly IC, a synthetic double stranded RNA analogue, induces behavioral suppression and brain Fos expression in mice.

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Behavioral suppression is a common symptom during viral infection though its mechanism is little understood. In this study, we aimed at establishing a mouse model of virally-induced behavioral suppression, and determining the brain loci possibly involved in behavioral suppression using Fos immunostaining. Spontaneous activity of mice was measured using infrared CCD camera. They were intraperitoneally injected with saline at 17:00-17:30 for 3 consecutive days. On the forth day, they were injected with a viral mimic, polyinosinic-polycytidylic acid (poly IC). Activity of mice was significantly suppressed for 2 days following poly IC. Treatment with diclofenac, a nonselective cyclooxygenase inhibitor, ameliorated behavioral suppression only in the early phase (up to 6 h). After 3 h of poly IC injection, significant increase in Fos expression was observed in several brain loci including ventromedial preoptic area, hypothalamic paraventricular nucl., lateral parabrachial nucl., and nucl. tractus soliatrius. Diclofenac also reduced Fos expression in these brain regions. These results suggest that poly IC-induced behavioral suppression consists of 2 phases, early prostaglandin(PG)-dependent and late PG-independent. Brain loci noted above are possibly involved in PG-dependent behavioral suppression.

P509 (3P3-180)

Prophylactic effect and detection against stroke by magnetic stimulation

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This study was to examine whether non-invasive neural stimulation using low repetitive transcranial magnetic stimulation (rTMS) had a prophylactic effect against stroke, and was designed to evaluate the functional response of central motor conduction time (CMCT) to various levels of blood pressure in malignant stroke-prone spontaneously hypertensive rats (M-SHRSP). M-SHRSP, which had severe levels of hypertension, were subjected to eddy current direction alteration of either 0.1 Hz or sham rTMS over the cortex. rTMS did not suppress the elevation of systolic blood pressure in M-SHRSP. rTMS retarded the progression of age-dependent development of stroke, and elongated the lifespan of M-SHRSP. This revealed the prophylactic effect of rTMS on the development of cerebral stroke in M-SHRSP. Next, single magnetic stimulation was applied to the vertex and C3-C4 vertebrae for transcortical and cervical stimulation, respectively. The cortical and cervical motor evoked potentials were detected from the gastrocnemius muscle. CMCT was calculated using the difference of latency in gastrocnemius response between transcortical and cervical stimulation. CMCT was not delayed with increased blood pressure, in contrast to that in normotensive rats. Just before a stroke, CMCT was prolonged to 2.21msec from the pre-stroke value of 0.41msec, and significant prolongation continued after the stroke in M-SHRSP. These results suggest that CMCT does not correlate with increasing blood pressure in hypertensive rats, but an abrupt prolongation of CMCT indicates the occurrence of corticospinal vascular lesions in M-SHRSP.

P510 (3P3-181)

NG2 proteoglycan expressing cells in the ischemic lesion of rat transient MCA occlusion: NG2-expressing microglia in the core transform into neuroectodermal cells in vitro

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Although NG2-chondroitin sulfate proteoglycan (NG2) has long been recognized as a marker for oligodendrocyte progenitor cells (OPCs), recent findings suggest that NG2 may be a marker of multipotent neural progenitors. On the other hand, microglial cells (MG) are also shown to express NG2 in pathologic brains. In the present study, we investigated the nature of NG2-expressing cells in the ischemic brains, whose middle cerebral artery was transiently occluded for 1.5 h. A huge number of amoeboid-shaped Iba1+ cells or MG accumulated in the ischemic core at 7 days post-occlusion (7 dpo), most of which were proliferating (or Ki67+) and NG2+, while proliferating NG2+/Iba1- ramified-shaped cells were present around peri-infarct region. Moreover, such NG2+ MG expressed nestin, GFAP and/or platelet-derived growth factor receptor α (PDGFRα) as revealed by immunohistochemistry, immunoblotting and/or RT-PCR. NG2+ MG were isolated from the ischemic core by treating the damaged tissue with trypsin-EDTA or EDTA alone. Most isolated viable cells were Iba1+ and often formed cell aggregates that were NG2+/nestin+/GFAP+. NG2+ MG had alkaline phosphatase activity in vivo and in vitro. The isolated cells differentiated into cells with neuroectodermal phenotypes that expressed β-tubulin III, GFAP or oligodendrocyte antigen. The present findings suggest that accumulated proliferating NG2+ MG potentially have the ability to act as neural progenitors.

P511 (3P3-182)

Impaired filterability of erythrocytes from patients with chronic hepatitis under combined treatment with interferon and ribavirin: Rheologic effects of eicosapentaenoic acid

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Treatment with interferon (IFN) and ribavirin (RBV) is effective for chronic hepatitis (CH). However, anemia is a serious adverse effect of this combination therapy and we found eicosapenaenoic acid (EPA) is effective to prevent this complication. Aim of this study is to investigate the effects of this therapy on erythrocyte filterability, and the protective mechanisms of EPA on anemia. Subjects included patients with CH (n = 24) and healthy controls (n = 5). EPA was administered to some patients (n = 10). Erythrocyte filterability estimated by nickel mesh filtration method was lower in patients than in controls (69.2 ± 10.8% vs. 80.5 ± 1.69%, $p<0.03$). In patients, filterability was further decreased by the combination therapy (57.8 ± 12.8%) and showed significant correlation with the development of anemia ($r = 0.54$), but not with MCV ($r = 0.21$) nor MCHC ($r = 0.36$). These suggest that impaired filterability arises from erythrocyte membrane damage. Filterability improved in responders of EPA for prevention of anemia (from 64.8 ± 6.6 to 77.4 ± 2.0%, $p<0.05$). In conclusion, patients with CH showed impaired erythocyte filterability, which is further reduced by the treatment with IFN and RBV, leading to hemolytic anemia. EPA restrained progression of anemia by improving erythrocyte filterability.

P512 (3P3-183)

The effect of acupuncture stimulation on the blood fluidity in rats: study I

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In Japanese Oriental medicine, the concept of "oketsu" is one of the most essential standard to diagnose patient's pathological state. Oketsu is considered as a stagnation of blood flow. For the therapy of oketsu, harbal medicine and/or acupuncture stimulation are often used and desirable results are obtained. However, the basic study regarding with the effects of harbal medicine or acupuncture stimulation on blood fluidity were very few. In this study, we attempted to clarify the effects of electroacupuncture stimulation on blood fluidity in rats using MC-FAN method. We selected four acupuncture points. Those were Saninko(Sp6), AshiSanri(S36), Meimon(GV4), and Naikan(P6). The stimulations were applied 1 hr a day for 2 days. The stimulation was applied at intensity to cause slight muscle twitch and at 1 Hz frequency. The blood was collected from inferior vena cava under urethane-chloralose anethsesia. Acupuncture stimulation to Saninko, AshiSanri, and Meimon augmented blood fluidity but stimulation to Naikan did not showed significant change. These results indicate that acupuncture stimulation is able to change blood fluidity and the effects of acupuncture stimulation on blood fluidity differ depending on acupuncture points.

P513 (3P3-184)

The effect of acupuncture stimulation on blood fluidity in the rats: study II

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The slow developing therapeutic effects of harbal medicine and/or acupuncture stimulation are well known. We have already reported that administration of harbal medicine for 1 week augmented blood fluidity in rats but single administration did not show significant effect. We attempted to investigate the effect of brief acupuncture stimulation (1Hz, 1 hr) on blood fluidity in rats in this study. Acupuncture stimulations were applied to AshiSanri, Goukoku, and abdominal muscle. The intensity was adjusted to cause slight muscle twitch. The blood fluidity was measured by MC-FAN method. Acupuncture stimulation to AshiSanri and Goukoku enhanced blood fluidity but to abdominal muscle did not. These results suggested that even brief acupuncture stimulation is useful for improvement of blood fluidity and the location of acupuncture stimulation may affect to the effect of acupuncture on the blood fluidity.

P514 (3P3-185)

The effect of adrenergic agonists on the blood fluidity of rats: *in vitro* experiment

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We have already demonstrated that some stressors impaired blood fluidity through the activation of adrenergic system in rats. Adrenergic alpha agonist and beta antagonist decreased blood fluidity. On the other hand, alpha blocker and beta agonist enhanced the blood fluidity. In the present study, we attempted to clarify the effect of adrenergic reagent *in vitro* system. Blood was collected from inferior vena cava into a test tube under pentobarbital anesthesia. Adrenergic reagent was added into the test tube. As a control the same volume of saline was added into the test tube. After 30 minutes of mixing, blood fluidity was measured using MC-FAN. Alpha agonist diminished blood fluidity. On the other hand beta agonist enhanced blood fluidity. These results were same as that obtained in the *in vivo* experiments, which suggested that the effect of adrenergic agonists on the blood fluidity was a direct action to the blood.

P515 (3P3-186)

SMemb-specific siRNA expression vector inhibited bound thrombin-induced upregulation of SMemb gene in cultured rabbit vascular smooth muscle cells

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We previously demonstrated that bound thrombin upregulated embryonic myosin heavy chain (SMemb) and plasminogen activator inhibitor type1 (PAI-1) mRNAs in cultured rabbit aortic vascular smooth muscle (VSM) cells. The present study was aimed to examine whether the bound thrombin-induced upregulation of SMemb and PAI-1 mRNA expressions was prevented by a knockdown of SMemb gene. We also tested whether the knockdown of SMemb gene inhibited DNA synthesis, cell proliferation and migration of VSM cells. We constructed siRNA expression plasmid (p*Silencer*^{TM}) vector targeting SMemb mRNA (ORF-2 siRNA). Northern blot analysis revealed that ORF-2 siRNA inhibited SMemb mRNA expression. Imuunohistochemistry demonstrated that SMemb protein expression was depressed in ORF-2 siRNA-treated cells. Bound thrombin-induced upregulation of SMemb mRNA expression was significantly inhibited at 48 hr after transfection with ORF-2 siRNA. The DNA synthesis, cell proliferation and migration of VSM cells were measured at 48 hr after transfection of ORF-2 siRNA using BrdU ELISA, tetrazolium salt WST-1 and FluoroBlok^{TM} Insert system, respectively. ORF-2 siRNA treatment did not inhibit bound thrombin-increased DNA synthesis and cell proliferation. ORF-2 siRNA treatment, however, tended to decrease the migration activities of VSM cells. Therefore, the knockdown of SMemb mRNA by ORF-2 siRNA may prevent bound thrombin-induced phenotypic modulation in cultured VSM cells.

P516 (3P3-187)

Molecular involvement of Homor in mouse heart failure model

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Homer is a scaffolding protein that exists in post synapse of neurons and also identified in heart. However, the roles of Homer in heart remains to be elucidated. In this study, we examined the characteristics and functions of Homer in heart. We identified that Homer1c and Homer2a/Cupidin (CPD) existed in heart and it linked with Ryanodine receptors (RyRs). In primary cultures of neonatal mouse hearts, Homer preceded RyRs on Z band distribution. As to muscle contraction, Homer and RyRs showed similar expression patterns, which suggested that Homer regulate the EC coupling. Then we made mouse heart failure model by ligation of left coronary artery and examined the dynamics of Homer in remodeling of congestive hearts. In our mouse heart failure model, RyRs reduced their expression in protein level and long form Homer showed similar patterns. In order to clarify these phenomena, we made GFP-CPD and GFP-CPD/N expressing recombinant adenoviruses and performed physiological studies with infected neonatal cardiac myocytes. As a result, Homer might be involved in remodeling of failing hearts from the aspect of Ca^{2+}dynamics.

P517 (3P3-188)

The voiding pattern in animal model of urinary bladder instability produced by bladder outlet obstruction in vivo.

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Bladder instability (Over active bladder) is one of the most common phenomenon in patients with lower urinary tract obstruction such as benign prostate hyperplasia (BPH) and urethral stricture. The cause of the bladder instability is, however, not clear at present. To investigate the phenomenon, an animal model of urinary bladder instability was produced by ligation of the rat proximal urethra uncompletely, and six weeks after the ligation, urinary volume and voiding behavior was evaluated by continuous unanesthetized cystometry. In the present study, we also evaluated continuous unanesthetized cystometry and investigated the effect of several drugs on bladder instability. Voiding behavior patterns of 25 obstruction model rats were compared with that of 10 normal rats and were divided into several voiding pattern used by continuous cystometry. The many model rats had premicturition contraction in storage phase but the model rats had several voiding patterns after the same operation methods. Voiding behavior were unstable at first, but become stable with time. The micturition interval were 365s±51s at 2250s after the beginning of measurements and 770s±104s at 7250s, respectively($p<0.05$) and attained plateau 2hours after. Therefore it is important to evaluate the quality of models and pay attention to the timing of administration of several drags on experiments.

P518 (3P3-189)

Impaired spatial learning in muscular dystrophy (mdx) mouse

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A non-progressive cognitive impairment is observed in about 1/3 of Duchenne muscular dystrophy (DMD) patients. Mdx mouse, an animal model of DMD, is reported to have impaired retention in a T-maze and a passive avoidance task, but not a spatial learning. We reinvestigated the spatial learning by means of the Morris water maze in weaning (3-4 wks-old, male), young (10-14 wks-old, male), old mdx mice (14-16 mo, female) and their age-matched control. In the Morris water maze, mice were given a total of three trials a day on 4 consecutive days. Escape latency, the time taken for the animal to locate and climb onto a hidden platform (7 cm diameter) after being placed in the water in a pool (86 cm diameter), shortened greatly during the training days in all of the three different age groups of the control. On the contrary, the escape latency shortened only a little in all of the three different age groups of the mdx mice. The velocity of swimming as determined by dividing the total swimming distance by the escape latency was much smaller in young and old mdx mice, and only a little in weaning mdx mice than the control. Administration of nicotine to the old mdx mice and the control before the exercise increased the velocity of swimming in the both but had little effect on their escape latency. The result indicates that mdx mice have impaired spatial leaning from the weaning period throughout all stage of life independently from the degeneration-regeneration of their skeletal muscle.

P519 (3P3-190)

Comparison of whole blood serotonin (5-HT) level between pre- and post-menstrual phases in human.

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Decreased serotonin is known to be associated with depression in human. The present study was conduncted to evaluate physiological basic of premenstrual syndrome (PMS). Whole-blood (WB) 5-HT levels in young female subjects were measured during the follicular phase (F) and premenstrual (PM) phase of the menstrual cycle. Subjects were required to record basal body temperature and to complete a symptom diary once each day. Mood States were evaluated by POMS questionnaire Score. WB 5-HT level in the healthy subjects with no or less PMS symptoms showed significant increase during PM phase, as compared with that during F phase. However, WB 5-HT level in those subjects with PMS symptoms was lower during PM phase than that during F phase. These results suggest that an impairment of 5-HT system during PM phase might be responsible for a development of PMS.

POSTERS
Miscellaneous—modeling & simulation, methodology, history, etc.

P520 (1P3-188)

Relation between the irradiation intensity of Nd:YAG laser and the suppressive effect on the conduction of Xenopus tactile nerve fibers

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For several years, we have studied the pain control effects of Nd:YAG laser in dental clinical treatment, and showed the considerable pain relief by this treatment. In previous reports, we have showed the direct effects of the Nd:YAG laser irradiation on nerve discharges of Xenopus tactile nerve fibers with the Chinese ink painting. Nerve discharges were reversibly suppressed with the laser irradiation. This result strongly suggests that the pain relief in clinical treatment is not only the Placebo effect but also direct effect on the conduction of the nerve discharges. In this study, we investigated the relation between the irradiation intensity and the suppressive effects on conduction of the Xenopus tactile nerve fibers. Dorsal surface skin of Xenopus was removed together with fine tactile nerve bundles. Chinese ink was painted on a dissected fine nerve bundle. From this bundle, nerve discharges with tactile stimuli on the skin surface were recorded by a silver electrode, and counted by the spike counter. After Nd:YAG laser irradiation with various pulse height and duration, tactile responses were periodically recorded with paying enough attention to stimulate the same surface area under the dissection microscope.The reversible suppression of nerve discharges depended upon the total energy rather than the pulse height of the laser irradiation.

P521 (1P3-189)

The modification of traditional device to record the force and length in small animal's isolated papillary muscle.

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We modified the measuring system to record force and length in small animal's isolated papillary muscles. The measuring system was constructed by pen-motor system, control system and circulated system. The Ag-AgCl electrode and constant voltage regulator were used for the stimulation. In the circulation, Tyrode solution was used. Other physiological parameters were set as standard conditions. Physiological data were converted into the digital signals and plotted on the paper.In the experiment of small papillary muscle, the frequency range is high rather than not small animals, and the noise level become critical factor because the signal level is so small. The system was fabricated in consideration of these points.As a result, it was able to record the force and length of the small animal's papillary muscle such as a gerbil or mouse by using the modified system.

P522 (1P3-190)

Suppressive effect of Juzen-Taiho-To on the lung metastasis of B16 melanoma cells *in vivo*

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Juzen-Taiho-To (JTT; Si-Quan-Da-Bu-Tang in Chinese) is one of Kampo (Japanese herbal) medicine,that consists of 10 component herbs, and frequently used for the treatment of depressed or weakened states including fatigue, appetite loss, anemia and anorexia in chronic disease. Although JTT is used for the prevention of metastasis of several cancers, such as colon and breast cancer, the precise mechanism by which JTT could prevent cancer metastasis. Therefore, the present study was carried out to examine the possible anti-metastatic mechanism of JTT in vivo C57BL/6 male mice were fed normal mouse chew contained 0.2% JTT or 1% JTT for three weeks. These mice were injected intravenously with 2×10^5 B16 cells, seven days starting JTT administrated. Metastasis of B16 in lung tissues were examined two weeks later.JTT at 1% (but not 0.2%) caused significant inhibition of B16 metastasis in the lung. This suppressive activity of JTT on metastasis was abrogated by the pretreatment of mice with anti-asialo GM1 antibody. These results strongly suggest that JTT enhances NK cell activity and results in inhibition of cancer metastasis *in vivo*.

P523 (1P3-191)

Physiological role for *C. elegans vps-45* in endocytosis

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Sec1/Munc-18 (SM) family members are important for various membrane trafficking pathways in eukaryotic cells, but their precise roles are still unclear. To elucidate unifying roles for SM proteins in multicellular organism, we have systematically isolated deletion mutations for *C. elegans* SM genes. We found that depletion of SM proteins resulted in severe lethality, suggesting that these proteins appear to be essential for development and viability in *C. elegans*. The putative null mutation of *vps-45* also showed a larval lethal phenotype and transgenic analyses using fluorescent reporters revealed that both pinocytosis and receptor-mediated endocytosis were impaired in *vps-45*. To investigate a physiological role for VPS-45 in endocytosis, we identified the cognate syntaxin for VPS-45, because extensive studies in several organisms have suggested that all SM proteins interact with members of syntaxin family (Q-SNAREs) and may regulate SNARE complex formation. We found that VPS-45 exhibits specific interactions with PEP-12 and TLG-2 among 9 syntaxins of *C. elegans*. To elucidate whether *vps-45* functions with the cognate syntaxins *in vivo*, we isolated the deletion mutations and found that even in the double mutant of these syntaxin genes did not exhibit a phenocopy of *vps-45* mutant. These results suggest that specific binding of SM proteins to syntaxins has probably important aspects, but SM proteins might also have syntaxin-independent functions in endocytic trafficking. Further studies for characterizing the functions of other genes that act in concert with *vps-45* are also in progress.

P524 (1P3-192)

Adenosine-induced neuroprotective actions in ventral horn neurons of the rat spinal cord.

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To date, four subtypes of adenosine receptors, A_1, A_{2A}, A_{2B} and $_{A3}$ receptor, have been cloned and characterized.Although it has been reported that adenosine has neuroprotective effects, such as reducing the mortality of neurons and improving neurological deficits following brain injuries, it is still unknown whether adenosine has a neuroprotective action in the spinal cord. In the present study, we investigated cellular actions of adenosine in spinal ventral horn neurons by using whole-cell patch-clamp recordings in spinal slice preparations.Application of adenosine significantly decreased the frequency of spontaneous excitatory postsynaptic currents (sEPSC) in all ventral horn neurons recorded.An adenosine A1 selective agonist, CPA, also reduced sEPSC frequency in all ventral horn neurons recorded.In the presence of an adenosine A1 antagonist. DPCPX, adenosine or CPA did not decrease sEPSC frequency in all ventral horn neurons recorded.In addition, application of adenosine and CPA also produced outward currents in most of ventral horn neurons tested. In the presence of DPCPX, those outward currents were significantly suppressed. This study has provided a cellular basis for an involvement of pre- and postsynaptic adenosine A1 receptor in the neuroprotective actions of adenosine in the spinal ventral horn. Adenosine A1 receptor agonists may serve as a potential pharmacological tools to reduce loss of motor function in spinal cord injury patients.

P525 (1P3-193)

Introduction of experiential studies to the education of physiology in a nursing school at medical university

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In nursing education, physiology stands as the basic science to understand the structure and function of the human body. But it holds difficulties because of low participation of scientific subjects (i.e. physics and chemistry) in the entrance examination, and early start of study of physiology after entrance to school usually with weak motivation for it. To assist for the students to learn physiology more easily and firmly, we introduced experiential (practical) studies. Among total class work time of 135 hours (5 units)of the subjects "Structure and function of the human body", 16 hours were assigned to practical learning. The items of Physiology practices includes "Elements of blood and understanding of anemia", "Measurement of composition of body with impedance method using InBody 3.0, "Spirometry and respiration", in which students studied on their own bodies. As for laboratorical works, they did "Preparation and dilution of aqueous solutions and introductory study on buffering solution" and "Excitability of nerve and muscle, and muscle contraction"(mainly demonstration by teacher). In addition, students had 4 hours of class work of anatomical study to investigate pre-dissected corpora of volunteers in Department of Anatomy, School of Medicine, Jichi Med.Univ. In this report, basic statistical data for body compositions of 300 subjects (288 females, 12 males) with permission by informed consent are presented. They consist of body weight, ICF, ECF, mass of protein, mass of bone, mass of body fat, mass of muscle, BMI, BMR and others.

P526 (1P3-194)

Intravital Ca imaging by fiber-coupled realtime confocal microscopy

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To study Ca signals in situ in realtime, we developed a fiber-coupled confocal microscope, and observed fluorescently-labeled cells in the brain of anesthetized rat, or cultured cells. The microscope system consisted of DPSS laser (488 nm, 200 mW), microlens-attached Nipkow disk scanner (CSU10, Yokogawa Co), and a lens-coupled imaging fiber unit. The imaging unit had over 20,000 of single mode fiber of 3 micron in diameter, which was scannned with Nipkow disk to form a confocally sectioned image. The effective NA value was approximately 0.2. The thickness of the sectioned image was < 5 micron as estimated from the HWHM of the fluorescence intensity measured with the focus shift (over a fluorescent bead of 10 micron in diameter). The fiber unit was 2.2 mm thick, for easier approach to the target inside the brain without a large physical damage. The confocal images were captured with an electron multiplying-CCD camera at over 30 F/s. For intravital imaging, we inserted the fiber unit into the brain, in which some neurons were labeled with Calcium orange. When the incidental face of the fiber unit was placed 3 mm below the brain surface, many bright spots and fibrous structures were clearly discernible. They showed fluctuation in fluorescence intensity partly dependent on the breathing and possibly on some neuronal activities. Such an in situ imaging technique is very promising for detailed studies on the relationship between neuronal structure and functions.

P527 (1P3-195)

Dynamic responses of the cranium and the cervical vertebrae to occlusal force

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To investigate the mechanical responses of the cranium and the cervical vertebrae during application of occlusal force to the teeth, occlusal force was applied to the teeth in a Japanese monkey fixed in a standing position under anesthesia, by electrically stimulating the central area of the bilateral masseter muscles. The following results were obtained:1. The sphenoid bone in the cranium was pressed by the zygomatic bone, which is anterior to the sphenoid bone, from the antero-superior direction, and was pressed by the temporal bone, which is posterior to the sphenoid bone, from the postero-inferior direction. Since the sphenoid bone is a single bone, and is anatomically located at the center of gravity of the cranium, the sphenoid bone was regarded as the mechanical center in the cranium.2. When the first molar on a unilateral side was forced to bite substances, the lamina of the vertebral arch of the second cervical vertebra on the non-working side was markedly extended in the supero-inferior direction. This suggested that the second cervical vertebral dens adjusts the cranial position to prevent the cranium from excess tilting to the working side. These results revealed that when occlusal force was applied to the teeth, the sphenoid bone controls the antero-posterior and bilateral movements of the cranium, and the second cervical vertebral dens controls the balance of the movement of the whole cranium.

P528 (1P3-196)

Evanescent wave illumination with a varied angle of light incidence to the interface for 3D observations of molecular events near the cell membrane

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The evanescence microscope with a high resolving power is a useful tool for the observation of molecular dynamics. However, an accurate tracking of molecules in the intracellular space is difficult because the evanescent wave for the fluorescence excitation is restricted only to the area close to the coverslip. To overcome this problem and to study the dynamic events in the cytoplasm, we modified the evanescence microscope with an ultrahigh NA objective lens. We employed a galvanomirror to aim and switch the laser beam rapidly at the back focal plane of an 1.65 NA objective near its periphery so that the penetration depth of evanescent wave varied depending on the beam-incidence angles at the interface. Under this microscope, trafficking of secretory vesicles was studied in MIN-6 cells expressing insulin-GFP. Stimulation of the cell with glucose induced a transient increase in GFP fluorescence, but evanescence images acquired alternately at a 0.5 s interval were slightly different in spite of the same field of view. This result reflects temporal and spatial dynamics of secretory vesicles prior to exocytosis. The ultrahigh NA lens provides a large window for evanescent wave illumination with a wide range of penetration depth, thus is useful for analyzing 3D events in the cell.