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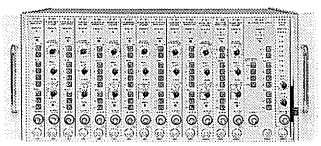
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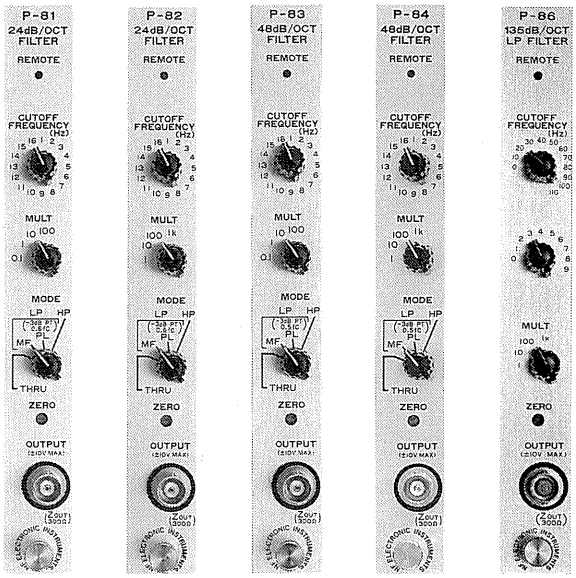
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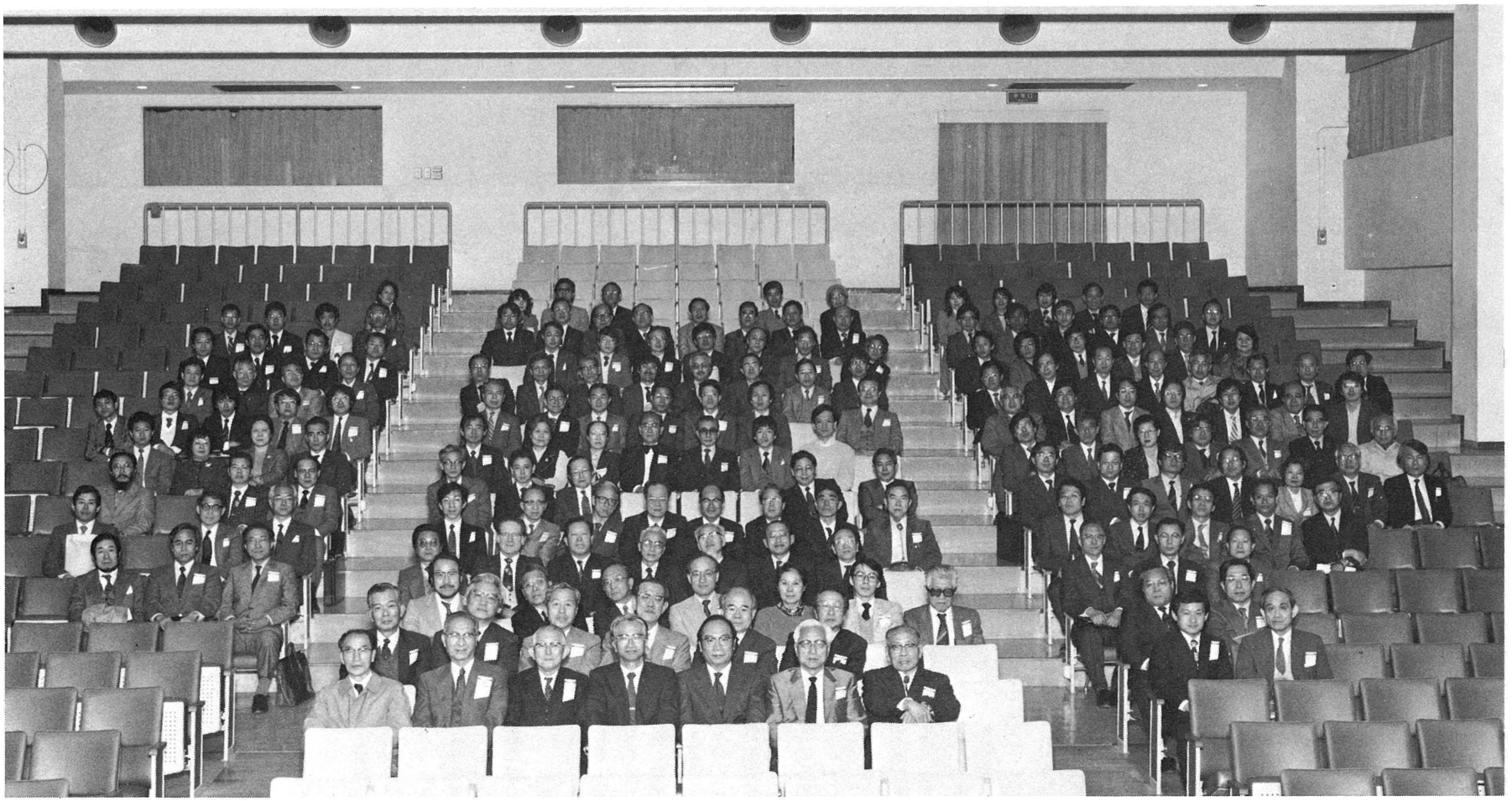
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第62回　日本生理学会大会　　昭和60年 3 月28～30日　於 久留米大学御井学館

第62回日本生理学会大会を終えて

第62回日本生理学会大会
当番幹事　嶺　巓　教　三・西　　彰　五　郎

足掛3年の間，計画し，準備してきた第62回日本生理学会大会を無事終了することができた．当番幹事をお引き受けした時，大会運営の企画が当番幹事に白紙委任されているということを生かして，何か従来の生理学会大会とは異なった企画をしてみようと考えた．そこで考えついたのは，従来，生理学会大会では行われていないシンポジウムを企画することであった．まず，シンポジウムのテーマとしては，この数年来の日本生理学会大会の演題を調べ，その数の多い研究分野，即ち，日本の生理学会員の数が多い研究分野を6つ選び出した．そして，その夫々の分野において活躍されている若手の教授の中のお1人に座長になっていただくことにした．生理学会には活発に研究されて業績を上げておられる若手の教授が多く，座長選定には苦労したが，当番幹事の独断として容赦していただきたい．6題のシンポジウムは3日間の学会期間中，1つの会場で毎日午前と午後1題ずつ行われ，合計30題の演題が発表された．シンポジウムと同時に他の8つの会場では一般口演の発表（3日間で合計552題）が行われていたので，シンポジウムのテーマと，同じ研究分野の一般口演のテーマと時間的に重ならないようにプログラム作成に当っては細心の注意を払ったつもりである．

今回の生理学会大会でシンポジウムとともに大成功であったと思うのは2題の特別講演である．九州大学医学部の後藤昌義教授のお世話で，国際生理学会の重鎮であるニューヨーク州立大学教授 C. Brooks 先生と，岡崎の国立生理学研究所の渡辺　昭教授のお世話で，日本生理学会の大先輩である米国国立精神衛生研究所の田崎一二先生の特別講演を催すことができたのは，大会当番幹事として感謝にたえないことであった．この2題の特別講演はともに非常にユニークな内容で格式高いものであった．Brooks 先生の講演は生理学者としての研究の進むべき道の示標を与えて下さったものであり，田崎先生の講演は，真に実験を愛し，研究に一生を捧げておられる先生のお人柄と心情がにじみでた研究成果のご発表であった．この2題の特別講演は満場の聴衆に強烈な印象を与えたものと確信している．この他，大会では大阪大学の中馬一郎教授のお世話により，生理学教育シンポジウムが開催され「生理学の新しい分野への教育的展望」について活発な討論が行われた．このシンポジウムが時間的に特別講演と重なったことは，プログラム作成上やむを得なかったこととはいえ，非常に申し訳なく残念であった．

一般口演の他，188題の演題はポスター展示により発表が行われた．学会発表の方法としてポスター展示を行うことの是非については，いろいろと意見があると思うが，私どもはポスター展示による発表の利点は充分に評価していいと思っている．

大会期間中，出席していただいた生理学会会員の方々の懇親会は予算の都合上催すことができなかった．しかし，研究分野に分れて11のグループディナーが一夜楽しく挙行され，研究交流と親睦の会がもたれたことは懇親会以上の成果が上ったものと考えている．

最後に，日本全国からはるばる九州の小都市久留米までおいでいただき，学会に出席していただいた方々には色々とご不便な点，ご満足できなかった点が多々あったと思われる．改めてここにお詫び申し上げる次第である．

THE 62nd ANNUAL MEETING
OF
THE PHYSIOLOGICAL SOCIETY OF JAPAN

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Kurume University School of Medicine

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SYMPOSIUM I

I. Synaptic transmission in the vertebrate retina.

S - I

SYNAPTIC TRANSMISSION IN THE VERTEBRATE RETINA. KANEKO, A. National Institute for Physiological Sciences, Okazaki 444

Phototransduction and processing of the visual information are the two main functions of the vertebrate retina, which is a thin nervous tissue (measuring about 200 μm) consisting of six different types of neurons orderly arranged in three layers. Since the retina can be maintained functioning even after isolation, it provides a compact and useful model of the central nervous tissue, and has been regarded as the "E. Coli of CNS". However, the analysis of the retinal circuitry is not a symple task.

Difficulty arises from complicated network of closely packed retinal neurons including bidirectional synapses (e.g., photoreceptor -- horizontal cells, bipolar -- amacrine cells, and amacrine -- amacrine cells) and electrical coupling (among photoreceptors, and among horizontal cells). Physiological, morphological and pharmacological methods, or their combination, have been the main techniques used to study the function of the retina: the application of superfine microelectrodes enabled intracellular recording from all types of retinal neurons responding to various types of photic stimuli or to extrinsic chemical compounds; and staining of recorded neurons with intracellular marker dyes provided not only a reliable basis for cell identification but also the precise morphology of specific neurons. Electron microscopic observation of Golgi-impregnated cells or of cells filled with an electron dense tracer material provided valuable morphological information. Studies on solitary retinal cells dissociated from the network have offered many pieces of important new information on the synaptic mechanisms of the retina, which are difficult to obtain from the whole retina preparation. In this symposium, the contributors discuss several key questions to understand the retinal functions. Some of them are as follows;

1. What is the transmitter substance released from photoreceptors?
2. How are the photoresponses of horizontal cells formed?
3. Is the feedback pathway present from horizontal cells to photoreceptors?
4. If so, what is the transmitter substance released from horizontal cells?
5. How are the color-opponent responses made?
6. How are ON and OFF responses of bipolar cells made?
7. What are the mechanisms underlying center vs. surround responses?
8. What is the role of amacrine cells in the formation of receptive fields of bipolar and ganglion cells?
9. How are ganglion cells differentiated in relation to their functions?
10. What is the transmitter substance mediating signals to ganglion cells?

S - I (1)

STUDIES OF SYNAPTIC MECHANISMS OF PHOTORECEPTOR-BIPOLAR TRANSMISSION IN THE RETINAS. SAITO, T. Dept. of Physiol., St. Marianna, Univ. Sch. of Med. 2095, Sugao, Miyamae-ku, Kawasaki.

Bipolar cells,second order neurons in vertebrate retinas,can be classified into depolarizing(ON) and hyperpolarizing(OFF) cells according to their response polarizity to white light spot in their receptive field center. Electrical membrane properties and photoreceptor conectivities of ON-and OFF-bipolar cells in the carp retina were studied by means of intracellular recording and intracellular injection of HRP. Light and electron microscopy revealed that these functionally different bipolar cells make synaptic contacts with both rods and cones. ON-bipolar responses mediated by rod inputs were generated by the ionic mechanism with a positive reversal potential, while those mediated by red cones were generated by the ionic mechanism with the negative reversal potential. OFF-bipolar responses were generated by the ionic mechanism with a positive reversal potential only, regardless the type of photoreceptors. Both ON-and OFF-cells can be divided into to subtypes (I and II) according to the relationship between the position of their dendritic processes and the synaptic ribbons in the receptor terminal. The type I ON-bipolar cell is characterized by a large cell body, a thick primary dendrite, and a big swelling on the axon terminal in the innermost part of the inner plexiform layer(IPL). Dendritic processes of this cell type make predominantly ribbon contacts with rods and non-ribbon contacts with cones. The type II ON-bipolar cell, having a large dendritic tree in the outer plexiform layer and a large ramification of the axon terminal extending over the inner part of the IPL, makes mostly non-ribbon contacts with rods and cones. Many of these type II cell processes, however, terminate very close to cone synaptic ribbons. The type I OFF-bipolar cell shows two varieties of axon terminal structure: a large terminal swelling or a large flat ramification of the terminal in the outermost part of the IPL. Type I off-center cells make predominantly ribbon contacts with rods and cones, and usually their dendritic process runs parallel to the synaptic ridge apex of cones. The type II OFF-bipolar cell, showing a large ramification of the axon terminal extending over the outer half of the IPL, makes mainly non-ribbon contacts with rods and cones. Results suggest that the type of synaptic contact made with receptors probably does not determine bipolar cell response polarity, and that there is no correlation between different ionic mechanisms and synaptic contact patterns.

The functional significant of the different synaptic contacts between photoreceptors and bipolar cells still remains to be determined.

S - I (2)

SYNAPTIC TRANSMISSION BETWEEN PHOTORECEPTORS AND HORIZONTAL CELLS: STUDIES USING SOLITARY NEURONS AS A NEW TOOL. TACHIBANA, M. and KANEKO, A. Department of Information Physiology, National Institute for Physiological Sciences, Okazaki 444

The interaction between photoreceptors and horizontal cells is supposed to provide a basic process of the center-surround antagonism in the receptive fields and the color opponency. We analyzed the effects of transmitter candidates on solitary photoreceptors and horizontal cells dissociated from enzyme-treated retinae. In this type of studies, solitary neurons are advantageous over the whole retina preparation in several aspects; lack of interference via neuronal circuit, no diffusion barriers nor uptake by glial cells, and successful application of voltage-clamp and patch-clamp techniques to analyze the ion-conductance mechanism.

1. Effects of L-glutamate (L-Glu), a candidate of transmitter released from photoreceptors, on solitary horizontal cells of Carassius auratus: L-Glu evoked membrane depolarization. The least effective dose was approximately 10 μM. The L-Glu sensitivity was diffusely distributed over the cell membrane. One hundred μM dose produced a maximal response, while higher dose rather decreased the response amplitude, perhaps due to the antagonistic effect of L-Glu itself. The reversal potential of the L-Glu-induced response was approximately 0 mV, which is close to that of the light-evoked response. L-Glu increased the membrane permeability to monovalent and divalent cations (with low selectivity for the ionic species) but not to anions. In addition, L-Glu had a blocking effect on the anomalous rectifier potassium channels. L-Glu reduced the number of available channels, but not affected the single channel conductance, open probability, or the open or close time constants.

2. Effect of γ-amino butyric acid (GABA), a candidate of transmitter released from the monophasic horizontal cell, on solitary photoreceptors of the Geoclemys reevesii: GABA sensitivity was high in red-sensitive cones and green-sensitive cones, but was extremely low in blue-sensitive cones and rods. The sensitivity was localized to the cone pedicle where cones make (bidirectional) synapses with horizontal cells. The dose-response relation was sigmoidal and the threshold dose was 10 nM. GABA increased the membrane permeability to Cl^- ions almost exclusively. The reversal potential of the GABA-induced response in in situ cones was estimated to be more negative than -40 mV, thus GABA induced membrane hyperpolarization.

In summary, these results support the ideas that photoreceptors make L-Glu-mediated excitatory (sign-conserving) synapses on horizontal cells, and that the monophasic horizontal cells make GABAergic inhibitory (sign-reversing) synapses on specific types of cones.

S - I (3)

THE CHOLINERGIC AMACRINE CELLS IN THE RABBIT RETINA

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The acetylcholine synthesizing neurons in the rabbit retina have been shown by autoradiography, to be positioned in roughly equal numbers on both sides of the inner plexiform layer. In an attempt to learn the shape of cholinergic amacrine cells, we have developed a method in which the cells are selectively injected with marker compounds after staining of their nuclei with a fluorescent dye. The fluorescent DNA stain 4,6-diamidino-2-phenylindole (DAPI) was injected intravitreally in amount of 0.5μg. Two days after, the retina was removed, fixed for approximately an hour in the mixture of 2% paraformaldehyde and 0.1% gultaraldehyde in 0.1M phosphate buffer, and mounted flat. For unknown reasons, the blue fluorescent dye is selectively accumulated by a certain group of neurons in the ganglion cell layer. Those cells were identified as cholinergic amacrine cells by their ability to synthesize acetylcholine and the escape from labeling by retrograde transport of dye. In a fluorescent microscope, the use of micropipettes filled with lucifer yellow CH allows penetration of the labeled neurons under visual control. It turned out that the neuronal membrane maintain its integrity after fixation of the retina so that lucifer dye injected into cell body could fill the dendrites well. Neurons of the retina stored at 4 C in 0.1M phosphate buffer containing antibiotics could be filled successfully 3 month after fixation.

More than a thousand DAPI labeled cells of the ganglion cell layer were successfully injected. The cells stained with DAPI were found to form a single morphological type, with uni-stratified dendritic arborization in a flat plane. They have two to seven primary dendrites, which branch repeatedly and form a round or slightly oval dendritic tree. The shape and size of the dendritic tree were continuously graded across the retina; the dendritic tree is narrower and the branching denser in the central retina, wider and sparser in the peripheral retina. They have very thin dendrites, especially in their distal third, abundant varicosities. A mirror symmetrical population of cells was also identified in the inner nuclear layer. From knowledge of the population density and the shape of the neurons, the array of dendrites within the retina was reconstructed. The overlap of dendritic field is an order of magnitude greater than for any other retinal neurons thus far described. Because the cells not only overlap greatly but branch profusely, a very dense plexus of cholinergic neurons is created. Since amacrine cells have inputs and outputs intermixed along a single process, many cholinergic dendrites that overlie any location on the retinal surface may have powerful local action on the target cells(motion sensitive ganglion cells are the substrate of the cholinergic amacrine cells).

S - I (4)

STRUCTURAL AND PHARMACHOLOGICAL BASES FOR PARALLEL PROCESSING OF SPATIAL AND TEMPORAL INFORMATION WITHIN THE CAT'S RETINA.

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In the last decade, it has been established that the retinal ganglion cells of the cat can be classified into W-, X-, and Y-cells according to their response properties to photic stimuli (Rodieck, 1979, Ann. Rev. Neurosci. 2:193-225). In contrast to W-cells, which contain several subtypes, X- and Y-cells are considered to be distinct and homogeneous functional classes which extract a spatial difference in luminance (X) and a temporal change in luminance (Y), respectively.

Anatomical investigations have also divided the cat's retinal ganglion cells into three classes; alpha, beta and gamma cells, and suggested a correlation with functional classes, i.e., alpha to Y-cell, beta to X-cell and gamma to W-cell, respectively (Boycott & Wässle, 1974, J. Physiol. 240:397-419). This correlation was directly proved by combining intracellular recordings of responses and injections of Lucifer yellow (Saito, 1983, J. Comp. Neurol. 221:279-288) or HRP (Fukuda et al., 1983, Neurosci. Lett. Suppl. 13:S125, Stanford & Sherman, 1984, Brain Res. 297:381-386) into the ganglion cells. X-cells, which showed sustained depolarization (on-center) or hyperpolarization (off-center) in response to a spot of light presented at the receptive field center, spread bushy dendrites within a narrow circular retinal area. On the other hand, on-center and off-center Y-cells, which showed transient responses to the spot stimulus, expanded sparsely branched thick dendrites far from the cell body. A narrow dendritic field permits the X-cells to analyze spatial contrast with a high resolution, whereas a wide expansion of Y-dendrites ensures temporal summation of inputs over a wide retinal area for keeping high sensitivity to a luminance change. It was also confirmed that on-center/off-center distinction is based upon the difference in dendritic lamification within the inner plexiform layer; on-center cells lamify the sublamina b, whereas off-center cells lamify the sublamina a.

X- and Y-cells are also different pharmacologically. It has been shown from the observation of effects of iontophoretically applied putative transmitters and their antagonists that aspartate works as an excitatory transmitter at on-center and off-center X-cells, whereas acetylcholine operates at both types of Y-cells (Ikeda & Sheardown, 1983, Vision Res. 23:1161-1174). Although there is a contradiction among authors, our observations on the effects of antagonists applied to the perfusing medium of the cat's eye-cup preparations suggested an involvement of different neurotransmitters in the surround mechanisms of X- and Y-cells; the surround inhibition of on-center X-cells was blocked by glycine-antagonist, whereas that of on-center Y-cells was blocked by GABA-antagonist (Saito, 1981, Brain Res. 212:243-248).

All of these observations clearly indicate the strict differences in structural and chemical designs for the achievement of parallel processing of different visual information within the retina.

SYMPOSIUM II

II. Active transports : secretion and absorption in the gastrointestinal epithelia.

S - II (1)

Ca^{++}-ACTIVATED IONIC CHANNELS AND THEIR ROLE IN FLUID SECRETION IN EXOCRINE ACINAR CELLS. MARUYAMA, Y. Ist. Dept. of Physiol., Tohoku Univ. Sch. Med., Sendai 980.

1. Two types of Ca^{++}-activated ionic channels have been identified so far on the basolateral cell membrane from exocrine glands with the help of "Giga-Seal" single channel current recordings. Experiments on the mouse and rat pancreatic glands first showed voltage-insensitive channels (30 pS) not descriminating Na^+ from K^+ (Na^+/K^+-channel), whereas on the salivary glands showed the presence of high conductance (200-250 pS), purely K^+-selective voltage-activated channels (LK^+-channel). LK^+-channels have been further characterized in acinar cells from the pig pancreas and the lacrimal gland. LK^+-channels are very Ca^{++}-sensitive, fully activated by 10^{-7}M $(Ca^{++})i$, while Na^+/K^+-channels require the unknown co-factors to maintain their activities in the physiological concentration of $(Ca^{++})i$. Both types of channels could be activated by various secretagogues acting via an increase in $(Ca^{++})i$. 2. Role of LK^+ channels in secretion (Salivary and pig pancreatic glands). There are several evidences for Na^+-K^+-$2Cl^-$ co-transport across the basolateral cell membrane being involved in isotonic NaCl fluid secretion. Effective regulation of the Cl-uptake into the cell could be achieved allowing K^+ to recirculate across the membrane via the LK^+-channels and the co-transporter. Accumulated cell Cl^- might be released to the lumen using the negative intracellular voltage generated by the operation of LK^+-channels. In fact recent experiments using whole cell voltage clamp demonstrated the presence of Ca^{++}-activated Cl^--channels, presumably localized in the luminal cell membrane. These features might be exactly the case in other fluid secreting epithelia such as in the trachea and colon. In such cases, again LK^+-channels will play a critical role in the uptake and the release of Cl^-. 3. Role of Na^+/K^+ channels in secretion (rat and mouse pancreatic glands). The membrane potential (Em) of these glands is assumed to be very close to the equilibrium potential of Cl^- (E_{Cl}) not only the absence but also the presence of agonists, which activate Cl^- and Na^+/K^+-channels via an increase in $(Ca^{++})i$. Assuming Cl^--channels to be located both on the basolateral and luminal cell membrane the uptake and release of Cl^- might be occured almost simultaneously due to the competition between Em and E_{Cl} (uptake when $Em > E_{Cl}$), release when $Em < E_{Cl}$). 4. Fluid formation. To achieve the formation of isotonic NaCl in the lumen, Na^+ should be also transported from serosa. The route for Na^+ is appeared to be in the paracellular pathways involving tight junctions at present. 5. Exocytosis-Endocytosis. New method to detect the small capacitance change (phase detection method) presumably due to endocytotic-exocytotic cycles has been introduced by Neher and Marty. Applying the method to the single rat pancreatic acinar cells demonstrated that $(Ca^{++})i$ is needed to cause the capacitance fluctuations exceeding 10 fF.

S - II (2)

RECEPTOR-MEDIATED Ca^{2+} MOBILIZATION UPON ELECTRICAL RESPONSES TO SECRETAGOGUES IN CULTURED INTESTINAL EPITHELIAL CELLS AND GASTRIC PARIETAL CELLS.

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The precise role of Ca^{2+} in stimulus-secretion coupling remains unknown in gastrointestinal epithelial cells. To assess this, electrophysiological studies were carried out in single cells using cultured intestinal epithelial cells and gastric parietal cells. Cultured Intestine 407 cells, derived from fetal human small intestine, responded with hyperpolarizations to putative intestinal secretagogues: acetylcholine (ACh), histamine, 5HT, ATP, VIP and secretin. The membrane became unresponsive after successive applications of these secretagogues. Such membrane desensitization to one of the secretagogues did not prevent the responses to other secretagogues. The ACh- and histamine-induced responses were blocked by muscarinic and H1 antagonists, respectively. The rat parietal cells in primary culture (Okada & Ueda, 1984, J. Physiol. 354, 109) also exhibited hyperpolarizing responses to putative gastric acid secretagogues: ACh, gastrin and histamine (plus isobutylmethylxanthine). Muscarinic and H2 antagonists inhibited ACh- and histamine-induced responses, respectively. Thus, it appears that the activation of independent receptors is associated with the electrical responses both in intestinal and gastric epithelial cells. An inhibitor of Ca^{2+}-activated K^+ channel, quinine, blocked all the hyperpolarizing responses in both cell types. Significant increases in cytoplasmic free Ca^{2+} were observed upon the receptor-mediated hyperpolarizing responses by means of Ca^{2+}-selective microelectrodes in intestinal epithelial cells and of the quin 2 fluorescence in parietal cells. Thus, it appears that the receptor stimulation brings about Ca^{2+} mobilization or Ca^{2+} influx into the cytoplasm, which in turn activates the K^+ conductance. Deprivation of extracellular Ca^{2+} suppressed ACh- or 5HT-induced responses in intestinal epithelial cells, and responses to histamine (plus IMX) in parietal cells, but did not those to other secretagogues. Caffeine, which presumably impairs the Ca^{2+} transport in ER, inhibited VIP- or secretin-induced responses in intestinal epithelial cell, and ACh-induced responses in parietal cells. Mitochondrial inhibitors preferentially suppressed gastrin-induced responses in parietal cells. Therefore, it is concluded that not only the extracellular space, but also intracellular Ca^{2+} stores (ER, mitochondria and so on) are the sources of Ca ions transported into the cytoplasm upon the receptor stimulation, and the main sites are different from each other depending on the receptor species. Since cyclic AMP-mediated secretagogues (VIP or secretin in intestinal epithelial cells, and histamine in parietal cells) and dibutyryl cyclic AMP also induced increases in the intracellular Ca^{2+} concentration, it is suggested that cyclic AMP is involved in the receptor-mediated Ca^{2+} mobilization or Ca^{2+} influx in both cell types.

S - II (3)

ION TRANSPORT IN PARIETAL CELLS. TAKEGUCHI, N. Faculty of Pharmaceu. Sci., Toyama Med. & Pharmaceu. Univ., Toyama, 930-01

Roles and pathways of ion transport of H, Cl and K in parietal cells have been greatly clarified for past ten years.

With regard to K: Gastric secretion in vitro gastric mucosa requests the presence of K in bathing media. Intracellular K concentration $(K)_{cell}$ of bullfrogs changes enzymatically as a function of medium (K) and the acid secretory rate is a function of $(K)_{cell}$ (Takeguchi et al., AJP, 1977 and 1979). The relation was also found in mammalian gastric glands (Berglindh, APS, 1978; Koelz et al., AJP, 1981). In Xenopus, K selectivity of the serosal membrane is a function of the acid secretory rate. The higher K selectivity accompanies the higher rate (Takeguchi et al., AJP, 1981). Acetycholine stimulation of acid secretion requires the presence of Ca in bathing medium, suggesting that Ach triggers Ca influx from medium to intracellular space (Berglindh, Sachs and Takeguchi, AJP, 1980).

H and Cl: Gastric H,K-ATPase found by Forte et al.(JCP, 1967) is a non-electrogenic $H \longleftrightarrow K$ antiport (Lee et al., BBRC, 1974 ; Sachs et al.; JBC, 1976). The pump resides in tubulovesicles at resting state. Although there are plenty of Mg, K, and ATP in intracellular space, the pump activity is arrested because the amount of K in the vesicles is limited due to the small rate of KCl influx across the vesicle membrane (the K conductance is high, but the Cl conductance is low.). At secreting state, the tubulovesicles fuse with the apical membrane and $H \longleftrightarrow K$ antiport function can operate when K is present in luminal space. For continued secretion of acid, K-recycling mechanism has been suggested to be present. Actually, gastric vesicles obtained from acid secreting apical membrane have high K and Cl conductances (Wolosin and Forte, FEBS Lett., 1981).

Cl: We found that closed Cl channel in tubulovesicles can be opened by S-S cross-linking of H,K-ATPase, suggesting that the increased KCl influx triggers fusion of vesicles with the apical membrane and affords the K-recycling mechanism, and that one to one secretion of H and Cl is possible (Takeguchi et al., JBC, 1983). Some benzimidazole compounds inhibit Cl channel as well as ATPase activity, suggesting that Cl channel is in one of the 100 kDa subunits of the ATPase. Pungent ingredient of curry and Wasabi, allylisothiocyanate, also inhibits Cl channel and ATPase activity (Takeguchi et al., BBRC, 1983).

(AJP, Am. J. Physiol.; APS, Acta Physiol. Scand.; JCP, J. Cell. Physiol.;BBRC, Biochem. Biophys. Res. Commun.; JBC, J. Biol. Chem.)

S - II (4)

CHARACTERISTICS OF COTRANSPORT OF ORGANIC SOLUTES AND CATIONS ACROSS THE BRUSH BORDER MEMBRANE OF SMALL INTESTINE. HIMUKAI,M.,TAKUWA,N.,SHIMADA,T.,MATSUMOTO,H. and HOSHI,T. DIV. OF BAS. NUTR.,NATL.INST.OF NUTR., Toyama-cho,Shinjuku-ku, Tokyo 162 and DEPT. OF PHYSIOL., FAC. OF MED.,UNIV. OF TOKYO, Hongo,Bunkyo-ku, Tokyo 113

Untill recently, it has been thought that the coupling ion of the cotransport is Na^+ in animal cells without exception. However, Takuwa et al. showed the presence of H^+ / oligopeptide cotransport by brush border membrane vesicles of rabbit small intestine. A marked overshoot uptake of glycylglycine(Gly-Gly) into the membrane vesicles could be demonstrated in the absence of Na^+ when an inward H^+ -gradient ($[H]_o > [H]i$) was imposed across the membrane. Moreover, Shimada et al. showed using a microelectrode technique that Gly-Gly -induced depolarization of luminal membrane was entirely independent of Na^+ in the incubation medium and greater at pH 6.0 than at pH 7.3 in Triturus small intestine. But, it is still unclear at present how the driving force of uphill transport of H^+ / oligopeptide cotransport is maintained in the enterocyte. As a possible mechanism, it can be postulated that the inward H^+ -gradient would be preserved by Na^+ / H^+ antiport mechanism. If so, the uphill transport of oligopeptides would be defined as tertiary active transport. Another unanswered question on the H^+ / oligopeptide cotransport is the disagreement with the results obtained from the experiments using everted intestines of guinea pig. Also in guinea pig everted intestine, carrier-mediated influxes of Gly-Gly and carnosine(β-Ala-His) are independent of Na^+. And, the addition of the dipeptide into the mucosal solution evoked a sudden and sustained increament of the mucosal negativity. However, the changes in short-circuit current (Isc) evoked by the peptides increased as Na^+ concentration in the incubation medium was increased. Although both dipeptides evoked small increases in Isc even in the absence of Na^+, the optimum pH of this apparently Na^+ independent increase in Isc was about 7(6.4-7.4). The peptide-induced change in transmural PD was entirely insensitive to amiloride which is known to be an inhibitor of Na^+ /H^+ antiport. Moreover, an unique electrical phenomenon was observed in peptide transport by guinea pig everted intestine. From a kinetical study of transport, it was clearly shown that Gly-Gly and carnosine were transported by a common carrier. But, the maximum change in PDt evoked by carnosine was about 2 times smaller than that of Gly-Gly. Such a discrepant relationship between the transport and transport-related change in PDt has not been observed in Na^+ / organic solute cotransport. These observations suggest that peptide-induced electrical phenomena are not simple in the intact epithelium of guinea pig small intestine. To elucidate the reason of disagreement, further studies are necessary.

SYMPOSIUM Ⅲ

Ⅲ. Central mechanisms of interactions among different hypothalamic functions.

S - Ⅲ (1)

CENTRAL MECHANISMS OF INTERACTIONS OF DIFFERENT HOMEOSTATIC SYSTEMS. INTRODUCTORY REMARKS.

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Interactions of different homeostatic systems have been frequently observed as the competition between different systems when two drives exist concurrently. Such interactions may be attributed to the physiological limitations such as, 1)use of common effector organs among different homeostatic systems, 2)influence of different functions on common physiological variables, and 3)difficulty to satisfy more than one drives with a single behavior at a time. In these respects, the thermoregulatory (TR) system is unique among major physiological systems. It has no specialized effector organs except for human sweat glands. Its actions may readily affect many physiological variables such as energy and water balance. And TR behaviors may compete with non-thermal behaviors. As an introduction of this symposium, discussions were made on the central mechanisms of such interactions of thermoregulation with osmoregulation, food intake/energy balance and non-thermal emotional behaviors. Reduced sweating and panting have been taken as the centrally regulated responses when confronted with both heat stress and reduced availability of water simultaneously. This response may be explained, at least partly, by our findings of decreased activity of preoptic warm-sensitive neurons by hyperosmotic stimuli. Whether hypovolemia also affects preoptic neuron activity is currently unknown. Heat and cold stimuli increases and decreases pituitary secretion of vasopressin respectively in mammals and birds. The salt gland, an extrarenal osmoregulatory organ in water birds, changes the activity during hypothalamic thermal stimulation. These thermal influences on osmoregulatory functions may be partly attributed to increased activity of paraventricular nucleus neurons to a rise in hypothalamic and skin temperature. Preoptic and skin thermal stimulations have been demonstrated to affect the activity of glucose-responsive neurons in the VMH and LH in a way which may explain the cold-induced hyperphagia and heat-induced hypophagia. Cold-induced non-shivering thermogenesis and diet-induced thermogenesis share at least partly the control mechanisms (VMH-sympathetic nerve-brown adipose tissue). In addition, the BAT thermogenesis was shown to increase in response to emotional stimuli like restraint. Thermoregulation, feeding control and emotion are thus interrelated closely with each other. In our study of TR operant behavior in the monkey, some preoptic neurons exhibited the responses which were dependent upon monkey's affective aspects of thermal sensation. Preoptic thermosensitive (TS) neurons responded to non-thermal emotional stimuli more frequently than non-TS neurons, and some TS neurons changed the activity during feeding operant behavior. The results, together with the previously demonstrated limbic and prefrontal connections with preoptic TS neurons, suggest the involvement of preoptic TS neurons in non-thermal motivated behaviors as well as TR behaviors. Thus a full understanding of homeostatic systems requires qualification and quantification of interactions among different homeostatic drives, which are formidable, but now accessible challenges.

S - Ⅲ (2)

INTERACTION BETWEEN BLOOD PRESSURE REGULATION AND BODY TEMPERATURE REGULATION. IRIKI, M. Yamanashi Medical College, Tamaho Nakakoma Yamanashi 409-38

Both cardiovascular and body temperature regulatory systems are important for maintaining the homeostasis of the body. Because the sympathetic and parasympathetic nervous systems compose the common effector pathway involved in both regulatory systems, thermal and circulatory stimulations must interact in their responses. The present experiments were performed to clarify the central interaction between body temperature and blood pressure regulations.

Firstly, the responses of skin and renal sympathetic nerve activities (SkSNA and RSNA) and heart rate (HR) to blood pressure change, which was represented as baroreflex curves, were determined during warming (warm environment with or without spinal warming) and during cooling (cold environment with or without spinal cooling). SkSNA baroreflex curve was almost horizontal, because SkSNA is barely effected by baroceptor inputs. The curve was shifted parallel to the lower SkSNA level during warming, barely interaction between thermal and baroceptor influences. RSNA baroreflex curve was shifted to the lower blood pressure side and the gain of the reflex was inhibited during warming, indicating the inhibitory interaction between both influences. The curve of HR baroreflex, which is controlled primarily by parasympathetic nerve activity, was shifted to the lower blood pressure side during warming, indicating the interaction between both influences. As presented above, the mode of the effect of thermal stimulation on baroreflex curve was different in each sympathetic or parasympathetic twigs.

Secondary, to clarify the central mechanisms of the interaction between both influences, the effect of thermal stimulation on HF baroreflex curve were investigated in 5HT deprived rabbits with intracisternal injection of 5,7-DHT (dihydroxytryptamine) 1-2 weeks before experiments. 5HT is well known to have an influence on both thermoregulatory and baroreceptor responses. HR baroreflex curves was not shifted to lower blood pressure during: this was not the case in intact rabbits. This result indicates that 5HT system in CNS contributes to the interaction between both influences, and further that the experiments using animals with the deprivation of certain neurotransmitter can be one of the useful methods to clarify the mechanisms of the central interaction of the various regulatory systems of the body.

S - Ⅲ (3)

ROLE OF PLASMA OSMOLALITY AND CARDIOVASCULAR SYSTEM IN CONTROL OF BODY WATER BALANCE: THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS AS AN INTEGRATIVE SITE OF AUTONOMIC AND NEUROENDOCRINE FUNCTIONS. YAMASHITA, H. Dept. of Physiol., Univ. of Occup. Environ. Health Med., Kitakyushu 807

For maintenance of homeostasis in body water balance, a broad range of endocrine and autonomic responses and behaviors are involved. A great deal of work suggests that the hypothalamus, in particular the paraventricular (PVN) and probably the supraoptic (SON) nucleus play a critical role in the coordination of such responses and behaviors. We have devoted our efforts to elucidate the neural and humoral integrative mechanism at the level of PVN and SON which coordinate these responses. One of the major humoral factors which control the body water balance is the plasma vasopressin concentration level. The role of cardiovascular receptors in control of vasopressin release was investigated. The results indicated that neurosecretory cells in the PVN and SON, which contain vasopressin-secreting neurons, receive inhibitory synaptic inputs from volume- and baroreceptors, and excitatory inputs from chemoreceptors. Such information was found to be mediated through a direct projection from the nucleus tractus solitarius or by way of the presumably A1 noradrenergic neurons in the ventrolateral medulla. Another factor which control the body water balance is through hemodynamic changes, which alter renal blood flow, glomerular filtration rate and is related to the renin-AII system. Recent anatomical studies have demonstrated that the PVN project directly to the spinal and medullary autonomic centers. The direct caudal projections from the PVN were confirmed electrophysiologically. Neurons which were located in or near the PVN and SON were antidromically excited by stimulation of the dorsomedial medulla and spinal cord. Thus those two humoral and autonomic factors were integrated by local circuits and chemosensitivity inside the PVN. Chemo- and thermo-sensitivity of neurons in the PVN and SON were examined in hypothalamic slice preparations. The results indicated that an increase in the osmolarity of the medium in the physiological range excited all SON neurons tested and inhibited 75 % of the PVN neurons. Noradrenaline and vasopressin were found to have excitatory effects on SON neurons mainly through the α_1-receptor and the V_1-receptor respectively. And angiotensin II had also excitatory effects on SON neurons. Even after reducing synaptic transmission by low Ca^{2+} and high Mg^{2+} perfusion medium, certain neurons still showed these chemosensitivity. We also found existence of warm-sensitive and cold-sensitive neurons in the PVN. From these results, I emphasized the view that the neuroendocrine system such as hypothalamo-neurohypophysial system and cardiovascular control system are very closely linked at the level of the PVN as well as the SON, thereby contributing significantly to maintenance of homeostasis of the body water balance.

S - Ⅲ (4)

REGULATION OF FEEDING BY ENDOGENOUS CHEMICAL SUBSTANCES. SHIMIZU, N. Dept. Physiol., Fac. Med., Kyushu Univ. 60, Fukuoka 812

Accumulating evidence suggests that some endogenous blood factors contribute to the induction of hunger and satiety. Kennedy's lipostat theory is based in essence on a weight control process which depends on free fatty acids (FFA) carried in the blood. Mayer's glucostat theory, likewise involves a metabiolite which, in this case, is blood born glucose. Both of them specifically modulate the neuronal activities of the lateral hypothalamic area (LHA; feeding center) and ventromedial hypothalamic nucleus (VMH; satiety center) in a reciprocal manner. Evidence obtained from lesions produced in various sites within the hypothalamus led to early conclusions that feeding behavior was controlled or could at least be modified within that general region. There was, of course, controversy as to whether this was due to specific control centers or to fibers of passage through the hypothalamus. Grossman and Grossman, with their kainic acid technique, managed to lesion neurons while leaving fibers of passage intact. This further supported the control center idea. The neural activity of chemosensitive neurons in the LHA and VMH is also affected by hormones such as insulin, glucagon and other substances characterized as intrinsic feeding-related components. The systemic levels of these endogenous substances fluctuate as the nutritional state of an animal varies. Endogenous sugar acids, 3,4-dihydroxybutanoic acid (2-deoxytetronic acid, 2-DTA) and 2,4,5-trihydroxypentanoic acid (3-deoxypentonic acid, 3-DPA), have been identified in the serum of fasted rats. A good correlation can be seen between the LHA and VMH chemosensitive neuronal activity and the feeding behavior changes elicited by these sugar acids. Injection of 2-DTA into the third ventricle of chronic rats suppressed food intake and neuron activity in the LHA. The same amounts of 3-DPA elicited feeding and increased LHA glucose-sensitive neuron activity. In contrast, these sugar acids had completely the opposite effects on the glucoreceptor neurons in the VMH which increase in activity upon direct application of glucose. 2-DTA caused excitation while 3-DPA an inhibition.

It can be concluded that neurons in the LHA and the VMH respond to blood-born glucose, and they may be responsible for controlling feeding behavior and possibly weight. Furthermore, these neurons are susceptable to other substances such as FFA, 2-DTA, 3-DPA and other factors which are related to feeding behavior.

It seems, in particular, that the chemosensitive neurons of the hypothalamus integrate information regarding those functions and thereby produce overall regulation of feeding behavior. It is also true that glucose directly affects neurons in the nucleus tractus solitarius, dorsal motor nucleus of vagus and vagal afferents in several peripheral organs. Therefore, endogenous chemical substances affect neurons that in turn control other factors, thereby creating positive or negative feedback loops. There is a possible hierarchical arrangement of neuronal networks that process chemical information.

S - Ⅲ (5)

INSTINCTIVE BEHAVIOR AND EMOTION. ONO, T., NISHINO, H., FUKUDA, M., NISHIJO, H. AND YAMATANI, M. Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01.

Feeding, drinking, sexual and temperature regulation are examples of instinctive behavior that is essential for animal survival and to maintain homeostasis, physiological equilibrium of temperature, water and energy in the body. Pleasant feelings and joy are produced by satisfaction of the instinctive or internal needs, or by perception of reward related stimuli such as food, water or the opposite sex, etc., and unpleasant feelings are produced by nonsatisfaction of instinctive needs or by perception of punishment related stimuli such as dangerous objects, enemy, etc. Emotion is the brain process of producing these pleasant and unpleasant feelings. Pleasant stimuli produce approach behavior, while unpleasant stimuli lead to attack or escape. Thus, pleasant and unpleasant feelings are crucial in biological judgement of whether a stimulus is rewarding (useful) or punishing (dangerous), and execution of the proper behavior in response to the environmental situation. It is thus clear that emotion includes learning, experience, memory and perception components, so animals can not achieve proper instinctive and emotional behavior without emotion. In these studies, single unit activity was analyzed to investigate roles of the lateral hypothalamus (LHA), amygdala (AM), prefrontal cortex (PFC), caudate nucleus (CD), globus pallidus (GP) and substantia nigra (SN) in sensory perception of various objects and environmental events, emotional association of sensory information, decision making, sensory-motor integration and execution of goal directed behavior during feeding, drinking and avoidance by monkeys. The experimental results were: The AM is involved in object evaluation (rewarding or punishing, useful or dangerous) and reward perception. The LHA is involved in food discrimination, drive to get reward, reward perception which are affected by learning or internal state such as hunger or satiety. The PFC might also be involved in these functions plus perception of the significance of the environmental situation, genesis of motivation, sustaining the motivational state and dicision making to produce integrated behavior. The CD is related to drive, sensory-motor integration and ingestion. The GP is important in motor coordination and ingestion. The SN might be involved in motor execution and ingestion. The AM-LHA-PFC system might thus be important in stimulus association with pleasant or unpleasant emotion, stimulus-reward association, emotional evaluation, sustaining motivation and decision making to produce motion. The PFC-CD-GP-thalamus (-motor cortex) or SN-system might be important in drive, sustaining motivation, sensory-motor integration, motor coordination, motor execution and ingestion. Co-operation among these systems is important for the emotional behavior to survive.

SYMPOSIUM Ⅳ

Ⅳ. Pathophysiological aspects of electrical activity and ionic channels in the cardiac muscle.

S-Ⅳ(1)

BETA-ADRENOCEPTOR MEDIATED INTERVENTION OF $\dot{V}$max AND SLOW CONDUCTION IN RESIDUAL FAST CHANNEL-DEPENDENT RESPONSE OF VENTRICULAR MUSCLE. ARITA, M. and HISATOME, I. Dept. of Physiol. Med. Coll. of Oita, Oita 879-56

We studied the mechanism of catecholamine-induced reduction of the maximum rate of rise ($\dot{V}$max), and changes in conduction velocity, using isolated guinea pig papillary muscle perfused with oxygenated Tyrode's solution of different K^+ concentrations (2.7-14.9mM), stimulated at a rate of 0.2Hz. In relatively high K_o (10.8-14.9mM), the $\dot{V}$max is composed of 2 components, i.e., the $\dot{V}$max,fast followed by $\dot{V}$max,slow. The $\dot{V}$max,fast is a measure of activation of residual (mostly inactivated) fast channel while the $\dot{V}$max,slow is that of slow channel. Isoproterenol (ISP) and noradrenaline depressed $\dot{V}$max,fast with an increase of $\dot{V}$max,slow in a concentration dependent manner (0.01-1μM), the effects being about 10 times greater in ISP than noradrenaline, while alpha-stimulant, phehylephrine (10μM) was without effects. The depression of $\dot{V}$max,fast became more remarkable with greater deporalization of the membrane, and which was attributed to the negative shift (2.2mV, $p<0.05$, $n=7$) of the curve relating $\dot{V}$max,fast to the resting potentials (Em) along the voltage axis; the slope of the curve remained unchanged. Similar shifts of negative direction were produced by an application of theophylline (4mM) or db-cAMP (3mM), indicating an intervension of cAMP on the ISP-induced shift of the $\dot{V}$max,fast-Em relationship. The ISP-induced shift was inhibited in the presence of beta-blockers such as pindolol (1μg/ml) or atenolol (10μg/ml), but not by an alpha-blocker, phentolamine (10μM) or calcium-antagonists, l-verapamil (1μg/ml) and D600 (0.4-10μM). Same results were obtained in very small preparations ($0.2mm^3$ or less) in which the cable complication may be negligible. The conduction velocity measured in 14.9 mM K_o ranged 30-40cm/s (slow conduction). ISP of increasing concentration (0.01-1μM) did not significantly change the conduction velocity but altered ionic channels in charge of the slow conduction from residual fast to slow channel. In the presence of l-verapamil (1μg/ml), ISP (0.1μM) rapidly decreased $\dot{V}$max,fast (without increasing $\dot{V}$max,slow) and produced total block of conduction. In the presence of pindolol (1μg/ml), all ISP effects on both $\dot{V}$max,fast and $\dot{V}$max,slow were dismissed with the conduction always depending on activation of residual fast channel. These results suggest that catecholamines block "residual Na channels" responsible for the slow conduction in K^+-depolarized ventricular muscle via stimulation of beta-receptor with resultant increase of cAMP, and that the block is not mediated by well-known effects of catecholamines on $\dot{V}$max,slow, i.e., increased Ca^{2+} influx or increases in Ca_i. Pretreatment a/o coexistence of Ca-antagonist may facilitate the deterioration of conduction related to $\dot{V}$max,fast inhibition by catecholamines, whereas beta-blockers might protect the conduction from such catecholamine-induced deterioration.

S-IV(2)

TRIGGERED-AUTOMATICITY OF DIFFERENT IONIC MECHANISMS IN GUINEA-PIG VENTRICULAR MUSCLES.

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Automatic activity of cardiac muscles has been seen under the physiological as well as the abnormal conditions. While the normal pacemaker activity is the limited properties to the specialized tissues of the heart, several sorts of abnormal automaticity have been observed in various cardiac preparations including the working myocardium. Delayed afterdepolarizations (DADs) and triggered-activity are one of a unique form of abnormal automaticity appearing in the specialized and in the working myocardium. We report the development of two types of DADs and triggered-automaticity which are formed by different ionic mechanisms in guinea-pig ventricular muscles. When muscle preparations were exposed to the low-K^+.high-Ca^{2+} conditions, they developed DADs with several membrane fluctuations after the driven action potential. These membrane oscillations were associated with the tension oscillations (after contractions). If the DADs became large enough to attain threshold, there appeared triggered-automaticity. Under the voltage clamp condition, the transient inward current (TI) forming the DADs were evoked upon repolarization from the preceding depolarization. The application of Co^{2+} quickly and reversibly depressed the TI irrespective of the modes of clamp depolarizations. Verapamil and diltiazem, however, blocked the TI with a use-dependent manner. The application of caffeine and procaine abolished the TI completely without eliminating the slow inward current. Thus, the TI under this condition was brought about by the mechanism related to the Ca^{2+}-release from the stores as well as to the Ca^{2+}-entry through the slow channel.

When Ba^{2+} (0.2 - 2.0 mM) was applied to muscle preparations, the resting potential of them depolarized to -70 - -40 mV. They developed afterhyperpolarization followed by a single DAD without aftercontraction. With increasing its amplitude, the DAD formed into spontaneous activity, which resembled the appearance of the slow diastolic depolarization. Under the voltage clamp condition, no TI was observed with Ba^{2+} application, but there appeared a bending on the outward tail current upon repolarization. This bending of the current as well as the DAD and automaticity were not blocked by the application of caffeine and procaine. The bending is most probably caused by differences in time-courses of a component a blocking action of Ba^{2+} on the K^+ current and of a deactivation of iK. A contribution of the slow inward current to form the Ba^{2+}-induced DAD was also indicated by the effects of Co^{2+} on eliminating the afterdepolarization and spontaneous activity, without abolishing both the afterhyperpolarization and the bending of the tail current.

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S-IV(3)

FLUCTUATION OF MEMBRANE POTENTIAL PRODUCED BY AUTONOMIC NEUROTRANSMITTERS IN MAMMALIAN VENTRICULAR MYOCARDIUM.

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The resting potential of ventricular myocardium depends mainly on the activity of anomalous rectifier K channels. In depolarized preparations, however, fluctuations of the resting potential due to other factors can be observed, especially under the influence of autonomic transmitters. The mechanisms of these phenomena were studied using isolated guinea-pig myocardial tissues. 1) In K(27mM)-depolarized ventricular muscle, catecholamines (CA) elicit a small depolarization of the resting membrane. This catecholamine-induced depolarization (CAD) is at least partly due to an increase in the slow channel conductance which is mediated by CA/β-receptor interaction (Ref. 1). Application of acetylcholine (ACh) during CAD causes a repolarization of the membrane, which is consistent with the concept of the adrenergic-cholinergic antagonism on the slow channel. However, removal of ACh in the presence of CA induces a transient increase in the depolarization (rebound). This rebound reflects an extra enhancement of the activity of β-receptor/slow channel system which develops following cessation of the myocardial muscarinic stimulation (Ref.2). Similar rebound phenomenon can also be detected in the behavior of the slow inward current in voltage-clamped Purkinje fibers treated with CA and ACh (Ref.3). Thus, such adrenergic-cholinergic interactions at the postsynaptic effector level may be one mechanism responsible for the classically-known post-ACh potentiation of the ventricular contractility or post-vagal tachycardia. 2) In K-depolarized ventricular muscles treated with CA, electrical stimulation (1-2 Hz) reversibly produces an oscillatory afterpotential (OAP) associated with each action potential, which eventually leads to a triggered activity. This OAP is enhanced by elevation of $[Ca]_o$ and reduction of $[Na]_o$ (50%), but inhibited by Mn (0.1 mM) and caffeine (2-5 mM). Thus the OAP under this condition resembles the other types of OAPs, such as those in digitalis-intoxicated myocardium. Mg ions (3-6 mM) also suppress the above OAP. However, unlike Mn, Mg increases the twitch tension, and, unlike caffeine which shortens the coupling time of the OAPs, Mg prolongs it. In addition, a marked positive staircase in tension develops during a train stimulation in the presence of 3-6 mM Mg. These actions of Mg cannot simply be explained by the mechanisms like those proposed for the inhibitory actions of Mn or caffeine on OAP. Our hypothesis is as follows. If the OAP reflects a certain membrane event related to the oscillatory release of Ca from SR, Mg may stabilize the function of SR so that the E-C coupling process is enhanced, i.e., at high $[Mg]_o$, Ca ions entering the cell during each action potential are sufficiently sequestered by SR and effectively released during subsequent beat, which also leads to a diminished OAP. However, it is unclear at present whether Mg acts extracellularly or intracellularly. (Refs. 1. J. Mol. Cell. Cardiol., 15, 555, 1983; 2. Am. J. Physiol., 247, H244.1984; 3. Jpn. J. Physiol., 34, 775, 1984)

S-IV(4)

MODULATION OF CALCIUM CHANNEL GATING BEHAVIORS IN CARDIAC MUSCLE. OCHI, R., HINO, N., OKUYAMA, H. Dept. of Physiol., Sch. of Med., Juntendo Univ., Tokyo 113

Since the open probability of the single Ca channels during depolarization is far less than one even at large depolarizations and depolarizations often fails to produce openings of the functional Ca channels, the macroscopic Ca channel currents could be enhanced markedly by modulating the voltage-dependent gating peocess and/or the process which determines the ratio of pulse containing sweeps. The single Ca channel currents were recorded from the isolated guinea-pig ventriculat myocytes by the cell-attached patch-clamp technique using 50 mM Ba-containing pipettes. When a dihydropyridine, BAY K 8644, was added to the superfusate, sustained openings with the open times more than ten times longer than control appeared. The open time histograms in the presence of BAY K 8644 were well fit by double exponential curves with the time constants of less than one ms as in control and those of about ten ms. The long openings occurred in clusters and short openings similar to those in control remained in some depolarizations also as clusters. The percentage of depolarizations with long openings in total depolarizations with openings increased with concentration increase and amounted to 57% at 1 μM. In the long open state both the latency-to-first-event and the interval between bursts were shortened in addition to the prolongation of the openings. The mean Ba currents were increased markedly by BAY K 8644. In the current voltage relationship the increase was relatively large at small depolarizations, because the open probability approached to one at large depolarizations during the long open state. Adrenaline (2μM) little affected the voltage-dependent gating process but affected the ratio of pulse-containing sweeps. The statistical natures of the occurrence of pulse-containing sweeps and nulls were analyzed by applying 150 ms pulses at 2 Hz. The percentage of depolarizations with Ca channel openings was 38 ± 15 % (mean ± SD., n=55) in normal Tyrode. It was 63 ± 16 % (mean ± SD., n=16) and was significantly higher than control in the presence of adrenaline. Pulse-containing sweeps and nulls appeared in clusters, respectively. The histograms of the times calculated from the stimulus frequency and the consecutive numbers of pulse-containing sweeps were fit by single exponentials with time constants of 1-2 sec in normal Tyrode, and of 1.5-4 sec in the presence of adrenaline. On the other hand the mean duration of consecutive nulls was 2-5 sec in control and 1-2 sec in the presence of adrenaline. The latency-to-first-event was longer just after nulls than after pulse containing sweeps both in the absence and in the presence of adrenaline. These results indicate that a time-dependent (metabolism-dependent) slow gating process exists in cardiac Ca channels. Adrenaline seems to modulate this process via membrane phosphorylation to increase the ratio of depolarizations associated with Ca channel openings and thus the macroscopic calcium channel currents.

S-Ⅳ(5)

K CHANNELS OF ATP-DEFICIENT CARDIAC CELLS.

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The giga-seal patch clamp electrodes were applied to enzymatically isolated single ventricular cells of guinea pig and whole cell current or the single channel current was recorded. When the cell was dialysed with various ATP-deficient internal solutions, the Ca current decreased in a dose-dependent manner to less than 10% of the control (5 mM ATP) at 0.5 mM ATP. A slight (ca. 25%) decrease of the slope conductance for hyperpolarizing current was observed. When a delayed rectification on depolarization followed by a marked outward current tail on repolarization was present, this time-dependent outward current was also depressed. An increase in a time-independent outward current was observed accompanied with marked current fluctuations.

K channels under the ATP-deficient conditions were examined with the open cell-attached (a modified inside-out) patch recordings. Two types of K channels were recorded; the inward rectifier K channel and the ATP-regulated K channel. When the ATP concentration on the inner side of the membrane was lowered from 2.0 mM to 0.25 mM, the open-state probability of the inward rectifier K channel decreased from 0.46 to 0.09 in the average of 3 experiments. While,activation of the ATP-regulated K channel was observed below 2 mM ATP. The maximum number of superpositions of single channel current was taken as the number of channels in the patch. The ratio of number of the inward rectifier K channels over the ATP-regulated K channels ranged from 0.5 to 1.75 with the average of 1.12 in 6 experiments, where both K channels were recorded from the same patch. Thus, it seems that the densities of both channels almost equal. The changes in these two channels well explained the above changes in the whole cell current. An increase of the intracellular Ca up to 100 uM decreased the open-state probability of both channels, but failed to activate a different class of K channel. Recording of the single channel current of the time-dependent K channel was not succeeded.

SYMPOSIUM V

V. Motor control.

S-V(1)

DESCENDING AND SEGMENTAL CONTROL OF THE NECK AND FORELIMB MOVEMENTS IN THE CAT.

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Dorsal neck motoneurones, splenius(SPL) and biventer cervicis and complexus(BCC) received disynaptic EPSPs from the tecto- and corticofugal fibres in the cat. These EPSPs were mediated by common reticulospinal neurones(RSNs) in Nucleus reticularis pontis caudalis (N.r.p.c.) and Nucleus reticularis gigantocellularis(N.r.g.) that projected their descending collaterals to the cervical enlargements and not to the lumbar segments(C-RSNs). C-RSNs in N.r.p.c. received predominant EPSPs from the tectofugal fibres and descended in the medial part of the ventral funiculus(VF), while those in N.r.g., receiving predominant EPSPs from corticofugal fibres, descended in the lateral part of VF or the lateral funiculus(LF). Monosynaptic excitatory connexion with neck motoneurones and muscle specificity of their connexions were revealed by the spike triggered averaging method: most of C-RSNs in N.r.p.c. had specific connexions with SPL, while those in N.r.g. with either SPL or BCC motoneurones. Intraaxonal staining of C-RSNs with HRP revealed that they gave off many collaterals along their descending course. In the brain stem, C-RSNs in N.r.p.c. distributed terminals in the VIth nucleus, N.r.g., N.r.ventralis(N.r.v.) and Nucleus prepositus hypoglossi, while those in N.r.g. gave off only a few thin collaterals in N.r.v. In C2-C3 segments, C-RSNs in N.r.p.c. gave off terminals in laminae IX, VIII and VII. C-RSNs in N.r.g. descending in the lateral part of VF and the ventral part of LF issued dense terminal branches in lamina IX and a few in laminae VI-VIII, but those descending in the dorsal part of LF gave off terminals mainly in laminae VI-VIII and very few in lamina IX. In the cervical enlargement, C-RSNs issued dense terminal branches in laminae VI-VIII but a few in IX. Based on these results, it was postulated that C-RSNs in N.r.p.c. may participate in eye-head coordinated movements, while those in N.r.g. in voluntary head movements. Forelimb motoneurones received disynaptic EPSPs from the cortico-, rubro-, tecto-, and bulbospinal tract fibres. These EPSPs were mediated by common interneurones located in C3-C4 segments (C3-C4 PNs). The motoneurones received polysynaptic EPSPs from the cortico- and rubrospinal tract via segmental interneurones located in the forelimb segments(Illert et al., 1977). The C3-C4 PNs were postulated to be involved in precise control of forelimb reaching a target, while the segmental interneurones were suggested to participate in grasping movements(Alstermark et al., 1981). In a lifting movement of a forelimb, postural adjustments were executed by 3 other limbs prior to and during the movements(Massion et al., 1975). C-RSNs descending in the dorsal part of LF are suggested to be involved in this postural adjustment, while information required for postural adjustment during head movement under normal standing may be given by spinal descending collaterals of C-RSNs that connect directly with neck motoneurones.

S - V (2)

NEURAL SUBSTRATES FOR MULTIPLE MUSCLE ACTIVATION: branching patterns in descending motor pathways and cerebello-thalamo-cerebral systems.

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The divergent projection of single long descending motor tract neurons, cerebellar nucleus(CN) neurons and thalamocortical(TC) neurons was analysed morphologically by combining intraaxonal injection of HRP with three dimentional reconstruction of stained axons. Vestibulospinal(VS), corticospinal(CS) and rubrospinal(RS) axons were impaled in the cervical cord, identified by their responses to stimulation of the vestibular nucleus, motor cortex(Mx) and red nucleus(RN), respectively, and then iontophoretically injected with HRP. The courses of the HRP-stained axons were reconstructed from 100um thick serial sections. In cats, the termical arborizations of CS axons were relatively compact and mainly confined to the intermediate zone (Rexed's laminae V,VI,VII) ;none were traced to motoneuron pools. Axon collaterals successively bifurcated in the gray matter and spread in a delta-like fashion, extending widely in an rostrocaudal direction(1-7mm). All CS axons had more than three collaterals and projected to widely separate segments. In monkeys, all CS axons sent at least one of their branches to the motor nucleus with the branches to the intermediate zone. The relation of the multiple arborizations of single axons to different motoneuron species was determined by retrograde labelling of brachial motoneurons by HRP injected into the nerves. The same axons innervated different motoneuron pools. Intraspinal trajectory of single RS axons was very similar to that of CS axons. In contrast, spinal branching patterns of VS axons were very different from those of CS and RS axons in that they ended in laminae VII,VIII and IX, that the rostrocaudal extension of single collaterals was narrow(200-800um), and that intercollateral distances were short. Intraaxonal staining of single interpositus and dentate nucleus neurons revealed that both axons sent axon collaterals to the RN before terminating in VL. Terminal boutons of a single axon spread rostrocaudally and were found on the cell body and the proximal dendrites of large neurons in RN. In VL, clusters of terminal boutons were widely distributed. Single TC axons from VL had two to four axonal bushes separated widely in Mx, each of which had a diameter of about 1mm. The result suggested that single CN axons might influence more than one efferent zone in Mx.

S-V(3)

NEURONAL ACTIVITIES IN THE PRECENTRAL MOTOR AND SUPPLEMENTARY MOTOR CORTEX OF ALERT, BEHAVING ANIMALS. TANJI, J. Dept. Physiol., Hokkaido Univ. Sch. Med. Sapporo, 060

In the vast literature on the experimental physiology of the motor cortex utilizing the technique of electrical stimulation one finds knowledge on the somatotopical organization as well as synaptic organizations of excitation and inhibition in its input and output circuitries. However, it was the technique of single cell recording from movement-performing animals, pioneered by Ricci et al. and by Evarts, which enabled one to ask questions as to how the motor cortex functions in voluntary motor activity. With this technique, studies on motor cortex neurons with respect to their (1) timing of premovement activity, (2) relation to movement parameters, (3) responses to sensory signals and their modifiability, (4) anticipatory activity before intended movements and (5) functional classifications have yielded prominent results, on which a brief overview will be attempted. In recent studies increasing attempts are made to (1) identify recorded cells and (2) look for functional roles in highly specified movement paradigms. Compared to the precentral motor cortex, knowledge on supplementary motor area (SMA) have been much less. It is recently performed single cell studies that started to provide informations as to how activity in this area takes part in controlling motor performance. Specific questions asked in these studies include those as to whether SMA is (1) really a motor area, (2) topographically organized, (3) related to proximal or postural control only, (4) specified for bilateral control system and (5) different from the precentral motor cortex. In summary, SMA neurons, while not as related to execution of simple movements as that in the motor cortex neurons, respond strongly to motor instructions that determine the way animals have to respond to forthcoming sensory signals.

S-V(4)

REORGANIZATION OF NEURONAL CONNECTIONS AFTER DAMAGE TO THE BRAIN: IN THE CEREBELLUM AND RELATED STRUCTURES.

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Functional deficits induced by brain damage recover with time although the degree of recovery varies largely depending on the site and extent of the damage, age, and training. The phenomenon of recovery is a challenge to the doctrine of the functional localization of the brain. In attempts to reconcile the phenomenon of recovery and the doctrine of localization, various explanations such as 'vicarious function', 'behavioral substitution', or 'retraining' have been proposed. These explanations require the occurrence of changes in synaptic transmission or neuronal connection or both as the underlying neuronal mechanisms. Such changes actually occur after damage to the cerebellum or related structures.

In the forelimb area of the frontal motor cortex (FM) of intact monkeys, a surface negative-depth positive (sN-dP) potential intimately related to hand movement occurs before the movement. This potential arises from activation of the cerebellar lateral nucleus. When the motor cortex was temporarily impaired by local cooling, a premovement sN-dP potential in the primary somatosensory cortex (SI) immediately became large and the monkey was able to execute the hand movement. Simultaneous cooling of FM and SI entirely paralysed the contralateral hand. Unilateral hemispherectomy of the cerebellum abolished the premovement potential in FM, which enhanced immediately the premovement potential in SI. The enhancement of the SI potential, marked at the beginning, became less with days, and so was the cooling effect of SI. Despite the decrease of potential enhancement in SI, recovery continued for 2-3 months judging from the shortening of the reaction time. The compensatory motor function of SI, thus, appears to work temporarily and likely to be replaced by another compensatory function which develops slowly outside the sphere of SI. There are changes of slower time-course which resemble a process of motor learning. The premovement sN-dP potential in FM which was abolished by destruction of cerebellar hemispherectomy never reappeared when both the lateral and interpositus nuclei were destroyed, but reappeared when the interpositus nucleus was spared. In the latter case, the potential reappeared at 11 days and increased till 3 weeks with concomitant recovery of function. There are changes involving anatomical alterations. Collateral sprouting of cerebellothalamic fibers occurred in kittens after neonatal hemicerebellectomy. Marked axonal regeneration, though infrequently, occurred in cerebellofugal projections after transection of the decussation of the superior cerebellar peduncle in kittens as well as in adult cats. In both cases the newly formed fibers began to mediate cerebellar outputs to the cerebral cortex in 2-3 weeks. Their final achievement, if indeed it be attained, will take months. To sum up, damage to the brain is followed by changes with immediate onset, changes of slower time-course, and changes involving anatomical alterations. Recovery of function appears to proceed in a sequence of these changes.

S - V (5)

NEURONAL CONTROL MECHANISMS OF POSTURE AND LOCOMOTION IN DECEREBRATE CATS AND IN INTACT CATS WITHOUT RESTRAINT. MORI, S. Department of Physiol., Asahikawa Med. Col., Asahikawa 078-11

In acute precollicular-postmammillary decerebrate cats, locomotion on a moving treadmill can be induced by stimulating the mesencephalic locomotor region (MLR) and the pontobulbar locomotor region (PLR). An important feature of electrically induced treadmill locomotion is that its success depends upon an appropriate level of postural muscle tone neither too little nor too much. It has been demonstrated that areas setting the level of postural muscle tone and thereby regulating the effectiveness of stimulation-induced locomotion are located in the midline of the pons. There is a dorsal inhibitory zone and a ventral excitatory zone, the latter being also capable of initiating locomotion when stimulated alone. In the identified alpha-motoneurons innervating extensor muscles in the hindlimbs, long-lasting membrane hyperpolarization, and depolarization with tonic firing was evoked by stimulating the dorsal zone, and the ventral zone of the pons, respectively, in accordance with the decreased and the increased levels of postural muscle tone.

In freely moving intact cats with stimulating microelectrodes implanted to each of the dorsal zone and the ventral zone of the pons, a series of postural changes were evoked. Within a few seconds from the beginning of stimulation to the dorsal zone, a freely moving cat ceased to walk maintaining a standing posture with a locomotor figure. With continuation of stimulation, it squatted and then laid itself down on the floor in a sequential manner. The cat continued to keep its last posture for several minutes even after termination of the stimulation. Stimulation to the ventral zone of the pons evoked almost an opposite series of postural changes. Within a few seconds from the beginning of stimulation, the cat changed from a lying to a squatting posture, and then started to walk during continuation of this stimulation. The pattern of induced locomotion was somewhat different from that of normal locomotion, and it was similar to that evoked in a decerebrate locomotor preparation on a surface of a moving treadmill. With the ventral stimulation, the cat was always "alerted", and "oriented" raising its head and looking around before the initiation of locomotion.

By stimulating the dorsal zone and the ventral zone in succession or in alteration, it was possible to make the cat adopt a variety of posture and locomotor movements. The major postural changes and the locomotor movements evoked in decerebrate cats seemed to be compatible with those evoked in intact cats. However, alerting and orienting behaviors were only observed in freely moving intact cats. Most previous studies aimed at elucidating the neuronal mechanisms of posture and locomotion from mechanical aspects, treating the decerebrate cat like a "robot". The studies in the freely moving, intact cats seem to offer a possibility to understand the neuronal mechanisms of posture and locomotion associated with the behavioral expression of the animals.

SYMPOSIUM VI

VI. The mechanisms of biosignal transduction at the cell membrane.

S-VI(1)

CATION CHANNELS ACTIVATED BY L-GLUTAMATE IN CULTURED HIPPOCAMPAL NEURONS OF THE RAT.
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The properties of the L-glutamate-activated ion channels were studied in cultured hippocampal neurons prepared from 17- to 19-day-old fetal rats. Some of the cultured neurons grew up to bear resemblance to young hippocampal pyramidal cells, and they became sensitive to L-glutamate about a week after plating. In the present study, these pyramidal neurons were investigated using two of the variants of the patch-clamp methods, namely the whole-cell recordings and outside-out patch recordings.

The relative permeability of the glutamate-activated channels to monovalent cations was determined from the reversal potentials of glutamate-induced currents. Although the selectivity among alkali metals was weak, K^+ and Cs^+ were more permeant than Na^+. Anion conductance could make very little contribution to the glutamate-activated current. The alkaline earth cations, Ca^{2+}, Sr^{2+} and Ba^{2+} were more permeant to the channels than the alkali metals. Using the constant field equation the permeability ratios of these divalent cations to Na^+ (P_x/P_{Na}) were calculated to be 2.4 (Ca^{2+}), 2.4 (Sr^{2+}), and 2.8 (Ba^{2+}), respectively. Mg^{2+} was much less permeant ($P_{Mg}/P_{Na} = 0.1$), and it markedly suppressed the glutamate-induced current in the potential range more negative than -40 mV. This effect of Mg^{2+} made the relationship between the glutamate-induced current and the membrane potential non-linear, the slope conductance being much smaller at < -40 mV.

In Mg^{2+}-free solutions, the value of the single-channel conductance detected by the outside-out patch recordings was 40 pS and the mean open time was 3 - 4 msec at 30 °C. In the presence of extracellular Mg^{2+}, single channel currents seldom appeared as simple square waves of the above conductance value, and they were chopped in bursts at < -40 mV. Nowak et al. have suggested that the blocking effects of Mg^{2+} could be partly explained by a fast channel block mechanism in cultured mesencephalic or striatal neurons of the mouse (Nature 307:462, 1984). This may be also the case in the present preparation.

Excitatory amino acid receptors have been classified into three groups, namely, N-methyl-D-aspartate (NMDA), kainate and quisqualate receptors. The cultured hippocampal neurons were sensitive to these three agonists. It is likely that glutamate activates the three receptors simultaneously in the cultured hippocampal neurons and that the single channel conductance (40 ps) detected above reflects the nature of NMDA receptor.

S - VI(2)

ACh RECEPTOR-ION CHANNELS AND THEIR PLASTIC MODULATION IN BULLFROG SYMPATHETIC GANGLION CELLS. KUBA, K., MINOTA, S., KUMAMOTO, E., TANAKA, K. & TSUJI, S., Dept. Physiol., Saga Med. Sch., Saga 840-01.

Although the characteristics of the acetylcholine receptor-ion channel complex (ACh-channel) in the skeletal muscle fibre were well analyzed, the ACh-channels in vertebrate neurones have not been fully characterized yet. We studied the properties of the ACh-channels in bullfrog sympathetic ganglion cells in situ and cultured ganglion cells, using two-electrode voltage clamp and giga-seal patch clamp methods.

The fast excitatory postsynaptic current (epsc) exhibited a double-exponential decay phase with time constants of 3.5 and 8 ms, which increased with membrane hyperpolarization. The miniature epsc also decayed bi-exponentially with similar two time constants. The slow component contributed about 20% to the peak amplitude in both epsc and miniature epsc. These results together with theoretical consideration (cf. Colquhoun & Hawks, 1977) suggest the existence of two types of ACh-channel with different open times in the ganglion cells.

The power spectrum of the current fluctuation induced by ACh in the ganglion cell in situ, which was pretreated by atropine (10 uM) and followed by wash (this procedure blocked the muscarinic action of ACh), was fitted by a double-Lorentzian function with cut-off frequencies of 5.2 and 59 Hz (30 and 2.8 ms in time constant). The single channel conductances (g) were calculated from the power spectrum, assuming a 20% contribution of the slow component to the 'total' mean current. They were 11.4 pS for the 'fast channel' and 10.9 pS for the 'slow channel'. The rate of desensitization of the ACh-channels in situ was slow with a half decay time of 15-60 s at a moderate dose.

The single channel currents induced by ACh were recorded from the ganglion cells cultured for 3-6 days or 3-4 weeks. The g and open time of the ACh-channel found at 3-6 days were 14-18 pS and 2.6-7 ms, resembling those obtained by the noise analysis in the cell in situ. On the other hand, They were 25-35 pS and 3 ms for the ACh-channels at 3-4 weeks. The current of the latter channel appeared in a bursting fashion and showed a strong voltage-dependent desensitization. The present results suggest that the properties of the ACh-channels in situ, or at early stages of culturing, resemble those of the extrajunctional type in the skeletal muscle fibre, while the ACh-channel at late stages of culturing is similar to the intra-, or peri-junctional type.

The quantal size of the fast epsp and the amplitude of ACh potentials in the ganglion cell in situ were potentiated over 3 h by repetitive antidromic stimulations. This potentiation was not seen by the stimulation applied in a Ca^{2+}-free solution. The intracellular injection of a chelating agent, EGTA, did not prevent the generation of potentiation. Since axon collaterals of the postganglionic axon were found to exist in the ganglion, it seems likely that the postsynaptic potentiation is generated by a substance released from the terminals of axon collaterals, rather than by the direct action of Ca^{2+} entered into the ganglion cell soma.

S-VI(3)

GABA-INDUCED Cl^- CURRENTS. AKAIKE, N. Dept. Physiol., Fac. Med., Kyushu Univ. 60, Fukuoka 812

Cl^- currents (I_{Cl}) in GABA-sensitive frog sensory neurons was separated from Na^+, Ca^{++} and K^+ currents by using internal perfusion technique which allows whole-cell voltage clamp. A concentration-clamp technique that permits rapid exchange (within 0.5 msec) of the external solution surrounding the internally perfused single neuron was employed. Drug-gated Cl^- channel currents were measured in 'inside-out' preparation by patch clamp technique.

Analysis of the variance of GABA-induced I_{Cl} fluctuations gave that the channel population N decreases exponentially with single-channel conductance γ in such a way that $\alpha_2 < \alpha_1 < \beta$ for γ and $\alpha_2 \gg \alpha_1 > \beta$ for N. The steady-state I_{Cl} is mediated by α_1 at low but by β units at high GABA concentrations, and the pronounced I_{Cl} peak at intermediate and high doses reflects the desensitization of α_1 and α_2 receptors, respectively. Patch-clamp recordings indicate two distinct classes of GABA-gated Cl^- conductances that appear to correspond to the α_1 and β types.

The presence of pentobarbital (PB) shifted the GABA dose-response curve to the left and increased slightly the maximum saturation current. PB prolonged the fast time constant (τ_{h1}) of desensitization of GABA-induced I_{Cl}. In patch clamp recordings, PB prolonged markedly the open time of GABA-gated single Cl^- channel current and shortened the closed time. The facilitatory effect of GABA-induced response by PB was several times greater than that of diazepam. Diazepam shifted GABA dose-response curve to the left without affecting the maximum saturation current. Diazepam prolonged both the open and closed times of GABA-gated single Cl^- channel currents.

Picrotoxin (PTX) suppressed the maximum GABA response without any change of affinity in a dose-dependent manner. Bicuculline (BIC) produced parallel shift of the GABA dose-response curve to the right. PTX did not affect the activation phase but selectively accelerated the rate of desensitization (inactivation) whereas BIC prolonged the desensitization onset. PTX shortened the open time of GABA-gated single Cl^- channel currents while BIC markedly reduced the number of events.

It remains to be examined how GABA agonists (e.g., muscimol), GABA potentiators (e.g., PB and diazepam) and GABA antagonists (PTX and BIC) might selectively act on the proposed subclasses of type-A GABA-gated receptor-ionophore complexes.

S - VI(4)

MECHANO-ELECTRICAL TRANSDUCTION AT THE ISOLATED VESTIBULAR HAIR CELL OF THE CHICK.

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Mechano-electrical transduction channel were studied in enzymatically dissociated isolated chick vestibular hair cells by using a whole cell recording variation of the patch voltage-clamp technique. The apical hair bundle was stimulated by a glass rod of which motion was monitored optically by using a photodiode. In response to stimuli of triangular waveform, the hair cell generated a current of triangular wave-form with occasional step-like appearing events. Control experiments confirmed that the current was generated only when the hair bundle was displaced towards the tallest stereocilium. The mechano-sensitive current was blocked by streptomycin and by neomycin. The blockage by streptomycin was clearly voltage dependent: The reduction of the current became larger with hyperpolarization of the membrane. This suggests that the positively charged antibiotic molecule plug the mechanically gated channels.

The permeability of the m-e.t. channel to various monovalent cations was determined from reversal potential measurements. Since a CsCl-EGTA intracellular medium was used, all the permeabilities were calculated relative to P_{Cs}. The sequence of permeabilities was Li(P_{Li}/P_{Cs}=1.39) > Na(1.22) ≥ K(1.17) ≥ Rb(1.12) > Cs(1) > Choline(0.33) > TMA(0.22) > TEA(0.17).

External Ca ions were indispensable for the recording of transduction current and Sr ions could replace Ca ions without loss of the transduction activity. The minimum $[Ca]_o$ for stable generation of the m-e.t. current was 20 μM in Cs saline. The m-e.t. current was observed in isotonic Ca and in Sr salines. Isotonic Ba, Mg and Mn salines were enriched with 1-2 mM Ca in order to generate the m-e.t. current. The permeabilities of the divalent cation relative to Cs were Ca(4.65) > Sr(2.82) > Ba(2.73) > Mn(2.50) > Mg(2.41).

Step-like m-e.t. currents were observed in Cs saline. The smallest step amplitude with clear resolution had a conductance of 49.7± 4.5 pS(mean±S.D., n=7 cells). This is very much likely to be an elementary m-e.t. channel conductance. The permeability coefficient of Cs ions calculated from the above conductance at -50 mV was 7.04×10^{-14} cm^3/sec. The m-e.t. potential was observed in the current-clamp experiment and the amplitude was 24 mV at membrane potential of -43 mV when using CsCl-EGTA intracellular medium. The potential change was smooth and indicated strong low-pass filtering by the membrane. The cell's input impedance ranged from 4.5-7.4 pF and the membrane time constant, calculated for voltages between -50 and -70 mV was in the order of 10 msec.

S-VI(5)

TRANSDUCTION MECHANISM IN CHEMORECEPTION.

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1)Receptor sites: Sugars and amino acids are received by specific receptor proteins. Acids, salts, bitter substances and odorants are not received by specific receptor proteins but the fundamental structure of the biological membrane is concerned with the reception of these stimuli. 2)Effects of ions on the responses: Elimination of specific ions such as Na, K, Ca or Cl from the solution perfusing the surface of the taste and olfactory organs did not affect the responses to chemical stimuli. The responses occurred under the conditions that the surface of taste and olfactory cells was perfused with 1 mM KCl solution. This indicates that an increase in ionic permeability across the receptor membranes does not contribute to the generation of receptor potentials. 3)Neuroblastoma as model for chemoreceptor cells: The neuroblastoma cell (N-18 clone) was depolarized reversibly by acids, salts, bitter substances and odorants. The depolarizations by these stimuli were similar to those in taste and olfactory cells. 4) Origin of membrane potential: In general, the membrane potential is composed of the diffusion potential within the membrane and two phase boundary potentials at the both side of the membrane. The phase boundary potential is composed of the surface potential (electric double layer potential) and "boundary potential" (potential in the membrane phase near the membrane surface). 5)Resting membrane potential: Selective K channels of taste and olfactory cell membranes seem to be closed at the physiological conditions. Probably resting membrane potentials of taste and olfactory cells are not determined by the equilibrium potential of K. 6)Origin of receptor potentials: Acids and salts lead to changes in the surface potentials. Bitter substances and odorants lead to changes in the "boundary potential". These changes in the phase boundary potential result in generation of the receptor potentials. This theory can explain well the folowing facts. a)Specific ions are not needed in generation of the receptor potentials. b)Depolarization occurs under the conditions that the receptor membranes are perfused with 1 mM KCl. c)Application of distilled water to the induces depolarization in the taste cells of certain animals no matter how ionic concentration of external medium is reduced. d)Depolarizations in response to certain stimuli occur without changes in the membrane resistance. e)The taste response to sugars, amino acids and distilled water in certain animals are suppressed by addition of electrolytes to the stimulating solutions. f)The taste response to salts are influenced by species of anions. 6)Generation of nerve impulses: Depolarization at the olfactory receptor membrane spreads electrotonically to the hillock of the olfactory nerve, which generates nerve impulses. The depolarization at taste receptor membranes spreads electrotonically to the synaptic area, which activates the voltage-dependent Ca-channel and in turn leads to an increase in Ca-influx into taste cells. This results in a release of a transmitter (probably norepinephrine) from taste cells.

NARRATE ORALLY AND POSTER EXHIBITION

1

On the application of beta-distribution upon a single nerve fiber
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This paper is a trial in order to deal with the electric phenomena of a single nerve fiber quantitatively.
The excitation of a single nerve fiber obeys binary notation, and the excitation generates with the probability of 1 or 0, and this basic manner is called as all-or-none principle.
Euler's beta-distribution is used in this research.
The normalized beta-distribution and gamma-function are expressed in slide.
These functions are able to forecast the promising future. For example, in this paper, we will discuss and calculate Weiss's formula, Tasaki's minimal gradient and Hill's stimulation theory of alternating current.

Literature

I.Tasaki:(Jap.J.physiol., I,1,1950)
A.V.Hill, B.Katz and D.Y.Solandt:(Proc.Roy.Soc.Lond., B 121,74,1937)

2

THE ISOLATION OF α AND NON-α CHAINS FROM HUMAN ADULT, FETAL, CANINE, PIG AND GUINEA PIG HEMOGLOBINS AND THEIR PHYSICOCHEMICAL PROPERTIES. TOMITA, S., SAKATA,S., OHGA,Y., KOHZUKI,H. AND ENOKI,Y. Dept. of Physiol., Nara Med. Coll., Kashihara, Nara 634

In order to get some informations as to why the varied oxygen affinities were found between bloods from various animals, we tried to isolate their α and non-α chains. The chains were separated on DE-32 or CM-32 column chromatography from their mercurated hemoglobins which were obtained by titrating with pCMB solution in 0.1 M phosphate buffer(pH 6.0) for canine, in 0.1 M acetate buffer(pH 4.6) for fetal and in 0.1 M acetate buffer containing 1.0 M KI(pH 4.6) for pig and guinea pig, and by incubating them for 24 hrs at 0° C. After demercuration, their regenerated SH groups, amino acids, pI, extinction coefficients, sedimentation coefficients and oxygen equilibrium properties were measured. The p_{50} values were pig > F > canine > guinea pig > A for tetrametric hemoglobins, pig ≃ guinea pig > canine > A(=F) for α chains, and pig > F > canine > A for non-α chains. Those results suggest that the oxygen affinity of tetrametric hemoglobin reflects, in large part, the oxygen binding properties of the constituent chains.

3

PROPERTIES OF HEMOGLOBIN BOUND TO RED CELL MEMBRANE (II)

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The effect of the red cell membrane on the oxygenation properties of three chemically modified hemoglobins was investigated. Intermediately cooperative Hbs, *N*-ethylmaleimide-treated Hb (NES-Hb) and iodoacetamide-treated Hb (AA-Hb), bound to membrane showed effects similar to those displayed by membrane-bound native Hb, i.e. lowered oxygen affinity and smaller response to organic phosphates; but very weak effects were observed for non-cooperative Hb, i.e. carboxypeptidase-treated Hb (des-His-Tyr Hb). The magnitude of oxygen affinity lowering was: NES-Hb > Native Hb > AA-Hb >> des-His-Tyr Hb. From the results of oxygenation experiments was inferred that: (a) the red cell membrane binds to deoxyHb more strongly than to oxyHb; (b) the difference in membrane binding affinity between oxy- and deoxyHb is closely related to the quaternary structure change in the Hb molecule occurring upon oxygenation. Binding experiments by means of ultrafiltration were also performed and proved that the red cell membrane actually binds to deoxyHb much more strongly than to oxyHb. The preferential binding of deoxyHb to membrane is inconsistent with previous results of Shaklai *et al.*, but is supported by the present binding experiments and a recent study by Walder *et al.*

4

COMPARATIVE STUDY OF TISSUE AFFINITY OF HEMATOPORPHYRINS AND PHEOPHORBIDES.

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Photoradiation therapy based on the administration of either hemotoporphyrin derivatives or Photofrin II recently gets reputation in cancer therapy, however the mechanism of their special affinity to cancer still remains unexplained. In the past two years we have measured comparatively tissue retention of kinds of drugs including porphyrins, pheophorbides and metal complex on golden hamsters bearing adenocarcinoma. A spectrofluorometer was used for quantification of retained drugs. In general, dimers and OH-rich compounds presented higher affinity. The dimer of 9-OH pheophorbid, one of the highest affinity group, remained in tumor at the concentration of 15μg/ml of tissue , 6μg in lung and liver, 3μg in muscle and 8μg in plasma at 24 hours after administration. Among metal complex, Ga and In groups showed high affinity, which were proposed to be a favorable localizer of tumor for X-ray or scintillation diagnosis. Co, Ni, Si, Mg, Cu and I groups belonged to lower affinity group.

The transition of the drups from blood to tumor cells was studied by three methods: centrifugal separation, PAG electrophoresis and gel filtration. Results implicated that the drugs immediately bound to plasma proteins and in tumor cells they bound to the proteins of about 300,000 mol wt to be retained at the maximal concentration for 48 hours.

5

^{1}H-NMR STUDIES ON STRUCTURAL TRANSITION OF BOVINE MERCAPTALBUMIN (BMA).

SOGAMI, M., NAGAOKA, S., ERA, S., KUWATA, K., WATARI*, H., SHIGA*, K. AND AKASAKA**, K. Dept. of Physiol., Sch. of Med., Gifu Univ., Gifu 500, *Natl. Inst. for Physiol. Sci., Okazaki 444 and **Dept. of Chemistry, Fac. of Sci., Kyoto Univ., Kyoto 606

Bovine mercaptalbumin (BMA) has various conformational states, such as E (pD 3.02), F(pD 4.03), N(pD 4.5-7.0) and B(pD 9.4) in 0.10 M KCl. An increase of BMA concentration in the F-form caused ^{1}H-NMP spectral changes (360 MHz) in parts of aliphatic and histidine signals which were substantiated by gel formation but not in the N-form. From the ^{1}H-NMR studies, the N- and B-forms showed the typical shape being ascribed to the rigid internal structure, while the F- and E-forms appeared to lose partly their rigid structure. In saturation transfer experiments, T_{IS} values of various side chains of the F-form obtained by f_2-irradiation at desired frequency, such as -2.45, 0.40, 0.90 and 7.13 ppm from TSP at $\gamma H_2/2\pi$ of 39 ~ 107 Hz were almost two-fold longer than those of the N-form. ^{1}H-NMR spectra and T_{IS} values of the F-form indicated that the rigidity in the intramolecular structure is at least partly lost in the N-F transition and the internal motion is liberated in the F-form (side chain melting or disorder).

6

THE ACID-INDUCED ISOMERIZATION OF SODIUM DODECYL SULFATE-BOVINE PLASMA ALBUMIN COMPLEX —— MOLTEN-GLOBULE STATE. ERA, S. ASHIDA*, H., NAGAOKA, S., KUWATA, K., SOGAMI, M., SHIGA**, K. AND WATARI**, H. Dept. of Physiol., Sch. of Med., Gifu Univ., Gifu 500, *Defence Med. Col., Tokorozawa 359 and **Natl. Inst. for Physiol. Sci., Okazaki 444

The acid-induced isomerization (the N-F transition) and expansion of sodium dodecyl sulfate-bovine plasma albumin complex (AD_m ; m, molar ratio of added sodium dodecyl sulfate to bovine plasma albumin ; $0 \leq m \leq 12$) were studied. The immobilization of tryptophyl fluorophors observed in the F-form of AD_0 was suppressed in the F-form of AD_{10}. The acid-titration analysis of AD_{12} showed non salt-bonding between carboxylate groups and cationic side chains in the F-form, as in the case of AD_0, indicating charged side chains being persumably mobile. ^{1}H-NMR spectra and cross relaxation times between irradiated and observed protons in the F-form of AD_{10} indicated the increase in the local motion. On the other hand, AD_{10} and AD_{12} did not show any significant changes in specific viscosity (Lovrien et al, 1967) and in CD-resolved secondary structure in the N-F transition. The F-form of AD_{10} or AD_{12} may therefore be the molten-globule state which has secondary structure similar to the N-form of the complex with fluctuating tertiary structure (side chains).

7

ION-EXCHANGE HPLC STUDIES ON THE N-A ISOMERIZATION OF BOVINE MERCAPTALBUMIN. KUWATA, K., ERA, S., SOGAMI, M. AND INOUYE*, H. Dept. of Physiol., *3rd Dept. of Int. Med., Sch. of Med., Gifu Univ., Gifu 500

In the alkaline pH region, bovine mercaptalbumin (BMA) isomerizes to the aged form (A-form) by the intramolecular SH, S-S exchange reaction, as shown in the following equation

$$N \rightleftharpoons A_1 + mH^+ \rightleftharpoons A_2 + nH^+ \rightleftharpoons \text{- - - - -}$$

where N and A_i are the N- and A-forms, respectively. The time course of the aging reaction of BMA at pH 8.6 without added salt at 25° obtained by ion-exchange HPLC was in good agreement with that by moving-boundary electrophoresis. The aging reaction was accerelated in glutathione oxidoreduction buffer. The effects of differing initial concentrations and ratios of GSH and GSSG on the aging reaction were similar to those on the extent of recovery of reduced hen egg lysozyme, reported by Saxena & Wetlaufer (1970). In D_2O which might strengthen the intra- and intermolecular hydrophobic and/or hydrogen bonds, the N-A isomerization was slightly suppressed. This indicates the existence of the global structural fluctuations which causes the N-A isomerization in D_2O as well as in H_2O.

8

IDENTIFICATION OF PLASMINOGEN ACTIVATORS IN XIPHOPHORIN FISH MELANOMAS. TAKAHASHI, K[1], WAKAMATSU, Y[2]*, OZATO, K[2]*, NAORA, H[1] AND MASUMURA, S[1] [1]Dept. of Physiol., Shimane Med. Univ., Izumo 693, [2]Biol. Lab., Yoshida Coll., Kyoto Univ., Kyoto 606

A monoclonal antibody to human urokinase-plasminogen activator was provided to quantitate, purify and identify antigens. By enzyme-linked immunosorbent assay and the determinaiton of the enzyme activity as well, higher level of the enzyme activities of the antigens was found in the F_1 and BC_1 hybrids carring melanosis, but not in parental platyfish and swordtails. Affinity purified antigens did reveal multiple molecular weight forms on SDS-polyacrylamide gels. They showed a specific molecular conversion of plasminogen to plasmin. The FITC-conjugated actibody located in the melanosis lesions. Thus, these results strongly suggest that active plasminogen activators are associated with the development of melanomas and might play a role in the invasiveness.

9

Which type of the protonated or unprotonated form at N atom of a ligand can bind to D-amino acid oxidase? NISHINA, Y., SHIGA, K., AND WATARI, H.* Department of Physiology, Kumamoto University Medical School, Kumamoto, 860 and *National Institute for Physiological Sciences,444

When D-amino acid oxidase is reduced by D-arginine in the presence of picolinate, the purple complex appears. The dissociation constants between the reduced enzyme and picolinate at various pH values were obtained by a spectroscopic titration at 600 nm. The constant was 35 μM at pH 7.0, at 25 °C. Judging from the pH dependence of the dissociation constant, we can conclude the picolinate is a protonated cation form at N atom. This conclusion is consistent with the previous results of the resonance Raman study of the purple complex with D-proline. Nicotinate is also a good ligand for complex formation with the reduced enzyme, but neither isonicotinate nor quinaldinate is a good ligand.

The resonance Raman spectra of the complex between o-aminobenzoate and the oxidized enzyme indicate that the amino group of the o-aminobenzoate is a neutral form.

10

ANALYSIS OF TERBIUM BINDING SITES ON TRYPTOPHAN. AIZAWA,K., SAKAI,M., SAITO,H., SAKAI,S., O'HATA,S., NISHIYE,H*, YAMAGISHI,H**, MATSUSHITA,K*** Dept.of Physiol. Tokyo Medical Coll. Shinjuku-ku Tokyo *Dept.of Physiol.. Juntendo Univ. Hongo Bunkyo-ku Tokyo **Lab.Membr.Biol. Natl.Inst.Physiol.Sci.,Okazaki ***NMR Appl. Lab.,JEOL.LTD Nakagami Tokyo

Terbium(Tb^{3+}) ions were known to be a sensitive environmental probe of Calcium binding site of proteins. Its fluorescence increase specifically upon Tb binding with Calcium binding proteins such as Troponin-C, Paralubumin and Calmodulin. The fluorscence of terbium which was excited by 225nm showed 4 peaks(490,545,585,620nm)in aqueous solution. But these emission peak disappeared by 290nm excitation. Tb fluorescence emitted by the excitation of 290nm in a squid giant axon added Tb ions. The enhanced fluorescence of the bounded Tb was sensitized by resonant energy which transefered from nearby aromatic residues in the Trp excited by 290 nm. The 270 MHz ^{1}H-NMR spectrometer and FT-IR spectrophotometer were used to study a Tb binding site of Trp in methanol solution. ^{1}H-NMR spectrum of OH and NH has a peak at 4.85ppm in the Trp solution. In addition of Tb, NH peak of Trp was shifted from 4.85 ppm to 4.60 ppm. Its spectrum intensity was related to a Tb concentration. The stretching vibration of NH group in the Trp solution has a large absorption peak at 3,000-3,600 cm^{-1}. In addition of Tb, these peak decreased broadly. The present experiments can be a support that the terbium binding sitet is a NH group on tryptophane in methanol solution.

11

CONFORMATION OF ANGIOTENSIN II. UYEDA*, M., IWAI, T., FUJIWARA, T. AND TANIMOTO, O. *Dept. of Physiology, Sch. of Med., Gifu Univ., Gifu 500 and Dept. of Applied Physics, Fac. of Eng., Osaka City Univ., Osaka 558.

For the estimation of peptide conformation by empirical force field methods, we deviced method of automatic glid search for main chain ϕ, ψ dihedral angles and their clustered probability using potential map, diminishing freedum of side chain dihedral angles as few as possible. We applied this method to search minimal energy conformation of Angiotensin II and Bradykinin, octa and nonapeptide hormone respectively. We found twelve different main chain conformations whose energy within 3 kcal/mole from minimal energy, -56 kcal/mole for Angiotensin II. For Bradykinin we got sixteen different conformations within 4 kcal/mol from minimum energy, -101.5 kcal/mol. The calculated CD spectra for obtained conformations by exciton apploximation are agree very well with experimental one. ϕ and χ_1 dihedral angles calculated from vicinal coupling constant $J_{HN\alpha}$ and $J_{\alpha\beta}$ also agreed well to obtained conformations. Self-consistent perturbation calculation (Pople 1968) also applied to these obtained conformations and satisfied result of calculated $J_{HN\alpha}$ and $J_{\alpha\beta}$ of agreement within almost 1 Hz of experimental one are obtained.

12

ANALYSIS OF THE ORINTED MULTIBILAYERS IN SLOW MOTIONAL DOMAIN BY SATURATION TRANSFER ELECTRON PARAMAGNETIC RESONANCE. Watari, H., Nishina, Y. and Shimoyama, Y.* Dept. Molecular Physiol., Natl Inst. Physiol. Sci., Okazaki 444 and *Dept. Physics, Hokkaido Univ. of Education, Hakodate 040

The saturation transfer electron paramagnetic resonance (ST-EPR) spectra was successfully applied to the spin labeled oriented multibilayer membrane of dipalmitoylphosphatidylcholine (DMPC) in order to explore molecular order and motion of the lipid membrane organisations. The spectra varies with an angle between the membrane film surface and applied magnetic field, yielding that the lipid membrane forms two-dimensional layered structures. The motional parameters were characterized by means of the relationship between the peak height ratio and rotational correlation time. Correlation times at temperatures below the gel-liquid crystalline phase transition point (ca. 313 K) were found to be in the range of 10^{-3} to 10^{-6} sec. The lipid motion in the multibilayer membranes at temperatures between 233 K to 273 K was determined by the correlation times. The integration method has been developed in order to determine the dynamical behavior of the lipid ensemble in the oriented membranes. Upon addition of cholesterol, an anisotropic structure of correlation times was found in the gel-phase of the multibilayer membrane.

13

^{1}H- and ^{23}Na-NMR STUDIES ON THE ACTIONS OF GLYCOSAMINOGLYCANS ON H_2O AND Na^+ ION. Mifune,T.,Kusaka,G.,*Seo,Y.,*Nishikawa,H.,Yamashita,F.,Sakakida,K., Dept. of Orthop.Surg. *Dept. of Physiol. Kyoto Pref. Univ. of Med. Kyoto,602.

Cartilage retains much amounts of extracellular water and cations such as Na^+ ion in its matrix. The matrix has fixed charged groups of glycosaminoglycans(GAG). In this study,the relations between the charged groups and water, Na^+ ion were analyzed by mean of variable pH relating to charge density, and ^{1}H- and ^{23}Na-NMR relaxation times of water and Na^+ ion in models of the matrix. Two groups of solutions as models were prepared. The one composed of sodium hyaluronic acid(HA) solutions and sodium chondroitin sulfate(Ch-S) solutions with a 0.15M NaCl in various their concentrations. The other, 1.6% HA solutions and 5% Ch-S solutions with 0.15M NaCl at various pHs. The relaxation times of H_2O and Na^+ ion were measured by JEOL PFT100 NMR spectrometer(^{1}H:100MHz, ^{23}Na:24MHz). Relaxation times(T_1 ,T_2) of water and Na^+ ion decreased as HA or Ch-S concentration increased. The decrease of T_1 and T_2 in Ch-S solution were larger than in HA solution. The relaxation times in Ch-S solutions showed minimums at pH 5 to 7. These findings indicated that Ch-S restricted mobilities of water and Na^+ ion more than HA and that the restriction was strongest near at physiological pH value in the matrix of cartilage.

14

QUANTUM CHEMICAL STUDIES ON THE TSH RELEASING MECHANISM BY THE THYROTROPIN-RELEASING HORMONE AND ITS ANALOGUES. SHINAGAWA, Y. & SHINAGAWA, Y. Dept. Physiol., Kyoto Univ. Fac. Med., Kyoto 606 & Dept. Physiol., Nippon Med. Sch., Tokyo 113

Quantum chemical approach using the semi-empirical molecular orbital method was done to the studies of the mechanism of the TSH(thyroid stimulating hormone)releasing action of TRH (thyrotropin-releasing hormone) and its analogues. The calculated molecules are pGlu-His-Pro-NH_2 (TRH), pGlu-Leu-Pro-NH_2, pGlu-Phe-Pro-NH_2, pGlu-Met-Pro-NH_2, pGlu-Gly-Pro-NH_2, pGlu-Val-Pro-NH_2 and pGlu-Thr-Pro-NH_2. Minimal energy conformations of pyro-L-glutamic acid, L-proline-NH_2, L-histidine, L-leucine, L-methionine and L-phenylalanine were calculated, then minimal energy conformations of TRH and its analogous molecules were calculated using the CNDO/2 methods and our geometry adjusting program. Several electronic indices in the frontier electron theory had significant correlations with the TSH releasing activities of those compounds. These indices are the ones at the oxygen, carbon and hydrogen atomic positions on pyroglutamic acid ring and the bridge moiety between pyroglutamic acid ring and proline ring, and a hydrogen atomic position on proline ring.

The electron accepting indices at the atomic positions of pyroglutamic acid ring and proline ring and the electron donating indices at the atomic positions of the bridge moiety were sorted as the ones with significant correlations.

15

DETECTION OF ACTIVE OXGEN FROM HPD USING LUMINOL.

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Conserning photodynamic mechanism of HPD, it is generally accepted that cytocidal effects are achived by singlet oxygen produced by HPD. We have performed studies to clarify the mechanism of active oxygen in photoradiation with HPD solution containing Luminol, because Aminophthalic acid which is the final substance of Luminol reaction has the same peak excitation wave length as HPD. Luminol and HPD were mixed in Menzel buffer solution and fluorescence was excited by 395nm wave length. The emission peak of Aminophthalic acid at 485nm decreased with time. The emission peak of Aminophthalic acid initially decreased in the first phase but increased later when HPD was added. This elevation of emission peak of Aminophthalic acid showes a production of active oxygen produced by HPD. This report describes the recognition of the release of active oxygen by photoradiation of HPD.

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INCORPORATION AND EXCRETION OF HpD IN CULTURED CELLS.

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We examined the incorporation and excretion of HpDI, HpDII and HpIX dihydrochloride in mouse fibrosarcoma cells(m-KSA) and normal lung cells of BALB/c mouse.Quantitative determination of intracellular quantity of HpD was performed by fluorescence microscopy using malti-channel analizer(OMA). Absorption spectra were recorded by the opal glass method using Hitachi 333 spectrophptometer. The results were as follows; 1) m-KSA cells predominantry bound HpDII. 2) In the fluorescence spectra of m-KSA cells and normal cells, the increase of peak-height at 660nm might be indicate intracellular aggregates of HpDI and HpDII, although this peak was not observed in HpIX. The measurement of absorption spectra was also supported the above result. 3) m-KSA cells seemed to have a heigher affinity for porpyrin aggregates than normal lung cells.

17

COMPARATIVE ^{1}H-NMR STUDIES ON THE WATER STRUCTURE IN SOFT CONTACT LENS AND PROTEIN GEL.

HAYANO, S., AMANO, H., TAKAHASHI, K., SOGAMI*, M., NAGAOKA*, S., ERA*, S., KUWATA*, K., WATARI**, H., SHIGA**, K. AND AKASAKA***, K. Dept. of Ophthalmol., *Dept. of Physiol., Sch. of Med., Gifu Univ., Gifu 500, **Natl. Inst. for Physiol. Sci., Okazaki 444 and ***Dept. of Chemistry, Fac. of Sci., Kyoto Univ., Kyoto 606

The water structure in soft contact lens (SCL) and modified bovine plasma albumin gel (BPA*-gel) were studied using 360 MHz ^{1}H-NMR spectra, $T_1(H_2O)$ and cross relaxation time ($T_{IS}(H_2O)$) obtained by irradiation at -4.00 ppm from TSP at $\gamma H_2/2\pi \sim 69$ Hz. In SCL, a sharp and a broad H_2O signals were observed at 4.74 ppm and 4.47-4.52 ppm, respectively. $T_1(H_2O$, 4.74 ppm) and $T_1(H_2O$, 4.74-4.52 ppm) were 2.7-2.8 sec and 0.56-0.63 sec, respectively. $T_{IS}(H_2O$, 4.74 ppm) and $T_{IS}(H_2O$, 4.47-4.52 ppm) were 1.5-2.9 sec and 0.35-0.37 sec, respectively. The more the water content in SCL was increased, the more the values of $T_1(H_2O)$ and $T_{IS}(H_2O)$ were increased. Though a sharp H_2O signal was decreased by soaking SCL into D_2O, a broad one was not affected. A broad one might therefore correspond to the strongly bound and free water in polymer matrix. On the other hand, H_2O signal in BPA*-gel did not show significant chemical shift. $T_{IS}(H_2O)$ in BPA*-gel were almost comparable to those for side chains, indicating the hydration of BPA* in the gel state being strong and rapidly exchangeable with free water.

18

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC STUDIES ON THE MERCAPT $\rightleftarrows$ NONMERCAPT CONVERSION OF HUMAN SERUM ALBUMIN.

SOGAMI, M., ERA, S., NAGAOKA, S., KUWATA, K., KIDA, K., SHIGEMI, J., MIURA(1), K., SUZUKI(1), E., MUTO(2), Y., HAYANO(3), S., SAWADA (4), S., KOSHIBA(5), T. AND OKA(6), T. Dept. of Physiol., (1)3rd Dept. of Int. Med., (2)1st Dept. of Int. Med., Sch. of Med., Gifu Univ., Gifu 500 (3)Hayatoku Hospital, Gifu 500, (4)Sawada Hospital, Gifu 500 (5) Dept. of Urology, Kitazato Univ., Sagamihara 228 and (6)2nd Dept. of Surgery, Kyoto Pref. Med. Col., Kyoto 606

High-performance liquid chromatographic (HPLC) analysis of human serum albumin (HSA) on GS-520 column (0.03 M sodium phosphate buffer, 0.15 M Na_2SO_4, pH 6.86) showed three peaks, the principal component corresponding to human mercaptalbumin (HMA), the secondary and tertiary components to nonmercaptalbumin (HNA). A mean value of [(HMA)/(HMA + HNA)], $\bar{f}_{HMA}$ for healthy male adults was 0.75 ± 0.028 (n = 28). In hemodialysis patients, $\bar{f}_{HMA}$ before dialysis and a mean value of [(f_{HMA} after dialysis)-(f_{HMA} before dialysis)], $\Delta\bar{f}_{HMA}$, were 0.41 ± 0.073 (n = 293) and 0.15 ± 0.086 (n = 291), respectively. In chronic renal failure (n = 28) and renal transplantation (n = 63), $\bar{f}_{HMA}$ values were 0.51 ± 0.096 and 0.61 ± 0.090, respectively. HPLC profiles of HSA in various liver diseases were quite different from those of normal healthy adults and these $\bar{f}_{HMA}$ were significantly lower than those of healthy male adults.

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THE HYDRATION OF PROTEIN IN COAGULATION AND FIBRINOLYSIS EXAMINED BY ^{1}H-NMR.

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Fibrinogen solutions (Soln) at the different concentrations of 1 to 100 mg/ml were made into fibrin gel by coagulation (Gel) and the gels were lysed to the sols by fibrinolysis (Sol). The Soln, Gel and Sol samples, each as an aliquot of 5 ml containing 10 % of D_2O, were taken into the 20 mm NMR sample tubes and examined on the T_1 and T_2 with Bruker's 80 MHz ^{1}H-NMR spectrometer. The hydration values (h, g H_2O/g protein) of those samples were calculated according to Kumosinski & Pessen (1982) and obtained each as 0.18, 0.31 and 0.07 at pH = 6.75, 0.23, 0.38 and 0.10 at pH = 7.75, and 0.26, 0.44 and 0.14 at pH = 8.75, for Soln, Gel and Sol, respectively.

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DIHYDROPYRIDINE BAY K 8644 POTENTIATES THE ADRENOMEDULLARY SECRETION INDUCED BY THE REVERSED Na-Ca EXCHANGE MECHANISM. YANO*, K., NISHIMURA, S. AND SORIMACHI, M. Depts. of Surgery* and Physiol., Kagoshima Univ. Sch. Med., Kagoshima 890

We have investigated the effect of BAY K 8644(K 8644) on the adrenomedullary secretion induced by Ca reintroduction under divalent cation-deficient condition. The responses to 0.5 mM Ca in the perfused cat adrenals were markedly increased in the presence of K 8644(0.1 μM). The increased response was antagonized by nifedipine, a dihydropyridine Ca channel blocker. When the responses to Ca were reduced after treatment of the gland with a medium lacking Na or containing Mg(1 mM), the amounts of catecholamines(CAs) released by the stimuli were markedly reduced even in the presence of K 8644. However, the responses became larger by K 8644 even in the presence of Mg when the control responses were restored after treatment with ouabain, K removal or metabolic inhibitors. Ca-45 uptake as well as CAs secretion by the isolated bovine chromaffin cells were significantly increased in response to Na-free medium containing 0.5 mM Ca. Since we previously suggested that the response to Ca reintroduction was due to Ca influx in exchange for internal Na, these results suggest that K 8644 somehow modifies Ca entry pathway to further increase CAs secretion.

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EFFECT OF Ca^{2+} ION AND ATP ON THE SECRETION OF TISSUE-TYPE PLASMINOGEN ACTIVATOR. SAKAI, T., KIKUCHI, H., *MATSUO, C., UESHIMA, S. AND MATSUO, O. Dept. Physiol. and *OB.GY., Kinki Univ. Sch. Med. Sayama, 589.

Human melanoma cell line (Bowes) secretes a large amount of tissue-type plasminogen activator (T-PA) into the culture medium. In the present study, the effect of adenosine triphosphate (ATP) and Ca^{2+} ion was examined to investigate the mechanism of T-PA secretion. The addition of Ca^{2+} ion up to 2.0 mM to the medium did not affect T-PA release, but the higher concentration of Ca^{2+} ion suppressed the T-PA release. ATP suppressed the T-PA release remarkably in a dose-dependent manner. These suppressive effects of Ca^{2+} ion and ATP were exaggerated by the simultaneous addition of two agents. The incorporation of ^{14}C-leucine was slightly inhibited by ATP, but not by Ca^{2+} ion alone. However, the simultanous addition of ATP and Ca^{2+} inhibited the ^{14}C-ieucine incorporation to about 80% of the control. Further, the simultanous addition of ATP and Ca^{2+} ion suppressed slightly c-AMP which increased under normal condition. These results suggest that extracellular ATP and Ca^{2+} ion may regulate cellular activity as to the T-PA secretion.

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PANCREATIC EXOCRINE RESPONSE VIA SECRETIN RELEASE INDUCED BY H^+ DEPENDENT AND NON-DEPENDENT PROCESS IN PIG. HARADA E., SHUTO B. AND NIYAMA M. Dept. Physiol., Fac. Vet. Med., Hokkaido Univ., Sapporo 060

The pancreatic exocrine response induced by intraduodenal acidification of various monocarbonic acids was investigated in anesthetized piglets. The flow rate of pancreatic juice and bicarbonate output bear a linear relationship to each other. The pancreatic response induced by lactate was increased dose dependently with the solution maintained lower pH than 3.8, but NaCl solution at higher pH than 2.0 did not occured any response. Responsibility of the exocrine secretion and secretin release induced by monocarbonic acids at pH 2.0 was as follows; formic acid > lactic acid ≫ acetic acid > propionic acid ≧ butyric acid. The pancreatic exocrine secretion via secretin release was evoked by intraduodenal infusion of trypsin inhibitor (pH 6.5, 200 mg/kg) and deoxycholic acid (pH 7.4, 40 μmoles/kg). It was suggested that secretin is released by H^+ dependent and non-dependent process from S-cells in pigs.

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FAST AXONAL TRANSPORT OF MEMBRANE PROTEIN AND ACETYLCHOLINE IN AN APLYSIA CHOLINERGIC NEURON: ITS MODIFICATION BY LOCAL COLCHICINE PERFUSION OR LOWERING TEMPERATURE. KOIKE, H., UMITSU, Y., MATSUMOTO, H., Dept. Neurophysiol., Tokyo Metropolitan Institute for Neurosciences. Fuchu City, Tokyo 183

Fast axonal transport of membrane protein and acetylcholine was examined by the intracellular injection of labeled amino acid and acetylcholine into a cholinergic giant neuron of Aplysia kurodai. It was modified either by lowering temperature of the perfusing solution or by the application of high concentration of colchicine, both of which were known to depolymerize microtubules. Movements of membrane protein and acetylcholine were stopped when the axon was locally cooled to about 5 °C. However, local application of colchicine (5mM in perfusing solution) only blocked the movement of membrane protein but little affected if any on the movement of acetylcholine. These results are suggestive for the contribution of microtubules to the fast axonal transport of different substances.

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Na^+/K^+-CHANNELS IN SUBMANDIBULAR AND LACRIMAL GLAND ACINAR CELLS FROM HAMSTER. MATSUMOTO, O., MARUYAMA, Y., IWATSUKI, N. AND NISHIYAMA, A. Ist. Dept. of Physiol., Tohoku Univ. Sch. Med., Sendai 980.

Using "Giga-Seal" inside-out, outside-out and whole cell clamp mode, we demonstrated three types of Ca^{++}-activated ionic channels (large K^+-channel, Cl^--channel and monovalent cation channel) in isolated submandibular and lacrimal gland acinar cells from hamster. Co-existence of those channels on acinar membrane has been already suggested in rat and mouse salivary gland, however, the properties of monovalent cation channels have not yet been studied in detail. We revealed that monovalent cation channels were exactly the same as those shown in mouse and rat pancreas (Ca^{++}-activated Na^+-K^+ non-descriminatory cation channels) from the views of their conductance, selectivity and temporal characteristics. Role of K^+- and Cl^--channels are discussed in the combination with Na^+-K^+-$2Cl^-$ co-transporter in electrolyte secretion. Tentative assumptions to explain the role of Na^+/K^+-channels are as follows i) depolarizing voltage generation to maintain the K^+-efflux via the large K^+-channels, ii) stimulation of Na^+-K^+ pump to maintain $(K^+)i$.

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RELATION BETWEEN OUABAIN-INSENSITIVE K^+ TRANSPORT SYSTEM AND MONOVALENT IONS IN HeLa CELLS. IKEHARA, T., YAMAGUCHI, H., HOSOKAWA, K., AND MIYAMOTO, H. Dept. of Physiol. Sch. of Med., Univ. of Tokushima, Tokushima 770

We made a kinetic study on furosemide-sensitive Rb^+ influx into HeLa cells. Double reciprocal plot of the influx against external Rb^+ concentration showed a linear relation between them. The Rb^+ influx was competitively inhibited by external K^+, showing that Rb^+ acts as a substitute for K^+ in the transport system. However, the transport system seems to slightly prefer Rb^+ to K^+. When the external Na^+ concentration was increased, the Rb^+ influx increased. From this experiment, we could obtain values of the apparent Km for both external Na^+ and Rb^+. The furosemide-sensitive Rb^+ influx was also increased as the external Cl^- concentration increased. Replacement of Cl by other anions such as NO_3 and SCN inhibited the Rb^+ influx, but sulfamate was not inhibitory. Using sulfamate for changing the Cl^- concentration, values of the apparent Km for the two Cl^- binding sites were determined. These results suggest that the furosemide-sensitive carrier system transports Na^+, K^+ and Cl^- in the ratio of 1:1:2 in HeLa cells as in other types of cells.

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POTENTIATION OF ISOPROTERENOL-STIMULATED TISSUE CYCLIC AMP BY CALCIUM IN RAT PAROTID
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Methacholine and carbamylcholine, in addition to their known inhibitory effect, augmented the effect of isoproterenol on parotid cyclic AMP accumulation. The significant augmentation by methacholine was obtained at 0.1 μM and the maximum effect was attained at 0.5 μM, but it required more than 10 μM to obtain the inhibitory effect. Atropine completely inhibited the effect of methacholine. Similar augmentation of the effect of isoproterenol on tissue cyclic AMP accumulation was observed by other cholinergic agonists such as oxotremorine, pilocarpine or muscarine and A23187. Oxotremorine and A23187 augmented the effect of isoproterenol in the presence of isobutylmethylxanthine. They, however, did not augment the effect of forskolin. Cholinergic agonists-and A23187-induced augmentation was completely abolished by the omission of calcium in the medium, but the effect of isoproterenol on tissue cyclic AMP accumulation was not. These results suggest that the cholinergic agonists-and A23187-induced augmentation is due to the activation of adenylate cyclase by influencing the coupling process between the regulatory and the catalytic subunits of adenylate cyclase and that it may be mediated by influx of extracellular calcium in to the cell.

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SEROTONIN-INDUCED CALCIUM INFLUX AND CYCLIC GMP FORMATION IN A GLIAL CELL LINE.
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Glial cells are multifunctional. For manifestation of a particular glial function, signal molecules are assumed to be transferred from neuron to glia. Using a rat glial cell line (C6BU-1), we have examined the possibility that neurotransmitters serve as the signal molecules. Upon an exposure to 1 μM serotonin (5HT), the cellular cyclic GMP content increased from ∿100 fmol/mg protein to ∿800 within 2 min. This increase was antagonized by 0.1 μM methysergide and metergoline, and depended on the dose of applied 5HT (ED_{50} of 5HT was 20 nM) and on the external Ca^{2+} level (ED_{50} of Ca^{2+} was 0.3 mM). Since we have previously reported an elevation of cytosolic free Ca^{2+} concentration ($[Ca^{2+}]_i$) induced by 5HT in the same cell, we studied the causal relationship of $[Ca^{2+}]_i$ rise and cyclic GMP formation. Externally applied 1 mM dibutyryl cyclic GMP caused no Ca^{2+} influx nor $[Ca^{2+}]_i$ rise. Ionomycin (1 μM) caused a rapid and marked rise of $[Ca^{2+}]_i$, but the cyclic GMP increase was slow and small (∿1.5 fold in 2 min). These results favor the view that the C6BU-1 glial cell possesses two separate 5HT receptor systems, one coupled with Ca^{2+} entry pathway and one coupled with guanylate cyclase, but the final conclusion was yet unreached.

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NONSELECTIVE CATION CHANNEL IN SEA URCHIN EGGS.
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The fertilization potential of a sea urchin egg consists of two transient depolarizations, that is, a quick depolarization and the following slow depolarization with a peak between +10 and +20 mV. We studied the ionic mechanism of this slow depolarization. At the peak of the slow depolarization, the membrane resistance decreased to 1/6 in comparison to that of an unfertilized egg, and the ratio of Na and K permeabilities (P_{Na}/P_K) was 1.1. Intracellular K^+ concentration did not change during generating the fertilization potential. These suggest that nonselective cation channels exist in a sea urchin egg and are activated at fertilization.

Recently, Ca-activated nonselective cation channels were found in cardiac muscles, pancreatic acinar cells and neuroblastoma cells. In sea urchin eggs, $[Ca^{++}]_i$ has been reported to increase transiently at the early stage of fertilization. Consequently, this increase may activate the nonselective cation cahnnels of a sea urchin egg. In other words, sea urchin eggs may also have Ca-activated nonselective cation channels.

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MEASUREMANTS OF THE TISSUE IMPEDANCE

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We measured the electrical impedance of the biological tissues by YHP 4192A Impedance Analyzer. The measured frequency was ranged from 5Hz to 13MHz. In cell suspension experiments, the cellular volume fraction has been measured by the electrical impedance. Thus the electrical impedance of the biological tissue is influenced by the ratio of the extra-cellular fluid (ECF) and intra-cellular fluid (ICF). The salivary gland secretes a large amount of saliva by the nervous stimulation. We measured the impedance of the dog submandibular gland during both of resting and stimulation. The electrical impedance changed during secretion. Without blood supply during stimulation, impedance locus change became more clear. And in the perfused gland with 1/2 Ringer + isotonic sucrose solution, the impedance locus enlarged. The impedance locus change suggested that the ECF of the gland decreased during stimulation. The light microscopic findings in the frozen section of the salivary gland showed that the ECF of the inter-lobular space decreased during stimulation without blood supply.

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QUANTITATIVE IMMUNOFERRITIN LOCALIZATION OF LEUCINE AMINOPEPTIDASE ON CANINE HEPATOCYTE CELL SURFACE.

TANAKA, K.*, OMORI, K., AND TASHIRO, Y. Dept. of Physiol., Kansai Med. Univ. Moriguchi 570

Canine liver was prefixed by perfusion with 0.6 % glutaraldehyde and the dissociated liver cells were prepared without losing their polarized structures. They were incubated with ferritin antibody conjugates against canine kidney leucine aminopeptidase (LAP) (EC.3.4.11.2) at the saturation level and the distribution of ferritin particles on the three surface domains of the hepatocytes were investigated quantitatively by counting ferritin particles. Our analysis clearly indicated that LAP exists exclusively on the bile canalicular surface and no significant number of ferritin particles were detected either on the sinusoidal front or on the lateral surface in agreement with the observations by Roman and Hubbard (J. Cell Biol., (98,1488-1496 (1984)). In our present analysis, however, the control level on the bile canalicular surface was less than 1 % of the experimental value. We conclude that LAP does not exist on the sinusoid-lateral surface but is exclusively localized on the bile canalicular surface.

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DEVELOPMENT OF QUANTITATIVE IMMUNOELECTRON MICROSCOPY AND IT'S APPLICATION TO THE INVESTIGATION OF MEMBRANE PROTEINS.

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Membrane proteins of the limiting membranes of various cytoplamic organelles and of the cytoplasmic membranes are occasionally heterogeneously distributed. Such heterogeneous distribution of the membrane proteins appears to be intimately correlated with the functions of the membranes. We have developed a quantitative ferritin immunoelectron microscopic technique and analysed the distribution of hepatocyte microsomal membrane proteins such as cytochrome P-450 and NADPH-cytochrome P-450 reductase, and hepatocyte cytoplasmic membrane proteins such as asialoglycoprotein receptors, 5'-nucleotidase, (Na^+, K^+)ATPase and LAP. The details of our method were explained and the limitation and generalization of our procedures were explained.

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LOSS OF INTERCELLULAR COMMUNICATION DURING THE DIFFERENTIATION IN 3T3-L1 PREADIPOCYTE.
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The change of intercellular communication during the adipose differentiation was studied in 3T3-L1 preadipocyte with microinjection of Lucifer Yellow CH into the cell. The number of dye-recipient cells and incidence of cell communication in preadipocytes were 10.4±0.5; 100%(mean±s.e.; incidence) (n=30). In the morphologically differentiated cells and adipocytes, these values were remarkably decreased in 0.63±0.22; 30% (n=30) and 0.13±0.09; 5.3% (n=38), respectively. Adipocyte also lost actin stress fiber and fibronectin. As cAMP induces the lipolysis of adipocyte and enhances the cell communication, 1mM dbc-AMP and 1mM caffein were added in the culture of adipocytes for 3 days. After this treatment, the incidence of cell communication in adipocyte was recovered to 42%. This recovery required new synthesis of protein. However, cAMP did not restore the disappearance of actin stress fibers and loss of fibronectin in adipocyte. These results might suggest that loss of intercellular communication was mainly caused by changes of gene expression during the differentiation and not by morphological changes.
This study was supported by Grant-in-Aid for Cancer Research (No.59015071) and Encouragement of Young Scientist (No.59770066) from the Ministry of Education, Science and Culture, Japan.

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CHARACTERIZATION OF INTERCELLULAR COMMUNICATION CAPACITY OF TRANSFORMATION-SENSITIVE BALB/C 3T3 CELLS.
ENOMOTO, T., SHIBA, Y., KANNO, Y., KAKUNAGA, T* AND YAMASAKI, H* Dept. of Physiol., Hiroshima Univ. Sch. of Dent., Hiroshima 734, Res. Inst. for Microbial Dis., Osaka Univ., Osaka 565 and IARC, France.

In order to study the relationship between intercellular communication and carcinogenesis, we investigated intercellular communication capacity of transformation-sensitive (3T3 A31-1-13) and -resistant (3T3 A31-1-8) clonal cells, using dye transfer method. At growing phase, Lucifer Yellow CH dye injected into a cell of both clonal cells was transferred to many neighboring cells and there was no significant difference between two clones in term of dye transfer capacity, indicating that both clonal cells have similar intercellular communication capacity at their growing phase. However, when these cells reached confluency, both clonal cells revealed remarkable differences of dye transfer capacity. Thus, dye transfer between 1-13 clonal cells was decreased at their confluency and this decrease continued up to termination of cultures, while 1-8 cells maintained high capacity of dye transfer at their confluency. These results suggest that intrinsic differences in the control of intercellular communication may be a determinant of the sensitivity of these cells to the induction of malignant transformation.

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CHARACTERIZATION OF A MONOCLONAL ANTIBODY TOWARDS CALCINEURIN, A CALMODULIN-STIMULATED MULTIFUNCTIONAL PHOSPHATASE.
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Calcineurin, a major calmodulin binding protein in brain was composed of two subunits ; α, Mr=60,000 and β, Mr=16,500. This protein was demonstrated to posess a metal ion-dependent and calmodulin stimulated phosphatase activity. It had been suggested that calcineurin had different conformational states depending on different metal ions Ni^{2+} and Mn^{2+}. We confirmed the hypothesis by using a monoclonal antibody towards calcineurin.

A monoclonal antibody, VA_1 which is specific for β subunit, inhibited Ni^{2+} -stimulated activity. This inhibition was not competitive with respect to substrate and changed both Vmax and Km value. Excess amount of Ni^{2+} did not relieve the inhibition, and had no effect on the interaction between VA_1 and calcineurin. Calcineurin-VA_1 immunocomplex is less responsive to calmodulin stimulation than VA_1 free calcineurin.

These results indicated that the active conformational states of calcineurin were metal ion-dependent, that the monoclonal antibody VA_1 affected the Ni^{2+} -induced confornational change of the enzyme.

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CELL CYCLE-DEPENDENT CHANGES IN CELL VOLUME OF L929 STRAIN. DOI, A., ITANO, T., MATSUI, H., OHMURA, Y., AND HATASE, O. Dept. of Physiol., Kagawa Med. Sch., Kagawa 761-07.

The changes in cell volume of L_{929} strain were analysed by a Coulter counter and a flow cytometer. The cells were synchronized by TN-16 (arresting at M-phase) and excess thymidine (arresting at S-phase).

The distribution of cell size in the synchronized cell population varied during the cell cycle. The cell volume in each phase of the cell cycle was estimated as follows: early G1; 990 μm^3, mid S; 1690 μm^3, G2 and M; 1973 μm^3, respectively. The cell volume did not changed for 5 hours after mitosis (early G1), it rapidly increased in late G1 and S phase. The duration time in each phase of the cell cycle was determined: G1; 9 hr, S; 7-8 hr, G2; 4-5 hr, M; 1 hr, respectively. Under conditions of exponential growth, the average cell size in a mix population remained constant (1260 μm^3).

The increase of the cell volume in the cycling cells was interpreted to depend to the amount of synthetic machinery available.

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DIELECTRIC PROPERTIES OF CULTURED EPITHELIAL-CELL (MDCK)-MONOLAYER THAT ENCOMPASSES A GELATIN DROPLET

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Our previous report dealt with the dielectric behavior of teleostean and amphibian embryos which was analyzed in terms of a shell model. In the present paper we evaluate the validity of this model approach by using a simplified culture system comprising a gelatin ball(1-2 mm in diameter) covered by a MDCK monolayer. Computer-controlled measurements over 10Hz-10MHz disclosed that dielectric dispersion, not detectable just after cell seeding, appeared after several hours in culture. A maximum dispersion of magnitude of some 10^5 and characteristic frequency around 10kHz was attained as the monolayer reached confluency. Theoretical analyses based on a single-shell model gave a low-frequency limit capacity for the monolayer(C_M) and a conductivity ratio of the gelatin phase to the external medium (K_i/K_a). C_M was 1.7 $uFcm^{-2}$ in good agreement with a separate dtermination on planar monolayers cultured on membrane filters. K_i/K_a was 0.67, which was consistent with that determined on gelatin balls alone.These results suggest that the single-shell model is applicable to spherical tissues covered with cell monolayers.

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TEMPARATURE DEPENDENCE IN COLONY FORMATION OF THE CELLS DERIVED FROM RABBIT AND HUMAN ORAL MUCOSA. KASAI, S., IKESHIMA, K., AND TERASAWA, T. Dept. of Physiol., Tohoku Dental Univ., Koriyama 963

Temparature dependence of epithelial cell proliferation in primary culture was investigated using newly developed temparature gradient incubator. Epithelial cells obtained from oral mucosa of adult rabbit or infant patient were cultured with or without feeder layer of Swiss 3T3 cells in MCDB 152 containing 10% FCS at some steps of temparature for 8 days. Colony numbers in each well were counted on the bottom surface after the staining with May-Grünwald and Giemsa solution. Results were following; 1) in rabbit cases, squamous cell colonies increased at 33-34°C more than 35-37°C with significant difference. On the other hand, the colonies consisted of small original type cells were increased at higher 35-37°C; 2) in patient, there was no remakable difference in their morphological feature between squamous cells and original type cells, which were suitable to call 'blast cells'; 3) the squamous cell colonies increased most at 34°C in patient, too, but those of the blast cell type were increased upto 37°C step by step, and significant difference was admitted between low 33-34°C and high 35-37°C.

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ROLE OF ZINC AND METALLOTHIONEIN IN PROLIFERATION OF ADULT RAT HEPATOCYTES IN PRIMARY CULTURE. Watanabe, K., Hasegawa, K., Ohtake, H.,*Tohyama, C. and Koga, M. Dept. of Physiol.,Dokkyo Univ. Sch. Med., Mibu, Tochigi 321-02 and *Env. Health Sci. Division, Natl. Inst. Env. Studies, Yatabe, Ibaragi 305

We have previously reported the relationship between metallothionein (MT) and cell proliferation in livers of neonatal, normal and partially hepatectomized rats. To understand the role of zinc and MT in proliferation, we used primary cultures of adult rat hepatocytes. Hepatocytes synthesized DNA and proliferated under serum-free conditions in KL medium supplemented with fibronectin, insulin and EGF. The increase in DNA synthesis was inhibited by addition of EDTA. This inhibition was completely reversed by further addition of zinc at a concentration much lower than that of EDTA. MT content in hepatocytes cultured in the control medium began to increase immediately after plating and reached a plateau at 30 h. At this time, MT content was 6-fold over 0 h value. EDTA almost completely suppressed the increase in MT content. When zinc was added to the EDTA containing medium, MT content increased with time and at 54 h it was 4-fold over 0 h value. When EGF was omitted from the control medium, both the increase in MT content and the DNA synthesis were inhibited. Based on these findings, it is assumed that the increase in MT content in the G_1 period is important in the regulation of hepatocyte proliferation.

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PARTIAL PURIFICATION OF A GROWTH FACTOR FROM MOUSE MAMMARY FIBROBLAST-CONDITIONED MEDIUM AND ITS APPLICATION TO MAMMARY CELL CULTURE. ENAMI, J., HATA,*M. AND KOGA, M. Dept. Physiol., Dokkyo Univ. Sch. Med., Mibu, Tochigi 321-02 and*Research Laboratory, Nitta Gelatine Co., Ltd., Yao, Osaka 581

Fibroblasts obtained from adult mouse mammary gland or from embryonic mouse mammary mesenchyme produce and secrete a growth factor(s) for primary monolayer cultures of normal and neoplastic mouse mammary epithelial cells. The growth factor has an apparent molecular weight of 100,000 daltons on a Sephadex G-200 column chromatography. It is distinct from EGF since ^{125}EGF binding to mammary tumor cells was not inhibited by the mammary-fibroblast conditioned medium. Based on these observations, conditioned medium from serum-free cultures of mammary fibroblasts was chromatographically concentrated and the growth factor was partially purified. The crude preparation maximally stimulated the growth of mammary tumor cells at 1-2 μg/ml. The growth-stimulating activity was lost after treatments with SDS, Triton-X-100, urea, mercaptoethanol, pronase, or 60°C for one hr. This preparation stimulated the growth and morphogenesis of normal and neoplastic mammary epithelial cells cultured embedded in collagen gels. These results suggest that mammary fibroblasts produce a peptide growth factor(s) and control the growth and morphogenesis of mammary epithelial cells.

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STUDY ON DIFFERENTIATION AND MATURATION SYSTEM OF THYMIC LYMPHOCYTES IN STREPTOZOTOCIN-INDUCED DIABETIC RATS. OKIMOTO,T., HASHIMURA,H., NIN, T., KIMURA,S., KINOSHITA,T. AND TABATA,T. Dept. of Physiol., and Dept. of Internal Med., Osaka City Univ. Med. Sch., Osaka-shi Abeno-ku Asahi-machi 1-4-54

Marked decreases in thymus weight and the number of thymic lymphocytes were found in streptozotocin(STZ)-induced diabetic rats. Histologically, the cortical lymphocytes surrounding the cortico medullary junction in the thymus seemed to be reduced selectively in the STZ-administered rats. In this anomalous thymus rosette-forming cells (RFC), which bound to guinea pig erythrocytes in the presence of fetal calf serum, were significantly decreased and pretreatment of the thymocytes with thymosin fraction 5 significantly enhanced RFC percent to near the control level. It was assumed that a maturational impairment of thymus cortical lymphocytes by insufficiency of hormonal secretion from thymus epithelial cells may be occured in STZ-administered rats. In addition, the thymocytes were separated into 3 subsets using discontinuous density gradient centrifugation method. Responsiveness of these subsets to soybean lectin (SBL) stimulation was examined. Enrichment of SBL responding cells and enhancement of SBL response by TF5 were found in the least dense subset of STZ-administered rat thymus. These results suggested that undifferentiated and precursor T cells increased in the thymus of STZ -administered rats and separated into the least dense fraction.

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STUDY ON THE FUNCTIONS OF THYMOCYTES AND THYMUS EPITHELIAL CELLS IN SPONTANEOUS THYMOMA RATS, BUF/Mna.

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In Buffalo/Mna (BUF/Mna) rats, thymomas which histologically resembled to human ones occur spontaneously at high incidence from 9 months of age. Therefore, BUF/Mna rats appear to be suitable for the research about not only pathogenesis of human thymomas but also the mechanism of differentiation and maturation pathway of lymphoid cells in normal thymus. Using Buffalo/Hoshino (BUF/Hos) rats as control, in which thymus normally develops, the authors obtained the results that Con A-responsiveness and rosette-forming capacity with guinea pig erythrocytes of BUF/Mna rat thymocytes were significantly greater than those of BUF/Hos rat ones in aged stage, while PHA-responsiveness of the former significantly reduced as compared to that of the latter in 6 months of age when thymomas could not be found yet. Furthermore, it was revealed that elution pattern of thymus epithelial cell culture supernatant of the former was much different from that of the latter in an analysis with high performance liquid chromatography. From these results, it was assumed that migration of cortical lymphocytes from cortex to medulla might be impaired by secretory disorder of thymic hormones from thymus epithelial cells in BUF/Mna rats.

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VASOACTIVE INTESTINAL POLYPEPTIDE NULLIFIES CYTOCHROME REDUCTION RESPONSE TO AFFERENT ACTIVATION OF THE PERFUSED BULLFROG BRAIN.

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Redox states of cytochromes in the cerebral hemisphere of the perfused bullfrog brain was continuously measured with aid of a scanning organ spectrophotometer and electrical activity was simultaneously recorded in the vicinity of the light guide of the spectrophotometer. Electrical stimulation(25 mA, 20 Hz, 5 msec in duration for 2 min) of the third dorsal root of the spinal cord induced parallel reduction of cytochrome $a(a_3)$, b and $c+c_1$ and concomitant burst activity in the cerebral hemisphere. Intracerebroventricular(i.c.v.)infusion of vasoactive intestinal polypeptide(VIP; 30 pmol/100g body weight) nullified reduction of cytochromes induced by the afferent electrical activation, but did not attenuate the burst activity. The i.c.v. infusion of the same dose of VIP per se had little influence upon reduction of cytochromes and burst activity induced by afferent activation. The present results provide an evidence that one of the physiological roles of the VIPergic neurons in the brain may be in balancing redox state of electron transport system of the neuron.

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WEAK PULSED MAGNETIC FIELD AND HEPATIC RESPIRATORY METABOLISM.

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Effects of weak, low frequency, pulsed magnetic field (PMF) on respiratory metabolism were studied using perfused rat livers. Livers from male Wistar rats(150-250g) were perfused via portal vein with 95%O_2-5%CO_2 saturated KHBB maintained at 37°C. Pulsed magnetic field (N) generated from a coil(165Ø,100T) connected to a dc power supplier intervened with a switching circuit which was driven with a pulse generator was applied to the material. Ranges used were 150-280Hz for frequency, 2.2-3.0 msec for duration, 25-68 gauss for magnetic force. Scanning spectrophotometry and NADH fluorometry were performed on liver surface. Effluent was monitored for pO_2 and analyzed for glucose, lactate, and pyruvate. [RESULTS] Oxygen uptake of liver was stimulated upon exposure to PMF, but the extents were varied according to the glycogenolytic, glycolytic, and mitochondrial metabolic control states. Hepatic sensitivity to glucagon was elevated upon PMF as evidenced by increases in glucose outputs.

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EFFECTS OF NONHOMOGENEOUS MAGNETIC FIELD ON CELL GROWTH AND DNA SYNTHESIS OF HeLa CELLS. SATOH, K., YONEZU, T., YAMAGUCHI, H., MIYAMOTO, H. AND KINOUCHI, Y.* Dept. of Physiol., Sch. of Med., and Dept. of Elec. Engin.* Univ. of Tokushima, Tokushima 770

A strong nonhomogeneous magnetic field can be produced by a device having a Sm-Co magnet in combination with a gilded iron needle (1 mm diameter). According to calculation by the finite element method, cells are exposed to the magnetic field of the strength of decreasing from 11 to 3 KGauss within 0.3 mm from the tip of the needle.We examined the effect of magnetic field on cell growth, DNA content and DNA synthesis of HeLa cells using microphotography,microfluorometry and autoradiography.The results are as follows. 1) Cell growth and the DNA content of cells inoculated from both growing and confluent cultures are not significantly affected by the magnetic field.2) although there seems to be slight difference of the labeled index by ^{3}[H]thymidine incorporation,the number of grains per nucleus is not significantly different between control and magnet-exposed cells. As the number of observed preparations is still small, the results does not yet lead to decisive conclusions of the effect of magnetic field on DNA synthesis.

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EFFECTS OF VARIOUS HORMONES AND THE DIFFERENCES BETWEEN STRAINS AND SEXES ON PROTEINASE F IN MOUSE MAJOR SALIVARY GLANDS. MURAI, T., KURIHARA, K., ATSUMI, T. AND UEHA, T. Dept. of Oral Physiol., Josai Dental Univ. Sakado-shi, Saitama 350-02

We have previously reported that sex difference was found in the amount of Proteinase F(Pro F) in submandibular gland(SMG) of ICR mice; Pro F in the female glands was much higher than those in the males and it was inhibited by androgens. In the present study, we determined the content of Pro F in major salivary glands for the effects of various hormones and the differences between strains and sexes by radioimmunoassay's method using Pro F purified from immobiline's method. The following results were obtained. 1)Pro F levels in sublingual gland(SLG) and parotid gland(PG) were only about 1/50-1/100 those in SMG. 2)Sex differences of Pro F levels in major salivary glands were found in almost strains except for DBA, however, in contrast to the higher levels of Pro F in ICR female mice, those in C3H female mice were lesser. 3)The levels, sex difference and the effects of various hormones on Pro F resembled between the SLG and the PG respectively. 4)T_4 has tendency to increase Prof F levels in almost all the cases except for SMG of ICR female mice.

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FRUCTOSE 2,6-BISPHOSPHATE IN RAT SUBMANDIBULAR GLAND. SUGIYA, H., YAMAGAMI, R., YAMAZAKI, T. and FURUYAMA, S. Dept. of Physiology, Nihon University School of Dentistry at Matsudo, 2-870-1, Sakaechonishi, Matsudo, Chiba 271

Regulation of Phosphofructokinase (PFK) in rat submandibular gland was non-Michaelis-Menten type at physiological pH. ATP played a dual role on PFK acting as a substrate and an inhibitor. Authentic fructose 2,6-bisphosphate (F-2,6-P_2) stimulated PFK activity from rat submandibular gland by relieving the inhibition by ATP and increasing the affinity for fructose 6-phosphate (F-6-P). The concentration of F-2,6-P_2 in rat submandibular gland was 8.22 nmole/g tissue, which was sufficient for the activation of PFK. When epinephrine, norepinephrine or isoproterenol injected intravenously to rats, the concentration of F-2,6-P_2 was increased. These results suggest that PFK is synergistically regulated by ATP, F-6-P and F-2,6-P_2, and F-2,6-P_2 is one of the important regulators in vivo for PFK in this gland.

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β-AGONIST INDUCED K^+ UPTAKE AND ITS FUNCTION IN THE SALIVARY GLAND FLUID SECRETION.
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Mechanism of K^+ uptake evoked by β-receptor stimulation and its role in fluid secretion were studied. 1) In measurements of net K^+ flux from the isolated superfused gland segments, isoproterenol (2.5×10^{-5}M, Iso) induced a transient K^+ uptake. The K^+ uptake was blocked either by ouabain (10^{-3}M, Oua) or furosemide (1.2×10^{-3}M, Fur). The K^+ uptake was markedly suppressed in a low Na^+ or Cl^--free solution. 2) In the measurements of membrane potential from the isolated segment acinar cells, application of salbutamol by an ionophoresis induced a membrane hyperpolarization after a latency of about 20sec which does not accompany change in input resistance. The hyperpolarization was sensitive to blockers and ionic condition in a similar manner to the K^+ uptake. 3) In the measurements of saliva flow and net K^+ flux from the perfused glands, Iso (10^{-6}M) induced small amounts of fluid secretion and a K^+ uptake. Both the salivary flow and the K^+ uptake was markedly reduced by Oua or Fur. These results indicate that β-receptor stimulation activates Na^+-K^+-Cl^- cotransport and Na^+-K^+ pump system, and that the Cl^- uptake linked to the K^+ uptake is an important step for the transcellular Cl^- secretion associated with saliva formation.

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THE K^+-CHANNELS FROM HUMAN LEUKEMIC T-LYMPHOCYTES STUDIED WITH THE HELP OF WHOLE-CELL PATCH CLAMP METHOD.
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1. Application of whole-cell voltage clamp to human leukemic T-lymphocytes cell-line (MOLT) demonstrated the presence of voltage activated-inactivated K^+-channels on the plasma membrane. The take off currents after the onsets of the various depolarizing voltage jumps from the holding potential of -60mV showed typical sigmoid shapes similar to those observed in K^+-channels from squid giant axon. During the experiments, some cells showed "rundown" of the channels presumably due to the decrease in the number of the channels. The cells having non- "rundown" channels were analysed using ensemble noise analysis and it was revealed that the number of the channels was about 300/cell and the single channel conductance was about 26 pS.
2. The K^+-channels from MOLT were irreversibly blocked by Quinin. Quinin suprresed the number of living cells during the cultivation and ^{3}H-thymidine uptake into the cells. These evidences suggest the K^+-channels ($(K^+)i$ - fluctuation) might affect the ability of cell division at chromosome level.

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ACTION OF TETRAETHYLAMMONIUM ON CALCIUM-ACTIVATED POTASSIUM CHANNELS IN PIG PANCREATIC ACINAR CELLS.
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The effects of tetraethylammonium ions on currents through high conductance Ca^{2+}-activated K^+ channels have been studied with the help of patch-clamp single channel and whole-cell current recording on pig pancreatic acinar cells. In excised outside-out patches TEA (1-2 mM) added to the bath solution virtually abolishes unitary current activity except at very positive membrane potential. TEA in a lower concentration (0.2 mM) markedly reduces the open state probability and causes some reduction of the single channel conductance. In inside-out patches bath application of TEA in concentration up to 2 mM has no effect on single channel currents. In whole-cell current recording studies TEA (1-2 mM) added to the bath completely suppresses the outward currents associated with depolarizing voltage jumps to the membrane potential of 0 mV. TEA (2 mM) added to the cell interior has no effect on the outward K^+ current. These results indicate that TEA acts on the outside of the membrane to block current through the high conductance K^+ channels.

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MEMBRANE POTENTIAL OF CYNOPS BLASTOMERE. II. CONTRIBUTION OF ELECTROGENIC Na/K PUMP TO THE MEMBRANE POTENTIAL. OHARA, A., DOIDA, Y., MURAYAMA, K., MARUNAKA, Y. & KITASATO, H. Dept. Biol. and Dept. Physiol., Shiga Univ. Med. Sci., Ohtsu 520-21, Japan.

Membrane potential (Em) of the egg of Japanese newt, Cynops pyrrhogaster, at the first cleavage stage was measured in solutions of various NaCl concentrations (NaCl, 60 ~120 mM; KCl, 2.0 mM; $CaCl_2$, 1.4 mM; HEPES, 2.4 mM; pH, 8.0). The egg which had been removed of its jelly coat and vitelline membrane was impaled with glass microelectrode. (1) At 1-cell stage before the first cleavage, Em was about -10 mV, being not affected by either changes in $[K^+]_o$ or addition of ouabain (0.1 mM). (2) During the first cleavage, when new membrane of the inner surface of blastomere was formed, the membrane gradually hyperpolarized, reaching to -80~-90 mV. The above phenomena of (1) and (2) were observed at any concentration of NaCl in solutions. (3) At 2-cell stage, effects of changes in $[K^+]_o$ and of ouabain varied according to NaCl concentrations. At 60~90 mM NaCl, the 10 times increase of $[K^+]_o$ depolarized the membrane by 30~40 mV, but ouabain was ineffective. At 100~120 mM NaCl, the increase of $[K^+]_o$ hyperpolarized and the decrease of $[K^+]_o$ depolarized the membrane. Ouabain depolarized the membrane by almost the same amount (20~30 mV) as in K-free solution. It is concluded that in the membrane of 2-cell stage blastomere, the contribution of electrogenic Na/K pump to Em becomes apparent when external NaCl concentration is raised to 100 mM or more.

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ENHANCEMENT OF ERYTHROID DIFFERENTIATION IN CLONES OF HUMAN LEUKEMIC CELL LINE K562 BY FETAL CALF SERUM. SAKATA, S., ENOKI, Y., TOMITA, S., KOHZUKI, H. and NAKATANI, A. 2nd Dept. of Physiol., Nara Med. Univ., Kashihara 634

Clone cells of K562 which are capable to synthesize spontaneously hemoglobin on a relatively high level were obtained by cell-cloning. The clone cells proliferated 25 times on day 6 in culture, and the growth rate was not affected by changing dose of fetal calf serum (FCS) from 5 to 30 %. On the other hand, the erythroid differentiation could be linearly enhanced by increasing dose of FCS and reached to a maximum after 4 days in culture. The enhancing effect of 30 % FCS on the erythroid differentiation in the clone cells was larger than that of 12.5 μM hemin, while in the wild type cells the relationship was reversed. There were no effects of erythropoietin (Epo) on the hemoglobin synthesis in both the clone cells and the wild type cells. When the various kinds of sera were added to the standard culture of the clone cells, only FCS had the enhancing effect. These results suggest that the spontaneous erythroid differentiation is not induced by hemin or Epo in FCS but FCS-specific substance(s).

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ROLES OF DIVALENT CATIONS IN ELECTROFUSION OF MOUSE LYMPHOMA CELLS. OHNO-SHOSAKU, T., OIKI, S. and OKADA, Y. Dept. of Physiol., Fac. of Med., Kyoto Univ., Kyoto 606

Mouse lymphoma L5178Y cells adhered by dielectrophoresis underwent cell fusion after the application of electric pulses. Changes in the extracellular Ca^{2+} concentration ($[Ca]_o$) markedly affected both the yield of fusion and viability of the cells exposed to electric pulses, without affecting dielectrophoretic cell adhesion. The fusion efficiency and cell viability increased with a $[Ca]_o$ increase, exhibiting a saturable relation to $[Ca]_o$. At the $[Ca]_o$ of lower than 10 μM, almost all the cells could not fuse while a large number of the cells were still viable. Over 0.2 mM $[Ca]_o$, most of the cells were viable and partook in cell fusion. The addition of Ca ionophore A23187 enhanced the fusion efficiency in the presence of 0.1 mM Ca^{2+}. Facilitating effects of Ca^{2+} on electrofusion could be substituted by Sr^{2+} or Mn^{2+} but not by Mg^{2+}, Ba^{2+} or Ni^{2+} at the concentration of 0.1 mM. Deprivation of intracellular Mg^{2+} after incubation of the cells in a Mg-free solution (3h, 0°C) rendered the cells very sensitive to pulse application. These Mg-deprived cells became irreversibly damaged after cell fusion. The addition of 1 mM Mg^{2+} or Ca^{2+} at the moment of pulse application prevented the cell damage. Thus, it is concluded that extracellular Ca (not Mg) ions more than 10 μM are indispensable for the cell fusion, and millimolar Mg (or Ca) ions are prerequisite to the resealing of membranes suffered from electrical breakdown.

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INFLUENCE OF $[Ca^{2+}]_o$ ON SECRETORY AND REDOX RESPONSES TO CCK-8 IN PERFUSED RAT PANCREAS. MATSUMOTO, T. & KANNO, T. Dept. Physiol., Fac. Vet. Med., Hokkaido Univ., Kita-ku Sapporo 060

Continuous stimulation with 50 pM CCK-8 induced an increase in pancreatic protein output with concomitant reduction of cytochromes, $a(a_3)$, b and $c+c_1$. The secretory and redox responses were completely abolished in the Ca-deficient environment. An almost linear relation was found between the magnitude of the protein output in response to 50 pM CCK-8 and $[Ca^{2+}]_o$ in the range 0.25-2.5 mM. Similar relations were also found between the reduction levels of cytochromes and $[Ca^{2+}]_o$. Continuous stimulation with 200 pM CCK-8 induced rapid and large protein output and marked reduction of cytochromes, and these responses were less in the Ca-deficient environment. The reduction of cytochromes was enhanced when $[Ca^{2+}]_o$ was increased from 0.25 to 0.5, 1.0 or 2.5 mM. These results are compatible with the view that the CCK-8-induced-reduction of the electron transfer system in the pancreatic acinar cell may coincide with uptake and retention of cytoplasmic excess Ca^{2+} by mitochondria.

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DIFFERENCE OF Ca^{2+} AND Na^+ DEPENDENCY AMONG SECRETORY RESPONSES TO VARIOUS CCK-8 CONCENTRATIONS IN RAT EXOCRINE PANCREAS. KAWAHARA*, J. & KANNO, T. Dept. of Physiol., Fac. Vet. Med., Hokkaido univ., Kita-ku Sapporo 060

In the isolated perfused rat pancreas, the pancreatic response(protein output and juice flow) induced by 5 pM CCK-8 were completely abolished by removing either $[Na^+]_o$ or $[Ca^{2+}]_o$. The pancreatic responses induced by 50 pM CCK-8 were decreased but still remained by removing either $[Na^+]_o$ or $[Ca^{2+}]_o$. The responses were nulified by removing both $[Na^+]_o$ and $[Ca^{2+}]_o$. The pancreatic responses induced by 1 nM CCK-8 were smaller than those induced by 50 pM CCK-8. The responses were not completely abolished by removing $[Ca^{2+}]_o$, and were not affected by removing $[Na^+]_o$. The responses, however, were completely abolished by removing both $[Na^+]_o$ and $[Ca^{2+}]_o$.

Based on these and other results, the roles of extracellular Na^+ ($[Na^+]_o$) and Ca^{2+} ($[Ca^{2+}]_o$) were discussed in pancreatic responses induced by CCK-8 at a physiological(5 pM), a maximal(50 pM), a supramaximal(1 nM) concentrations.

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TUMOR PROMOTERS POTENTIATE THE ADRENOMEDULLARY SECRETION BY ACETYLCHOLINE OR HIGH K POSSIBLY VIA THE SPECIFIC RECEPTORS. SORIMACHI, M., NISHIMURA, S. AND YANO, K. Dept. Physiol., Kagoshima Univ. Sch. Med., Kagoshima 890.

We have tested the effects of some tumor promoters on catecholamine(CA) secretion from the perfused cat adrenals. 12-O-tetradecanoylphorbol-13-acetate(TPA) alone did not stimulate CA secretion, but prior exposure to this agent for 15 min increased the responses to 15 mM KCl or ACh(10 μM) to 325 ± 43 % or 239 ± 35 % of the preceding controls, respectively. These potentiated responses were markedly reduced by nifedipine, a Ca channel blocker. PDD(1.5 μM) or teleocidin B(0.1 μg/ml), which is also an active promoter in mouse skin, increased the responses to 15 mM KCl to 209 ± 14 % or 249 %, respectively. On the other hand, 4-α-phorbol(2.7 μM) or 4-β-phorbol-13-acetate (2.5 μM), which is far less active as a tumor promoter, little affected the responses to 15 mM KCl(100 % or 117 ± 16 %, respectively). Particulate fraction of the cat adrenal medulla was found to possess the specific binding sites for ^{3}H-phorbol-12,13-dibutyrate(Kd, 4 nM). Its binding activity was markedly inhibited by TPA or PDD, but not by inactive promoters. These results suggest that active promoters modulate the secretory mechanism of the adrenal medulla via the phorbol ester receptors, though the further studies are required for elucidating their precise mechanisms.

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ROLE OF PROLINE IN PROLIFERATION OF ADULT RAT HEPATOCYTES IN PRIMARY CULTURE.
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Adult rat hepatocytes in primary culture proliferate under serum-free conditions in KL medium supplemented with fibronectin and hormones (insulin, EGF, glucagon, etc.).

Omission of only proline from KL medium inhibited DNA synthesis by 95%. Proline, therefore, is an essential nutrient for hepatocyte proliferation in primary culture. Ornithine-oxo-acid aminotransferase activity decreased during primary culture. This causes hepatocytes to require exogenous proline.

Since proline is one of the major components of collagen, the possibility whether collagen is involved in the induction of DNA synthesis was tested. When hepatocytes were cultured in a proline-free medium using collagen (type I & IV) coated dishes, proliferation was not observed, indicating that proline is not replaced by exogenous collagen. Addition of collagen synthesis inhibitors such as cis-hydroxyproline, -dipyridyl or d-penicillamine to a medium containing proline not only inhibited DNA synthesis by 98%, but suppressed protein synthesis by 50%. In a proline-free medium, protein synthesis was also decreased to 50%. These results suggest that above chemicals or proline omission inhibited DNA synthesis not by speciffically suppressing collagen synthesis, but by suppressing general protein sythesis.

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ION DISTRIBUTION IN PIG PANCREATIC ACINAR CELLS DETERMINED BY X-RAY MICROANALYSIS.
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Recently, it was reported that the membrane potential of pig pancreatic acinar cells hyperpolarized by the stimulation of muscarinic reagents and/or CCK-PZ and at that time the K^+ channels on the baso-lateral plasma membrane opened. It is, however, unknown whether the K^+ channels on the luminal plasma membrane open and the K^+ secretion occurs.

Using freshly frozen thin sections of normal and stimulated (CCK-PZ, 10^{-11} M, 1 min incubation) pig pancreas, electron probe X-ray microanalysis was performed to determine the intracellular (cytoplasm and secretory granules) and intraluminal elemental concentrations of exocrine acinar cells.

Three dimentional observations of pig pancreatic acinar cells were also perfomed using ultra-high voltage electron microscopy (Hitachi H 1250M, Natl. Inst. Physiol. Sci.).

Electron probe X-ray microanalysis showed that by the stimulation, the Na^+, Cl^- and Ca concentrations increased and K^+ concentration decreased in the cytoplasm, while the Na^+, Cl^- and K^+ concentrations increased in both the secretory granules and the lumen.

Stereoscopical obserbations of high voltage electron micrographs revealed that many micovilli are present in the lumen. Some K^+channels or pump on microvilli are discussed.

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PROTEINS SYNTHESIZED IN CNS OF XENOPUS EARLY EMBRYOS.
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Basic plan to form the central nervous system (CNS) of Xenopus is laid down at the earliest stage of development. So far, however, little is known of the molecular mechanisms under lying the formation of the CNS rudiment.

Using albumin (m.w. 68,000) covalently labeled with fluorescein isothiocyanate (FITC) as a lineage-linked tracer, CNS was shown to comprise cells descended mainly from the blastomere of dorsal-animal region at blastula stage. If we injected FITC-albumin into the paticular blastomere (D 1.1.1.1) at the 64 cell stage, the tracer was found only in cells in the CNS rudiment.

Embryos allowed to develop to an appropriate stage were transferred into the medium free of divalent cations and subjected to surgical removal of vitelline membrane. As a result all the cells of embryos were dispersed to single cells. The suspension containig 10^4-10^5 cells per ml was applied to the cell sorter which recognizes and sortes fluorescence containing cells.

Radioactive proteins in the cells sorted from the embryos which had been labeled with ^{35}S-methionine were separated by two dimensional gel electrophoresis and detected by autoraciography. Several peptides were found to be specific to the cells descended from blastomeres at the animal-dorsal region.

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Chromosome analysis of glucagon-producing ascites tumor cells(GOAT). M,IMAGAWA., H,SAITO., S,SAKAI., K,AIZAWA. and S,O'HATA. Dept.of Physiol., Tokyo Medical College, Shinjuku-ku, Tokyo.

We reported the establishment and properties of a glucagon-producing ascites tumor cell line(GOAT) obtained by inter-peritoneal injection of glucagonoma(GOP) cells to Syrian hamsters. Serial transplantation of GOAT cells produced ascites tumors, usrually in 4 weeks, and caused hyperglucagonemia in 8 hamsters. The ascites fluid had extremely high immunoreactive glucagon(IRG) levels. Cultured GOAT cells also secreted considerable amounts of IRG into the medium. The normal hamster complement contained 44 chromosomes distributed into 21 pairs of autosomes and 2 sex chromosomes. GOAT cells were observed the modal chromosome number was 62. Trypsin technique was used for banding pattern. In GOAT cells, X was shorter than normal X and small chromosomes were probably translocated from long chromosomes. In some cases,long chromosomes were disappeared and small chromosomes increased. C band technique had shown that one-half of hamster X chromosome consisted of darkly stained heterochromatin. In GOAT cells, it was difficult to identify X. It might be suggested that X was absent in GOAT cells.

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STIMULATION OF CAMP FORMATION BY GLUCAGON EXTRACTED FROM IT-1 TUMORS. SAKAI, S., SAITO, H., IMAGAWA, M., AIZAWA, K., O'HATA, S., SATO, J.*, SUDA, N.* and KANAZAWA, M.* Dept. of Physiol. and Int. Med.*, Tokyo Med. Coll., Tokyo 160

It is known that glucagon is synthesized by way of large precursor molecule and that conversion of the precursor to glucagon is accomplished by selective proteolytic cleavage of proglucagon. Immunoreactive glucagons (IRGs) with various molecular weights were obtained from acid-EtOH extracts of glucagon-producing tumor cells (IT-1) in Syrian hamsters by gel-filtration on antiglucagon Sepharose 4B and Bio Gel P-30. The biological activity of various IRGs was examined by measuring the release of glucose and cAMP from hamster liver cells in monolayer culture. Liver cells (3×10^6) were incubated in RPMI 1640 medium with standard glucagon ($0\text{-}10^{-6}$ M) or IRGs extracted from tumors for 1 hr. Stimulative effect of standard glucagon on glucose release and cAMP formation reached a plateau at 10^{-8} M. IRG^{3500} extracted from IT-1 tumors caused remarkable dose-dependent stimulations of cAMP formation and glucose release. $IRG^{20000>}$ and IRG^{9000} stimulated cAMP formation faintly.

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STIMULATION OF α-AMINOISOBUTYRATE UPTAKE AND Na,K-ATPase ACTIVITY BY NOR-EPINEPHRINE IN ISOLATED RAT SUPERIOR CERVICAL SYMPATHETIC GANGLIA. ANDO, M. and NAGATA, Y. Dept. of Physiol., Sch. of Med., Fujita-Gakuen Health Univ., Toyoake, Aichi 470-11

Active uptake of non-metabolizable α-aminoisobutyric acid (AIB) into isolated superior cervical sympathetic ganglia (SCG) excised from adult rats was considerably stimulated by the addition of norepinephrine (NE, 50 μM) to the medium during incubation for 2 hrs at 37°C. In SCG axotomized one week prior to the examination, in which most of the ganglionic neurons had degenerated and reactive proliferation of the glial component was in progress, the NE-evoked AIB uptake was much more pronounced, and was significantly diminished by the addition of not α_2- or β-adrenergic blocker but α_1-blockers (phentolamine, 10 μM, prazosin, 10 μM), compared to both intact and denervated SCG. Ganglionic Na,K-ATPase activity was greatly stimulated by NE both in intact and operated tissues. These results suggest that NE-induced stimulation of active AIB uptake occurs in the glial cells rather than the neurons with possible alteration of membrane properties mediated by α_1-adrenoceptor, associated with a change in activity of the transport enzyme, Na,K-ATPase, in the SCG.

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SODIUM NMR STUDIES ON THE RELATION BETWEEN INTRACELLULAR AND EXTRACELLULAR SODIUM CONCENTRATIONS IN FROG SCIATIC NERVES. NISHIKAWA, H., SEO, Y., Dept. Physiol., Kyoto Pref. Univ. Med., Kyoto 602

Intracellular Na^+ concentration($[Na]_{in}$) in the frog sciatic nerve was measured by using Na-NMR with the chemical shift reagent ($Dy(PPPi)_2^{7-}$). Isolated nerves were soaked in a frog Ringer's solution with the shift reagent for 2 hours, and then in a isotonic solution containing ChCl and Na^+ at lower concentration than normal for 8 hours. Action potential and NMR spectrum were observed every 2 hours. Na^+ and K^+ concentrations of the nerves were measured with flamephotometer. $[Na]_{in}$s were 23.5, 15.6, 12.9 and 10.6 mM/kg w.w. when the nerves were souked in 71.7, 21.0, 11.5 and 0 mMNa^+ isotonic solutions respectively. The reducing rates of $[Na]_{in}$ depended upon $[Na]_{out}$. When the nerves were soaked in a Na-free isotonic solution for 8 hours, $[Na]_{in}$s were 19.9 and 10.6 mM/kg w.w. with and without 1 x 10^{-4}M Ouabain respectively. The reducing rate of $[Na]_{in}$ in the absence of Ouabain was about twice as large as in the presence of it. Action potentials were not observable in 4 hours in the solution with Ouabain, but were observable still in 8 hours in the solution without Ouabain. These facts indicated that there was the "interspace" outside near cell membranes in which Na^+ concentrations were maintained higher than $[Na]_{in}$ and $[Na]_{out}$ by Na^+ active transport in oxon and Schwann's cell membranes.

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MECHANISMS OF THE STIMULATION OF PLASMA MEMBRANE Na,K-ATPase ACTIVITY BY INSULIN. KANBE, M., MARUNAKA, Y., MURAYAMA, K. and KITASATO, H. Dept. of Physiol., Shiga Univ. of Med. Sci., Ohtsu, 520-21

The mechanisms of the regulation of Na,K-ATPase activity by insulin were studied using partially purified plasma membrane vesicles obtained from skeletal muscles of *Rana catesbeiana*. The Na,K-ATPase activity of plasma membrane was increased approximately 2-fold by pretreating muscles with insulin. Monensin, an inhibitor of intracellular transport, completely inhibited this effect of insulin, but actinomycin D did not. Although the dependency of the Na,K-ATPase activity of intact plasma membrane was saturable with respect to Na concentration, the dependency of plasma membrane prepared from insulin stimulated muscles which had been pre-exposed to ouabain was not saturable even at 100mM Na. The change in the relationship between ATPase activity and K concentration was similar to that observed in relation to the activity and Na concentration. These results suggest that the stimulating effect of insulin on Na,K-ATPase is mediated by facilitating the intracellular transfer of membrane-transport proteins which have already synthesized and stored somewhere in intracellular space and that the characters of the Na,K-ATPase newly incorporated to the plasma membrane are different from the original Na,K-ATPase.

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Cl AND K ACTIVITIES IN THE MOUSE LACRIMAL ACINAR CELLS AND THE EFFECTS OF SECRETAGOGUES.
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Using double-barreled ion selective microelectrodes, intracellular Cl^- and K^+ activities (A^i_K and A^i_{Cl}, resp.) of the mouse lacrimal acinar cells in vitro were determined in both resting and secretory states. In the resting state A^i_{Cl} was 31mEq/l which was 1.4 time higher than the equilibrium activity predicted from the membrane potential (V_m) of -41mV. Addition of acetylcholine (ACh, 1μM) hyperpolarized V_m to -63mV and decreased A^i_{Cl} to 20mEq/l which was still two times higher than the equilibrium activity. Similar changes in V_m and A^i_{Cl} were evoked by phenylephrine (10μM) and A-23187 (5μM). A^i_K was 90mEq/l in the resting state and was also decreased by ACh to 84mEq/l in 5min. Application of a steeper Cl gradient across the basolateral membrane by superfusing low-Cl Ringer decreased V_m by a few mV and decreased A^i_{Cl}. DIDS (1mM) significantly hyperpolarized V_m and inhibited A^i_{Cl} decrease produced by low-Cl Ringer applications, but ACh-induced A^i_{Cl} decrease was less effected by DIDS. It is suggested that there are two pathways for Cl exit out of the acinar cells. One is across the basolateral membrane, is not sensitive to ACh and is inhibitable with DIDS. The other is located on the luminal membrane and is predominant in the secretory sate.

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THE EFFECTS OF CHOLINE ON FLUID SECRETION BY THE PERFUSED SALIVARY GLAND.
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The secreto-motor action of choline on salivary secretion has been studied in the isolated perfused mandibular gland of the rat. Choline Ringer solution (Na was substituted with choline) induced a salivary secretion in 10-100 mmol/l comparable to acetylcholine (ACh: 0.05-1.0 μmol/l) at similar Na concentrations. Continuous infusion of choline did not lead to a fall-off in the secretory response ("tachyphylaxis": The secretory responses induced by ACh always show tachyphylaxis.). The secreto-motor action of choline (46 mmol/l) was blocked completely and reversively by atropine (5 μmol/l), furosemide (1 mmol/l), a removal of Ca from perfusion and blocked 90% by amiloride (1 mmol/l), but not by propranolol (2.5 μmol/l) and phentramine (2.5 μmol/l) administered together. When ACh (1 μmol/l) or an additional Ca (10 mmol/l) was infused on a choline perfusion, a tachyphylaxis was elicited. The results suggest that choline and ACh act on the same population of muscarinic receptors and that an excessive build-up of free cytosol calcium now remains as the most likely cause of tachyphylaxis.

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MECHANISM OF Na^+-INDEPENDENT TRANSPORT OF GLYCYLGLYCINE (DIPEPTIDE) ACROSS INTESTINAL AND RENAL BRUSH BORDER MEMBRANES.
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Glycylglycine (Gly-Gly) transport across intestinal brush border membrane is Na^+-independent but electrogenic (Himukai et al., BBA 687:170, 1982 & 732:659, 1983). To clarify the mechanism of such peptide transport, its dependence on pH gradients and electrogenicity were studied in rabbit intestinal and renal brush border membrane vesicles (BBMVs). Gly-Gly uptake was significantly stimulated by lowering extravesicular pH (pH_o); the maximum stimulation was seen at 5.5-6.0. The stimulating effect was abolished by FCCP, a protonophore, indicating that a proton gradient, but not low pH_o per se, is stimulatory. Valinomycin-induced inside-negative K^+ diffusion potential also stimulated the uptake. Gly-Gly added to the vesicular suspension containing 2 μM diS-C_3 (5) caused a rapid and significant increase in fluorescence intensity, indicating the occurrence of depolarization of the membranes. In the presence of a sufficiently large proton gradient, overshoot uptake of Gly-Gly was observed. These findings indicate that Gly-Gly is transported by a H^+-coupled cotransport mechanism and that the transport can be classified into the tertiary active transport.

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Na^+-INDEPENDENT H^+-DEPENDENT CELL MEMBRANE DEPOLARIZATION AND RESISTANCE CHANGE INDUCED BY DIPEPTIDES IN INTESTINAL EPITHELIAL CELLS. EVIDENCE FOR H^+-COUPLED COTRANSPORT OF DIPEPTIDES. SHIMADA, T., MATSUMOTO, H., TAKUWA, N. and HOSHI, T. Dept. of Physiol., Faculty of Med., Univ. of Tokyo, Tokyo 113

Glycylglycine (Gly-Gly) transport across the brush border membrane of newt intestine was examined electrophysiologically. Although Gly-Gly mainly exists in the form of zwiterion in the physiological pH range, its transport was electrogenic. Gly-Gly evoked depolarization of the cell membranes and a decrease of the input resistance which was measured with double-barrel intracellular microelectrode, were completely Na^+-independent. Similar Na^+-independent depolarization and resistance change were also observed for L-carnosine. On the contrary, L-glycine evoked cell membrane depolarization and decrease of the input resistance only in the presence of Na^+ in the medium. When pH of the luminal perfusate was 6.00, the size of the depolarization increased by 24% as compared with at pH 7.30, while at pH 8.30, it decreased by 27% as compared with control. Removal of Ca^{++} from the perfusion solution and the presence of 1 mM EGTA did not affect Na^+-independent depolarization evoked by Gly-Gly. All results obtained seem to corroborate the possible mechanism of H^+-linked cotransport of dipeptides across the small intestinal brush border membrane.

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SUGAR-EVOKED TRANSMURAL POTENTIAL DIFFERENCE IN THE SMALL INTESTINE OF EXPERIMENTAL DIABETIC RATS IN VIVO. TAKEUCHI, T.*,† HIJI, Y., MASHIBA, H.*,† Dept. of Physiol., Int. Med†, Tottori Univ. Sch. Med., Yonago 683

Sugar-evoked transmural potential difference (ΔPD) in the small intestine of diabetic rats was investigated in vivo. Experimental diabetes with fasting blood glucose level of 100-400mg/100ml were produced by the intraperitoneal injection of streptozotocin (45mg/kg). PD was also measured using rats with acute hyperglycemia induced by intravenous infusion of 40% glucose solution for 4 hours. Magnitude of ΔPD by D-glucose was found to be higher in both jejunum and ileum of diabetic rats than normal ones. Kinetic analysis showed that these maximum PD's for D-glucose were significantly large in diabetic rats, whereas there was such a significant change in neither jejunum nor ileum of glucose-infused rats. Magnitude of the ΔPD generated by 50mM sucrose was large in both jejunum and ileum of 7 days after streptozotocin injection, and in ileum of glucose-infused rats. The present results of increased active hexose transport and elevated disaccharidase activity in experimental diabetes may be suggestive on understanding physiological function of the patients with diabetes.

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A NEW TECHNIQUE FOR MEASURING INTESTINAL WATER ABSORPTION. ANDO, M. Lab. of Physiol. Fac. of Integrated Arts & Sci. Hiroshima Univ. Hiroshima 730

In order to measure net water flux across the leaky epithelia, 3 kinds of method had been employed, but they had a defect respectively. Among them, only a perfusion method enabled to last experiment for long time by supplying O_2 continuously. In the perfusion method, however, net water flux had been calculated from alteration in concentration of reference substance such as PEG, and it had been reported recently that such a calculation was not reliable(Barmada et al., 1983). By modifying perfusion method, a new method was developed; net water flux calculated as difference between perfusion rate of pump into and the collecting rate through the isolated gut. In the present apparatus, perfusion rate was constant for more than 4 hr, and the net water flux was greater after perfusing the serosal side in everted intestine of the seawater eel than after perfusing the lumen(non-everted). In the everted intestine, net water flux was not affected by altering perfusion rate from 0 to 0.5 ml/min. In most cases, however, net water flux and serosa-negative PD in normal Ringer solution containing 5 mM glucose decreased gradually even under constant perfusion rate, suggesting that some substances which are essential to maintain high water and ion absorption across the seawater eel intestine are lost from the epithelium during perfusion. L-alanine seems to be one of such substances.

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CO_2 AND ACID SECRETION IN GUINEA PIG COLON.
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Colonic mucosa isolated from guinea pig showed the ability to acidify the mucosal bathing solution and to alkalinize the serosal bathing solution. Addition of methazolamide (10^{-4}M), a carbonic anhydrase inhibitor, to both mucosal and serosal bathing solutions caused reduction in both mucosal acidification and serosal alkalinization rates by the same extent. Effects of unilateral application of another carbonic anhydrase inhibitor, acetazolamide, to either mucosal or serosal bathing solution were not different each other, suggesting the intracellular location of carbonic anhydrase. Increase in CO_2 tension of gas from 0 to 5% in either mucosal or serosal bathing solution resulted in a small but significant increase in mucosal acidification rate. In conclusion, CO_2 either derived from mucosal metabolisms or supplied exogenously is hydrated to release H^+ and HCO_3^- through the activity of carbonic anhydrase in the mucosal cell. H^+ is then pumped out into the mucosal bathing solution while HCO_3^- is delivered into the serosal bathing solution.

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STIMULATIVE EFFECT OF PROPIONATE ON ION SECRETION IN RAT COLON.
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The luminal chemical stimuli on colonic ion-transport is still unclear. The application of propionate, one of major products in gut microbial fermentation, from luminal side evoked a phasic increase in transmural potential difference (lumen negative) and short-circuited current (SCC) in the rat colon in vitro. Increases in SCC were accompanied by increases in transepithelial conductance. There was a sigmoid dose response between changes in SCC and log dose of propionate. The half effective dose of propionate was approximately 0.06 mM. The SCC response to propionate were inhibited by the serosal ouabain (1 mM) or bumetanide (0.03 mM), but not by the mucosal amiloride (0.1 mM). Replacement of chloride ion in the serosal bath by gluconate and sulphonate ions abolished response to propionate which reappeared after chloride re-addition. Unidirectional 36Cl flux measurements indicate that effect of propionate on SCC is due to Cl secretion. Atropine (0.1 μM) or quinuclidinyl benzilate (10 nM) from serosal side partially inhibited the effect of propionate. Mianserin (0.5 mM) or cyproheptadine (0.05 mM) from serosal side inhibited SCC response to propionate. These results suggest that propionate stimulated colonic chloride secretion, probably via mediations of acetylcholin and serotonin.

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ROLE OF Na/Ca EXCHANGE MECHANISM ON THE PROXIMAL TUBULAR TRANSPORT OF Na AND Ca.
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Micropuncture experiments were performed on perfused bullfrog proximal tubules using double-barreled Ca- and Na-selective microelectrodes. A partial substitution of peritubular NaCl with mannitol caused an increase of intracellular free Ca, $(Ca)_i$, and an inhibition of Na and water reabsorption was observed (elongation of half time of shrinking droplet, Gertz's method). At the same time, peritubular membrane potential (E_M) was depolarized and intracellular Na, $(Na)_i$, was raised moderately. The procedures for increasing $(Ca)_i$ (e.g., an addition of Ca or Ca-ionophore, A23187, to the peritubular perfusate) also caused a depolarization of E_M and a rise of $(Na)_i$. Amiloride, known to inhibit the entry of Na across the luminal membrane of tight epithelia, caused a hyperpolarization of E_M and a decrease of $(Na)_i$.

These results suggest the existence of a Na/Ca exchange mechanism on the peritubular cell membrane, which plays an important role on tubular reabsorption of Na and Ca. Our experimental evidence demonstrates that the elevated $(Ca)_i$ does not reduce amiloride sensitive Na entry across the luminal membrane, and increases back leak of Na across the basolateral membrane, inhibiting tubular reabsorption of both Na and Ca. Thus, a Ca-extruding mechanism other than Na/Ca exchanger (presumably ATP dependent Ca pump) might co-operate to prevent the rise of $(Ca)_i$ and maintain the net transport of Na and Ca.

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EACH MEMBRANE RESISTANCE AND CELL-TO-CELL COUPLING OF BULLFROG PROXIMAL TUBULAR CELLS.
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Using cable analysis, we determined resistances of basolateral (R_{bl}), apical membrane (R_a), and paracellular shunt pathway (R_s) in doubly-perfused bullfrog kidneys. The value of ρ_c was considered as an indicator of openness in gap juctions. Cell Ca chemical concentration ($[Ca]_i$) was determined with double-barreled Ca selective microelectrode. 1) From cell and luminal cables, we determined the each resistance as follows; ρ_c= 2,179 ± 338 Ωcm (Means ± SE, N=11), R_s= 662 Ωcm^2, R_a= 1,037 Ωcm^2, and R_{bl}= 467 Ωcm^2. $p[Ca]_i$ was determined 6.38 ± 0.15 with membrane potential (E_M) of -56.8 ± 2.9 mV (N=8) (this mean value = 4.17 x 10^{-7} M). 2) In respiratory acidosis (from 1.5 % CO_2 to 5 % CO_2), λ_c was significantly reduced from 255 ± 52 x 10^{-4} cm to 194 ± 48 x 10^{-4} cm (N=11, $p<.001$) and ρ_c was significantly increased from 1,051 ± 181 Ωcm to 2,001 ± 396 Ωcm (N=11, $p<.05$). $p[Ca]_i$ was not significantly changed from 6.69 ± 0.47 to 6.67 ± 0.51 (N=5, NS) in 5 min period treatment (the mean values = 2.04 x 10^{-7}, 2.14 x 10^{-7} M, respectively). The present study suggests that gap juction closes significantly in respiratory acidosis, and that the closure may not be induced by the changes of cell Ca.

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EFFECTS OF α-HUMAN ATRIAL NATRIURETIC PEPTIDE ON SODIUM TRANSPORT AND OSMOTIC WATER FLOW IN THE TOAD BLADDER.
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Synthesized α-human atrial natriuretic peptide (α-hANP), at 10^{-6} M, failed to inhibit short-circuit current and basal and 10 mU/ml vasopressin-stimulated osmotic water flow in the bladder either pretreated with cyclooxygenase inhibitor, or preincubated with arachidonic acid, a substrate of prostaglandin E_2. These results indicate α-hANP to have no direct effect on sodium transport and water permeability in the bladder, and thus possibly does not suppress vasopressin-stimulated water flow with an increase in prostaglandin E_2 production.

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Effect of LTH and T_4 on the SCC of the isolated frog skin.
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Epidermal and dermal application of T_4 (10^{-6} M) did not affect on PD and SCC of the tadpole and adult frog skin under both Cl- and SO_4-Ringer's conditions. Epidermal application of LTH (10 μg/ml) did not alter PD or SCC of the skin under the same conditions, either. However, dermal application of LTH induced increase in PD and SCC. Parcent increase in SCC induced by LTH in Cl-Ringer's solution was larger than that in SO_4-Ringer's solution. Effect of LTH on SCC appeared 10 min after the application and it reached maximum value after 30-40 min and then it gradually decreased. When SCC increased by LTH, there were three types of change in electric parameters of the skin: the first, both E_{Na} and R_{Na} decreased (2/8 cases); the second, both E_{Na} and R_{Na} increased (3/8 cases); the third, E_{Na} increased and R_{Na} decreased (3/8 cases). Second shot of LTH was no longer effective on SCC.

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INTERCELLULAR ELECTRICAL COUPLING BY DYE TRANSFER METHOD IN FROG SKIN.
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Intercellular dye coupling in frog skin of Rana japonica was examined using a fluorescent dye, Lucifer Yellow CH, which has reported to be permeant through gap junctions. The epithelial cells were impaled with dye-filled(5%) microelectrodes, whose resistance ranged from 100 to 300 megohms. After recording stable intracellular potential,at least 10 minutes, the dye was iontophoretically injected (3nA to 10nA) under open circuited (o.c.) or short circuited (s.c.) condition. Fluorescent recoveries from skins injected under o.c. condition were in 1 or 2, at most 3, cells 30 minutes after injection. Recoveries from s.c. skins increased with time. 5 minutes after injection, only a few cells were stained. While 30 minutes after injection, the dye spread across more than 100 micrometers laterally. It is suggested that frog skin epithelial cells are not highly electrically coupled under normal condition.

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Influence of exogenous lactate on the anaerobic glycolytic activity in muscle.
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The effects of exogenous lactate on the intracellular pH (pHi) and the velocity of the energy production from anaerobic glycolysis in muscle were determined by ^{31}P- and ^{1}H-NMR. When lactate was added to the perfusate, the pHi decreased by -0.04 to -0.05 pH/hr. The pHi is resistant to the exogenous lactate. On the other hand, the content of creatine phosphate decrease by the addition of lactate to perfusate. The rate of decrement of creatine phosphate (ΔPCr) was higher when pH value of the perfusate was lower with the same extracellular lactate concentration ([Lac]e). The ΔPCr was increased by the increment of [Lac]e, and saturated with [Lac]e of 20 mM at external pH of 6.4, when the ΔPCr was about -0.7 mmol/kg muscle/hr. This change corresponds to about 20% of the total energy production of anaerobic resting muscle.

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INCORPORATION OF (Na,K)ATPase INTO ORGANIC SOLVENT FREE PHOSPHOLIPID BILAYERS.
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Phospholipid bilayers free from organic solvent were made on patch-clamp pipettes according to CORONADO & LATORRE (1983) with slight modifications. These membranes were stable and their electrical resistance was 2~10 Gohm. In order to characterize an ion channel property of (Na,K)ATPase, the enzyme was incorporated into the bilayer membrane. In the presence of NaCl, KCl, and $MgCl_2$(but without concentration gradient of salt across the membrane) and in the absence of ATP, no channel current was observed on application of transmembrane potential gradient below 30mV. However, when the potential gradient in excess of 30mV was applied, some channel current appeared. In several experiments , a pattern of conductance fluctuation between two discrete levels was observed. These two levels may correspond to an open or a closed state of the ionchannel of the enzyme. The minimum conductance was about 40 pS. These phenomena were not found with the enzyme preincubated with ouabain.

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EXPERIMENTAL STUDIES FOR THE ELECTRO-KINETIC PHENOMENA PREDICTED BY THE CIRCUIT MODEL

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By the network thermodynamic approach, it is available to synthesize the circuit model of the membrane transport system. The electro-kinetic phenomena, like as the electro-osmosis and the streeming potential, have been predicted by the model. In this paper, we report the evidences of electro-osmosis and streeming potential on the frog skin, the frog tongue mucosa and the frog gastric mucosa. Experiments were carried out using the modified Ussing chamber. When the alternative electric current which caused the fluctation of the membrane potential (ca.1V,0.01Hz) was applied across the membrane, we could observed the synchronized pressure fluctuation (ca.5-60 mmH_2O,0.01Hz) in the frog tongue mucosa and the frog gastric mucosa. When the alternative pressure was applied across the membrane by the tuning fork et al (ca.50-200 mmHg,100Hz and 12Hz), we could observed the synchronized potential fluctuation (ca. 1-15 mV,100Hz and 12Hz) in the frog skin. These electro-kinetic phenomena assumed to have some roles in the real epithelial transport system.

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STUDIES OF INTESTINAL SUGAR COTRANSPORT USING FLUORESCENT PROBES

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A fluoroprobe method using a potential-sensitive dye, diS-C_3-(5)(diS), has been applied to depict the sugar cotransport process in isolated chick enterocytes in suspension. All of glc, α-methylglucoside and 3-O-methylglucose exibited Michaelis-Menten type relationships between $\Delta F/Fo$ and [sugar] or [Na]. This indicates that this type of transport kinetics always holded within the sugar and Na concentration range examined, irrespective of the involvement of basolateral facilitated transport pathways and/or enhanced glycolysis. The induced glycolysis was shown to cause a pHi decrease as revealed by the use of a pH-sensitive dye, acridine orange. Sizable pHi decreases which were NaF-inhibitable, were caused by 10 mM addition of glc, mannose and fructose, when the supplementation of media with mannose (10 mM) was omitted. In this case the pHi decreases by the latter two sugars resulted in diS fluorescence decreases indicative of cell hyperpolarization. However, when the mannose supplementation was made as usual, pHi decreases became small and diS fluorescence decreases were almost suppressed. Therefore we conclude that, under the usual experimental conditions where mannose supplementation was made, a pHi decrease, if any, made no significant contribution to the diS fluorescence change. These results reinforce the usefulness of the potential probe method which is expected to provide a new means of characterizing electrogenic cotransport processes in enterocytes.

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POSSIBLE RELATIONSHIP BETWEEN BILIARY EXCRETION OF OUABAIN AND LATERAL MOBILITY OF SURFACE MEMBRANE PROTEINS OF RAT HEPATOCYTES...A STUDY USING A FRAP (FLUORESCENCE RECOVERY AFTER PHOTOBLEACHING) TECHNIQUE.

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Intravenously administered ouabain is known to be efficiently excreted into the bile without any biotransformation in the rat. The mechanism for its hepatobiliary excretion, however, remains totally unknown. We investigated in the present study whether the lateral mobility (diffusion) of plasma membrane proteins of rat hepatocytes has any regulatory role in the ouabain excretion. Four-month-old male Wistar rats were given orally 7 doses of spironolactone (each 10 mg/100 g b wt) in the period of 4 days. Spironolactone pretreatment enhanced significantly the biliary excretion of ouabain as well as the bile flow rate. The same treatment also increased by 40 % the diffusion constant of surface membrane proteins as assessed by a FRAP apparatus (Mikroskopie, 41:12, 1984) using an autofluorescence developed by the treatment with a H_2O_2 solution. The parallel increase in diffusion constant and ouabain excretion suggests the possibility that membraine protein movement has a regulatory role in the biliary excretion of ouabain.

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MECHANISM OF NaCl TRANSPORT INTO CSF BY THE CHOROID PLEXUS EPITHELIUM: A STUDY WITH Cl-SELECTIVE MICROELECTRODES.
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Choroid plexus epithelium of Rana catesbeiana transports Na, Cl and HCO_3 from the blood to the ventricle. Using double-barrel Cl-selective microelectrodes intracellular Cl activities (A_{Cl}) of the epithelium have been studied under various conditions. In the normal HCO_3-buffer solutions, the epithelial cells accumulated Cl twice as high as predicted for the passive distribution (24 versus 12mEq/l). In HCO_3-free NaCl Ringer the A_{Cl} increased from 24 to 30mEq/l, and the Cl activity in the subepithelial compartment was about 10mEq/l lower than that predicted for equilibrium. Removal of Na from the Ringer completely abolished uphill accumulation of Cl in the cells. In HCO_3-free NaCl Ringer addition of furosemide (0.6mM) to the ventricualr side had little effect on both apical membrane potential (V_m) and A_{Cl} while the addition to the serosal side decreased A_{Cl} toward the equilibrium level with little change in V_m. In normal HCO_3-buffer solution addition of IBMX (1mM) to the ventricular side depolarized V_m followed by an increase in A_{Cl} toward the equilibrium level. We conclude that at the serosal border of the epithelium Cl is taken up via a Na-coupled uphill transport mechanism and leaves the cells across the apical membrane down the electrochemical potential gradient. IBMX increases apical membrane Cl permeability.

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ACTIVE K^+ ABSORPTION IN GUINEA PIG COLON.
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We have previously shown the presence of H^+/K^+ exchange pump at the mucosal membrane of guinea pig colonic mucosa. To elucidate whether or not this H^+ pump is also responsible for active K^+ absorption, unidirectional K^+ fluxes from mucosal to serosal bathing solution (J^K) were determined in isolated colon under short-circuit conditions. Changes of J^K (ΔJ^K) were compared with those of H^+ secretion rates (ΔJ^H) in the following experimental conditions (in $\mu mol/cm^2 \cdot hr$).
1) 10^{-4}M ouabain in the mucosal solution: $\Delta J^K = -1.30 \pm 0.11$, $\Delta J^H = -1.02 \pm 0.11$.
2) 5×10^{-6}M ouabain in the mucosal solution: $\Delta J^K = -0.94 \pm 0.11$, $\Delta J^H = -0.65 \pm 0.15$.
3) 10^{-4}M methazolamide, a carbonic anhydrase inhibitor, added to the serosal solution: $\Delta J^K = -0.42 \pm 0.06$, $\Delta J^H = -0.58 \pm 0.09$. Thus, under all these conditions ΔJ^K and ΔJ^H were not different each other. In conclusion, H^+ pump at the mucosal membrane of guinea pig colonic mucosa not only causes the mucosal acidification but also plays an important role in active K^+ absorption.

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EFFECT OF OUABAIN AND SITS ON H^+ TRANSPORT MECHANISM ACROSS THE PERITUBULAR MEMBRANES OF BULLFROG KIDNEY.
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To study the renal tubular acid-base balance, the intracellular pH (pH_i) and the peritubular membrane potential (E_M) were simultaneously determined with double-barreled pH microelectrodes in the bullfrog proximal tubule perfused in vitro. Male bullfrogs were anesthetized, and both kidneys were exposed from the ventral side. The luminal and peritubular perfusions were carried out with Ringer solutions with or without transport inhibitors (ouabain and SITS). The peritubular ouabain (10^{-5} M) lowered the pH_i by 0.12 with a depolarization of $E_{M\ Peri}$ by 5.4 mV in 1 min administration, whereas SITS (5×10^{-4} M) produsedd a rise in pH_i by 0.09 with a hyperpolarization $E_{M\ Peri}$ by 5.4 mV in 5 min application. Thus, ouabain causes an elevation of intracellular Na^+, and this results in a cellular acidification with the suppression of Na^+-H^+ exchange mechanism. A delayed effect of SITS could be explained initially by a blockade of a neutral HCO_3^--Cl^- exchange, and later by an inhibition of HCO_3^- exit at the basolateral membrane.

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PROPERTIES OF THE IONIC CHANNEL WHICH COME INTO PROMINENCE AT METAPHASE IN CELL DIVISION CYCLE. MORIMOTO, K., *SATO, E. and *ITO, S. Dept. Physiol. Med. School and *Dept. Biol. Fac. Sci., Kumamoto Univ., Kumamoto

Single white macroblastomeres isolated from morula embryos of newt show the cyclic changes in the membrane resistance, potential, capacity, and the responsiveness to applied current according to the progress of cleavage cycle. PVA (poly vinyl alcohol) hold the cell membrane in the point correspond to metaphase in cell cycle. In such membrane, membrane potential to applied current quickly reach the peak potential and decay with exponentially during continuation of the currents. After cessation of the current, the potential promptly drop to about +25 mV , and then the potential make a hump on the way back to resting membrane potential. Membrane current under voltage clamp is composed of slowly rising outward current and the linear leakage current. This outward current was not affected by the concentration of Na, K and Ca ions in the external solution. But, Co ion (1 mM) depress the outward current. The current was also affected strongly by pH in the external solution. From the above observation, we discussed the possibility of the existence of proton current in the response.

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STOCHASTIC MODEL OF Na CHANNEL WITH THREE STATES. ASHIDA,H. AND TATSUNO,J. Department of Physiology, National Defense Medical College, Tokorozawa, Saitama 359

Three states model of Na channel, which was proposed by Aldrich et al. (1983) mainly with respect to the neuroblastoma was formulated stochastically and various observable quantities were calculated from this model. In this model which assumes Na channel can occupy one of the three states that are resting, open and inactivated, and each channel is independent, when the patch contains many Na channels, probability distribution of the numbers of the channels that occupy each of these states is a time dependent multinomial distribution and the distribution of the first passage time from resting state to open state becomes an exponential decay function with higher components. The average of channel current which is product of the unit current of the single channel and expectation of the number of the channels that are in open state, has a time course with a single peak and approaches to zero when time goes to infinity but no time delay appears in the current rise. Using various relations deduced from these distributions, it is possible to estimates all of the parameters involved in both the model and the experiments by patch clamp method from the observable quantities. Not only transition rates but also number of channels in the patch are determined by the average and variance of the channel current and by the distribution of the first passage time from resting state to open state.

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MODIFICATION OF CA-BLOCK OF SQUID AXON NA CHANNELS BY EXTERNAL PERMEANT CATIONS. YAMAMOTO, D. Lab. of Neurophysiol., Mitsubishi-Kasei Inst. Life Sci., Tokyo 194.

The mechanisms of cation permeation through Na channels were studied with internally perfused squid axons. Instantaneous current-voltage (I-V) relation, current-ion concentration relation, and ionic selectivity were measured in the presence of different permeant cations externally. In Na or Li media, the I-V curve was linear between +60 and -40 mV, but the current decreased at more negative potentials. The I-V curve became more linear with lowering the external Ca. In NH_4, the I-V curve saturated at -100 mV. In guanidine or formamidine, the I-V curve was more linear. Inward Na channel currents saturated at high cation concentrations. The apparent K_d's determined from the conductance-concentration curves at -60 mV were (mM): Na 278, Li 247, NH_4 174, guanidine 111, and formamidine 103. The permeabilities of NH_4, guanidine and formamidine relative to Na decreased with increasing concentration and/or depolarization. The shape of the I-V curve is determined by the relative affinity of permeant ions and blocking ions (Ca) at the binding site, and the ionic selectivity depends on the energy barrier profile.

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EFFECTS OF INTRACELLULAR DIVALENT CATIONS ON Na CHANNEL ACTIVATION AND INACTIVATION OF SQUID GIANT AXONS. YAMAGISHI, S., FURUYA, K. and KUKITA, F. Dept. of Cell Physiology, National Inst. for Physiol. Sci., Myodaiji, OKAZAKI 444

Effects of 10 species of divalent cations on Na channel activation and inactivation of squid axon membranes were examined by intracellular addition to the 100 mM K solution. By internal addition of divalent cations ranging 0.1 - 10 mM, prominent lowering of threshold potentials and decrease in amplitude of action potentials were observed in many species, which corresponded to the shift of voltage dependence of inward current to low voltage direction and suppression of peak inward current (I_{Na}). I_{Na} suppression of Cd and Be ions were 40 times stronger than that of other divalent cations. Concerning the effect of suppression on Na inactivation, divalent cations could be classified into three groups, i.e. (a) strongly suppressed group; Cd and Be ions, (b) moderatly suppressed group; Zn and Ni ions, (c) non suppressed group; Ba, Mg, Mn, Sr and Ca ions. Divalent cations seem to affect on the activation and inactivation sites of Na channel by different manners.

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EXPRESSION OF SODIUM CHANNELS IN XENOPUS OOCYTES BY INJECTION OF mRNA ISOLATED FROM RAT BRAIN. HIRONO, C., YAMAGISHI, S. AND SUGIYAMA, H. Natl. Inst. Physiol. Sci., Okazaki 444

Xenopus oocytes were microinjected with mRNA isolated from rat brain or electric organs of Electrophorus electricus. After 3 or 4 days, functional sodium channels were induced in the oocytes. When veratrine (0.5mg/ml) was applied, spontaneous repetitive spikes and action potentials were observed. The action potentials were sensitive to Na ions, scorpion toxin, tetrodotoxin and local anethetics. Thus the mRNA-induced sodium channels have the same properties as those in nerve and muscle. The rat brain mRNA was fractionated into four fractions by sucrose gradient centrifugation. A fraction (30S-46S) exhibited much more potency to induce the functional sodium channels in the oocytes than any other fractions and addition of any other fractions to this fraction did not enhance the potency of this fraction. These results indicate that this fraction has all mRNA necessary for the expression of the channels and that mRNA of smaller sizes may not be required.

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CHANGES IN PHYSIOLOGICAL PROPERTIES OF Na-CHANNEL DURING NEURONAL DIFFERENTIATION OF MOUSE NEUROBLASTOMA CELLS. Kamei, K and Miyake, M., Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo 060

Mouse neuroblastoma N-18 cells come to extend their neurites and generate all-or-none action potentials when serum concentration in culture medium is reduced below 2 %. Thus, these cells are usefull model system for investigating the neuronal differentiation from neurobalsts to matured neurons in mammal. In this system we observed the developmental changes in the physiological property of Na-channels, in addition to an increase in the population of the channel present in each cell.

Most cells in different developmental stages elicited anode break Na action potentials by hyperpolarizing stimulation exceeding a certain magnitude. The relationship between the maximal rate of rise of the action potentials and the conditioning membrane potential at the end of the preceeding hyperpolarization serves as a conventional measurement of the steady state inactivation curve of Na-channel. The mean values of half inactivation potential ($E_{0.5}$) thus obtained are -136 mV in undifferentiated stage, -89 mV in early developed stage(7-10 days after differentiation), and -69 mV in fully developed stage. This result indicates that the non-functional Na-channels in undifferentiated cells became functional in association with the neuronal differentiation. This notion was also confirmed by the results of Na-uptake experiments using scorpion toxin (a specific opener for the inactivation gate) and veratridine.

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THE MECHANISM OF INHIBITION OF Na INACTIVATION PROCESS BY SEA ANEMONE TOXINS IN THE CRAYFISH GIANT AXON. WARASHINA, A. AND FUJITA, S. Dept. of Physiol. and Dept. of Anesthesiol. Niigata Univ. Sch. of Med., Niigata 951

Toxin actions of four different sea anemone toxins from Anthoplerua (AP-A and -C), Anemonia (ATX II) and Parasicyonis (PaTX), and a scorpion toxin from Leiurus (LqTX) in crayfish giant axons were compared. These toxins commonly induced maintained Na currents during depolarizing pulses by suppressing the Na inactivation. The intensity of toxin-induced maintained current was utilized an index for analyzing the toxin binding to Na channels. Decreases in the association rate and increases in the dissociation rate were observed, in different extents with the respective toxins, either by elevating pH in the external medium or by imposing membrane depolarizations. The role of positive or negative charges of the toxins on the toxin binding properties was assessed using PaTX and ATX II derivatives in which amino or carboxyl groups were modified by incorporations of propionyl or glycine ethyl esters, respectively. No direct relation between ionic charges and toxin actions was obtained. AP-A, AP-C or LqTX that had bound to Na channels was replaced by PaTX in a slower rate compared with the binding rate of PaTX to normal Na channels. To interpret these results, a tentative model in which the respective toxins occupy slightly different positions in a cleavage of Na channel to exert their toxin action was considered.

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MECHANISM OF THE Na CHANNEL BLOCK WITH GUANIDYL-SIDE ARMED CYCLAM(G-cyclam). SEYAMA, I., YAKEHIRO, M. & YAMAOKA, K. Dept. Physiol., Sch. Med., Hiroshima Univ., Hiroshima 734.

A novel synthetic compound, G-cyclam, has a structural specificity common to drugs blocking the Na channel: a terminal guanidinium group is attached to cyclam through a long chain of alkyl group. The interaction of G-cyclam with Na channels was studied in squid axons isolated from Doryteuthis bleekelei. A combined method for internal perfusion and voltage clamp was used in all experiments. G-cyclam specifically suppresses the Na current when applied to the internal surface of the squid axon membrane without affecting the K current. G-cyclam induces inactivation after the sodium inactivation has been removed by the treatment of axon with pronase. G-cyclam-induced inactivation follows a single exponential time course, implying first order kinetics. The rate constant for the forward reaction(k) is independent of the membrane potential but that for the backward reaction(l) is dependent on it. Reduction of external Na concentration does not affect the dependency of l on the membrane potential. The relationship between l and the membrane potential(V) is expressed as $l=0.5 \times \exp(-0.014x V)$. Thus, dose-response curve for the steady state block is voltage-dependent, being more smaller with increasing depolarization, where $K_D = 7.0 \times 10^{-6}$ M at 0 mV.

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EXCITABILITY UNDER THE MONOIONIC CONDITION WITH MONOVALENT CATION. Terakawa, S. Natl. Inst. Physiol. Sci., Dept. Cell Physiol., Okazaki 444

Electrical excitability was demonstrated in the squid axon perfused intracellularly and extracellularly with the same solution containing a single species of salt. Previously this was attained using divalent cations and now using monovalent cations. An inward current applied in a constant mode induced repetitive action potentials. An inward current applied under the voltage clamp condition oscillated periodically. Neither EDTA used as anion nor TTX applied externally eliminated the response. The electrical response was accompanied by the electroosmotic water flow through the membrane. The water flow occurred in the same direction as the current flow. These conditions and behaviors were very similar to that of the Teorell model, suggesting that the conformational change may not be necessary in channel activities. However, the response could be induced in the absence of pressure gradient across the membrane. Besides, the response could not be induced by an outward current. These differences between the squid axon and the model imply that the ionic channel has a site works in an asymmetrical manner.

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EFFECT OF K^+ ON K-CHANNEL IN A SQUID GIANT AXON

KUKITA, F., YAMAGISHI, S. Lab. Membrane Biol. National Inst. Physiol. Sci., Okazaki

It is well known that a large amount of K^+ accumulates near the outer surface of axolemma of squid *(Doryteuthis bleekeri)* giant axon during a nerve activity, especially during the voltage clamp experiment. We could prepare a K-accumulation free axon by using the outward osmotic water flow. In this preparation, K-channel has a higher sensitivity to K^+ applied outside. The measured selectivity of K-channel for K^+ against Na^+ was much higher than reported values. During the voltage clamp with a long depolarizing pulse of 8 ms, we could keep $[K]_o$ and V_K constant. As the consequence we could measure the real activation kinetics of K-channel without a distortion due to the time-dependent V_K change. The time course of K-channel activation followed the Hodgkin-Huxley scheme i.e. $g_K\ n^4$ better than that measured in a usual preparation, in which this time course followed $g_K\ n^6$ at 10°C.

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RELATIVE ANION CONDUCTANCES OF THE AXON MEMBRANE OF THE SQUID, Sepioteuthis.,

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The electric conductances for various anions relative to Cl^- of the squid axon membrane were measured. Giant axons from squid, Sepioteuthis lessoniana were internally perfused with a 100 mM TEA-phosphate solution and bathed in 100 mM Ca-salt solutions (or Mg-salt solutions) containing 0.3 μM TTX. The external anion composition was altered. The currents under voltage clamp show a large amount of outward rectification which is due to the anion influx across the chloride channel of the membrane (Inoue, 1985, J. Gen. Physiol., 85). The magnitude of the outwardly rectifying currents depended on the species of anion. Though the anion conductances were greatly affected by temperature and by pH, the relative conductances kept their own value against the changes in the environmental conditions. The sequence and values of the relative conductances of anions examined are as follows: NO_3^- (1.80) > I^- (1.40) > Br^- (1.07) ≥ Cl^- (1.00) > $MeSO_4^-$ (0.46) > $H_2PO_2^-$ (0.33) > CH_3COO^- (0.29) > SO_4^{2-} (0.06).

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DEVELOPMENT OF EPIDERMIS-SPECIFIC ANTIGENS IDENTIFIED WITH MONOCLONAL ANTIBODIES IN AN ASCIDIAN EMBRYO.

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Tissue-specific monoclonal antibodies were produced by immunizing mice with homogenized tissues of tadpole larvae. Hybridomas producing antibodies against tunic (C1), tunic and epidermal cells (2C5) were obtained. C1 and 2C5 antibodies are regarded as markers of epidermal cell. Development of these antigens was studied and compared with that of ionic channels previously analysed in epidermally differentiating cleavage-arrested 1-cell embryos. C1 and 2C5 antigens appeared around the external surface of epidermis at about 45 hr after fertilization at 8°C, when Ca channel of differentiated type appeared in a cleavage-arrested 1-cell embryo. Application of actinomycin D (AD), a RNA synthesis inhibitor, from an early stage of development before 25 hr completely inhibited the appearance of C1 and 2C5 antigens. However, when AD was applied after 28 hr, small amounts of both antigens were detected after 2 days culture. These AD sensitive stages are similar to those for Ca channel and anomalous rectifier channel. The results suggest that mRNA synthesis for tunic antigens and that for ionic channels start simultaneously, and that secretion of tunic antigens and incorporation of functional ionic channels into plasma membrane also occur simultaneously.

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EFFEXTS OF X-IRRADIATION ON IONIC CURRENTS IN THE MOUSE OOCYTE.

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Effects of X-irradiation on the excitability of mouse oocytes were investigated using two-electrode voltage-clamp techniques. Mice were irradiated with single doses of X-rays (1,000-10,000 rad) and oocytes were collected from the ampulla of the oviduct for the study at 0.5-50 h after irradiation. The bathing medium was Na^+-free, 20 mM Ca^{2+}solution, because the excitability was exclusively dependent on Ca channels. The resting potential was not significantly affected by irradiation, while the input resistance was only slightly decreased by a large amount of X-rays. The potential of the threshold and the peak of the inward Ca^{2+} current were almost unchanged. The time-to-peak, the half inactivation time and the inactivation time constant of the Ca^{2+}current were prolonged, indicating the altered kinetic properties of the Ca channels. As for the steady-state inactivation curve, the value of $V_{h=1/2}$ was shifted to the depolarizing direction, and the slope of the curve became steeper. The peak amplitude of the maximum inward Ca^{2+} current was reduced. These changes appeared more severely, depending upon the dose of X-rays and time after irradiation.

The present study may be useful for considering the structure, properties, and turnover of the Ca channels.

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ROLE OF CYCLIC AMP IN THE PENTYLENETETRAZOLE-INDUCED BURSTING ACTIVITY IN SNAIL NEURONS. FURUICHI, H., KISHII, K., IMAI, S., ONOZUKA, M. and SUGAYA, E. Dept. of Physiol. Kanagawa Dental College, Inaoka-cho, Yokosuka

To determine the role of intracelluar cyclic AMP in the manifestation of PTZ-induced bursting activity, the effect of cyclic AMP was examined from the viewpoint of intracellular calcium concentration. Combined application of dibutyryl cyclic AMP and isobutylmethylxanthine (IBMX) showed phasic oscillation of membrane potential. Intracellular injection of guanylylimidodiphosphate (GMP-PNP) combined with extracellular IBMX application showed bursting type firing followed by long-lasting hyperpolarization. By voltage clamping analysis, increased outward current was observed by intracellular injection of GMP-PNP combined with exctracellular IBMX application. Extracellular application of $CoCl_2$ did not change this effect. The outward current increase by cyclic AMP injection was reduced after 20-30 min application of caffeine. The intracellular cyclic AMP injection provoked increased intracellular calcium concentration according to direct measurement using calcium ion selective microelectrodes.

The above findings suggest that the role of cyclic AMP in the provocation of PTZ-induced bursting activity is indirect and cyclic AMP plays a trigger role in the intracellular calcium related phenomena.

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DOES MAITOTOXIN ACT ON CALCIUM CHANNELS ? KURODA, Y., YOSHII, M. # TSUNOO, A.*#and NARAHASHI, T.*#, Dept. Neurochem.,Tokyo Metropol. Inst. for Neurosci., Tokyo 183, Japan and #Dept. Pharmacol.,Northwestern Univ. Med. Sch., Chicago, IL 60611, U.S.A.

Maitotoxin (MTX), isolated from the dinoflagellate Gambierdiscus toxicus (Yasumoto, 1980), has recently been suggested to activate Ca channels. However, it remained to be seen whether voltage-sensitive Ca channels were affected by this toxin. To clarify this point, experiments were performed with Ca channel currents of neuroblastoma cells (N1E 115) using the whole cell clamp technique. Under the condition in which Na and K channels were blocked, Ca channel current as carried by Ba^{2+} ions was evoked by a step depolarization to -20 mV from a holding potential of -80 mV. MTX (10^{-9} g/ml) did not cause a significant change in either amplitude or time course of the Ba^{2+} current. Instead, an inward holding current (at -80 mV) began to increase 10-20 sec after application of MTX and developed progressively at a rate of -0.4 nA/10 sec from the control value of -0.1 nA. This MTX-induced current was completely blocked by 1 mM La^{3+}. Verapamil (0.1 mM) also prevented the development of the steady current. MTX does not seem to act on normal voltage-sensitive Ca channels in the neuroblastoma cells.

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THE DETECTION OF INTRACELLULAR FREE CALCIUM CHANGES IN SINGLE CELLS BY MEANS OF VIDEO-FLUORESCENCE MICROSCOPY. OZAKI, K.[1]*, KUDO, Y.[1], MIYAKAWA, A.[2] and OGURA, A.[1] [1]Department of Neuroscience, Mitsubishi-Kasei Institute of Life Science, Machida, Tokyo 194 and [2]Department of Pharmacy, College of Science and Technology, Nihon University, Tokyo 101

A fluorescence microscope coupled with an image intensifier was used to examine the intracellular free calcium changes in individual NG108-15 (neuroblastoma × glioma hybrid) and C6 Bu-1 (clonal astroglioma). The cells at a density of 10^5/ml were plated on silicon rubber-walled glass coverslips. Twenty-four hours later, the cells were loaded with the calcium-sensitive indicator quin 2 and were set on the fluorescence microscope. The output of the image intensifier tube was observed with a calnicon video camera (Hamamatsu Photonics). The changes of the intensity of fluorescence from cells can be determined by measuring the brightness of the video image with a photodiode. The elevation of the intracellular free calcium concentrations was detected by this method when the cell was exposed to 1 μM ionomycin, 10 ng/ml maitotoxin, or 1 μM serotonin.

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Ca CHANNELS IN EMBRYONIC SKELETAL MUSCLE CELLS IN CULTURE. KANO, M., WAKUTA, K., SATOH, R. Dept. of Physiol., Kitasato Univ. Sch. of Med., Sagamihara, Kanagawa

Long-lasting action potentials with overshoot were evoked in embryonic chick skeletal muscle cells developing in cell culture after removing Na^+, Ca^{2+} and Cl^- from bathing solution and adding Ba^{2+} and TEA^+ to it. The rising phase of this potential showed two steps. These two components of action potential can be operated by Ca channels since both components were abolished by Co^{2+} and D 600. The maximum rates of rise of these two components of action potential were decreased as a function of number of days in culture. To record the currents through these Ca channels using voltage clamp techniques, spherical myoballs were produced by incubation in a culture medium containing colchicine. A suction pipette was used to pass current and perfuse internally with K-free solution. The inward current was separated into two components, one was activated at about -40 mV and the other at about -10 mV. The time-course of the current differed significantly between the two channels, the rate of inactivation was substantially slower in high threshold channel than in low threshold one. These results suggest that the embryonic chick skeletal muscle cells have two types of Ca channels having different membrane potential dependence and the densities of these channels decrease during muscle cell development.

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RELEASE OF NERVOUS SYSTEM-SPECIFIC PROTEINS FROM ISOLATED RAT SUPERIOR CERVICAL GANGLIA IN Ca^{2+}-FREE OR VERATRIDINE CONTAINING MEDIUM. MIWA, M., TAKAHAMA, K., ANDO, M., and NAGATA, Y. Dept. of Physiol., Sch. of Med., Fujita-Gakuen Health Univ., Toyoake, Aichi 470-11

Neuron-specific γ-enolase isozyme was released from isolated superior cervical sympathetic ganglia (SCG) excised from adult rats during aerobic incubation at 37°C for 1 to 2 hrs in a Ca^{2+}-free EGTA (1 mM) medium. The release was also observed after addition of either Ca^{2+}-channel blockers (nicardipine, 10 μM) or Na^+-channel opener (veratridine, 50 μM). Calcium-ionophore (A-23187, 2 μM) or Na^+-channel antagonist (Tetrodotoxin, 2 μM) blocked the releasing effect of γ-enolase from the SCG, caused by nicardipine or veratridine. However, the release of non-neuronal α-enolase was not affected by the incubation in either a Ca^{2+}-free or veratridine containing medium. Astroglia-specific S-100 protein in the SCG was only released by the incubation in a Ca^{2+}-free EGTA medium. These results suggest that membrane depolarization produces the release of γ-enolase from neurons, and S-100 protein from glia in isolated SCG.

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Voltage clamp studies of the membrane currents in the thrombocytes of the newt. Kawa, K. and Obata, K. Dept of Pharmacol., Gunma Univ. Med. Sch., Maebashi 371.

The whole-cell membrane of the thrombocytes of the newt, Triturus pyrrhogaster, were voltage-clamped with patch electrode. Depolarization over -30mV evoked transient K^+ currents which decayed completely during 150 msec stimuli. The currents were blocked by 1 mM 4-aminopyridines. The monovalent cation selectivities of the K^+-channels were estimated from shifts in reversal potential as $Tl(1.68) > K(1) > Rb(.89) > Na(.03)$. In the presence of Ca^{++}(1.8mM), the thrombocytes on contact to glass slip can be activated to spread spontaneously. In the activated cells, depolarization evoked K^+ currents with negligible decay during 150 msec stimuli, which suggested that the inactivation gate of the K^+ channel have been modified remarkably. The monovalent cation selectivities of the K^+ channels, however, showed no significant changes after the activation.

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A SURVEY OF IONIC CHANNELS IN THE CULTURED CELLS DERIVED FROM PANCREATIC ISLETS WITH THE PATCH-CLAMP METHOD. MARUNAKA, Y., MURAYAMA, K., OHARA, A., KITASATO, H. and KANBE, M. Dept. of Physiol. and Dept. of Biol., Shiga Univ. of Med. Sci., Ohtsu, 520-21 Japan

To clarify the change of ionic permeability in pancreatic B cells underlying the glucose-induced depolarization followed by a burst of spikes, we surveyed ionic channels in the cultured pancreatic B cells using single channel recording techniques with patch electrodes filled with 120 mM KCl + 13 mM $CaCl_2$. Pancreatic islets of mice were isolated by exposure to collagenase from the exocrine tissue and cultured for 2-3 weeks in RPMI 1640 medium containing 10% fetal bovine serum. B cells were identified by Aldehyde-Fuchsin staining. We found three types of ionic channels. 1) One of them was a voltage-dependent (probably Ca^{2+}-) channel which opened in response to depolarizing clamp pulses. 2) The second was a channel which was open for about 45% of the time at the resting or more hyperpolarized membrane potentials. A reversal potential obtained from the I-V relation was very close to the resting membrane potential and the single channel conductance from the slope was 12.8 pS. The results suggest that this channel is Cl^- channel. 3) The third was a (probably K^+-) channel that was open with the mean single channel current of 1.6 pA and open probability of 0.182 at the resting membrane potential.

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THE OPTICAL ACTIVITY SIGNAL RECORDED FROM A SQUID GIANT FIBER. WATANABE, A., National Institute for Physiological Sciences, Okazaki 444.

A transient change in optical activity of a squid giant axon was found when the axon was excited. The external medium was artificial sea water containing 0.1 mM of the dye N-(4-Dimethylamino-3,5-dinitrophenyl) maleimide. The axon was illuminated with a quasi-monochromatic light. A polarizer and a photoelastic modulator were employed so as that the azimuth of the E vector of the incident light changed periodically from -45° to +45° in reference to the longitudinal direction of the axon. The transmitted light was passed through an analyzer and received with a photodiode. The photocurrent from the photodiode was rectified by a lock-in amplifier. The change in output of the lock-in amplifier was further amplified by an AC amplifier and fed to the input of a signal averager. Several thousand stimuli were delivered to the axon cooled around 0 °C and the resultant change in lock-in output was accumulated in order to increase the signal-to-noise ratio. An optical activity signal appeared as a sharp increase in levorotation at the rising phase of the action potential when the wavelength of the incident light was around 470 nm. The size of the increase was about 10^{-5} degree, and the duration was 0.5 - 1 ms. When the azimuth of the transmission axis of the polarizer was rotated by 90° the the direction of the signal was reversed.

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REAL-TIME OPTICAL MAPPING OF NEURONAL ACTIVITY IN EARLY EMBRYONIC GANGLION. SAKAI, T., HIROTA, A., KOMURO, H., KAMINO, K. Dept. Physiol. Tokyo Ika-Shika Daigaku, Sch. Med. Tokyo 113.

The answers to many questions in developmental neurophysiology require understanding of the organization of integrated functions in the central nervous system. However, the use of microelectrodes cannot usually provide simultaneous recording from many sites on the arborization of single nerve cells. Using a voltage-sensitive merocyanine-rhodanine dye together with a 10×10 element photodiode matrix array, we have monitored electrical neuronal activity in the semilunar ganglia of early developing chick embryos. The electrical membrane potential response we monitored simultaneously from 96-100 adjacent areas in the ganglion, with the stimulation of the afferent nerve fibers (the ophthalmic division or the maxillo-mandibular division). In the 4-7-day old embryonic ganglia, we could identified the areas in which is functionally connected by the ophthalmic division, the maxillo-mandibular division, and both the divisions. In addition, applying the stimulation of the ophthalmic division or the maxillo-mandibular division, evoked electrical activity was optically detected from the brain (myelencephalon) region. These results demonstrate the feasibility that multiple-site optical recording can be used for real-time mapping of spatio-temporal pattern of neuronal activity and for developmental mapping of functional organization in the early embryonic central nervous system.

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THE EFFECT OF BOVINE PLASMA ON THE LOCAL ANESTHETIC ACTION OF PROCAINE IN THE RAT SCIATIC NERVE IN VITRO. NODA, K.*† TANIGUCHI, M.*, MAEYAMA, I.*† SATO, T.*†† Dept. of Physiol., Orthopedics† Anesthesiol††, Tottori Univ. Sch. Med., Yonago 683

The effect of organic acids and nucleotides on the local anesthetic action of procaine in bovine plasma was compared with that in Locke's solution. The anesthetic potency of procaine has been known to be enhanced by propionate or salicylate mixed. This enhancing effect in bovine plasma was observed to the same extent as that of Locke's solution. While efficacy of procaine itself was to be greater in bovine plasma than in Locke's solution. This became much more notable in filtrated plasma in which larger molecules than 10000M.W. were sieved off. Nevertheless, when 0.1% ATP was added to plasma or filtrated plasma, such a great efficacy of procaine was almost completely reduced in both cases, whereas no infulence in Locke's solution. The efficacy was slightly decreased by 0.1% 5'-AMP, but not affected by 0.1% adenine in filtrated plasma. These results suggest that there may exist a certain factor in bovine plasma having a molecular weight below 10000 which intensifies the anesthetic potency of procaine.

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SELECTIVE INHIBITION OF SODIUM CURRENT IN SQUID GIANT NERVE BY FATTY ACID: AN ANALYSYS BY NON-LINEAR LEAST SQUARE METHOD. HORI, H., HORIE, H. and TAKENAKA, T. Dept. of Physiol. Yokohama City Univ. Yokohama 232

Fatty acids inhibit the excitability of the nerve membrane. For example, 2-decenoic acid, having 10 carbon atoms, suppresses the early inward (sodium) current but not the late outward (potassium) current. To study this mechanism, the parameters of the kinetics were calculated by computer curve fitting using the data of the physiological experiments. We calculated sodium and potassium currents directly from voltage-clamping current by using non-linear least square method following the Hodgkin-Huxley equations and obtained g'_{Na}, g'_K, τ_m, τ_h and τ_n.

An application of 1.58 mM 2-decenoic acid decreased the peak sodium current to 38 %. Under this condition, from the calculated results, it is showed that τ_m becomes twice value in almost all voltage stages as compared with the control. And g'_{Na} values in small test pulse are decreased. From g'_{Na} and τ_m values, α_m and β_m were calculated. Voltage dependency of α_m and β_m was sifted about 10 mV. But there is little change in other parameters.

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EFFECTS OF FATTY ACIDS ON THE MEMBRANE FLUIDITY OF CULTURED CHICK DORSAL ROOT GANGLION NEURONS. TAKENAKA,T.,HORIE,H.,KAWASAKI,Y.*Physiol. Yokohama City Univ. Sch. of Med.,Yokohama, Mitsubishi-Kasei Inst. of Life Sciences.

The effect of fatty acids on the membrane fluidity in tissue cultured neurons of chick embryo dorsal root ganglion was studied by fluorescence recovery method. Lateral motion of the lipid was measured by observing the fluorescent probe, 5-(octadecylthiocarbamoylamino)fluorescence,F18. The effective lateral diffusion coefficient of the membrane was around $0.30 \times 10^{-8} cm^2/sec$ in control cells, $0.42 \times 10^{-8} cm^2/sec$ in 2-decenoic acid treated cells, and $0.35 \times 10^{-8} cm^2/sec$ in valeric acid treated cells. From these results it is concluded that effective mobilities of the membrane complex increased about 40% by the external application of 2-decenoic acid, while valeric acid increased it only 12%. From the physiological results that 2-decenoic acid inhibits the Na-channel, it is suggested that this increase in the membrane fluidity might affect the Na-channel.

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LATERAL DIFFUSION OF MEMBRANE LIPIDS CHANGES WITH AGING IN C57BL MOUSE DORSAL ROOT GANGLION NEURONS FROM A FETAL STAGE TO AN AGED STAGE. HORIE,H., KAWASAKI,Y*.,TAKENAKA, T. Physiol. Yokohama City Univ.Sch. of Med., Yokohama, Mitsubishi-Kasei Inst. of Life Sciences, Tokyo.

The membranes of dorsal root ganglion(DRG) neurons dissected from different age groups of C57BL mice, from 18-day fetal to 30-month-old mice, were labeld with the fluorescent analog of fatty acids, F18, after a day in culture. The fluorescent probe specifically labeled the cell surface. The lateral diffusion of F18(5-(octadecylthiocalbamoylamino) fluorescein) was measured with a Fluorescence Photobleaching Recovery (FPR) method. The value, $(0.34 \pm 0.07) \times 10^{-8} cm^2/sec$, of the lateral diffusion coefficients of F18 labeled to 18-day-old fetal mice rapidly decreased and reached $(0.22 \pm 0.07) \times 10^{-8} cm^2/sec$ in a 3-day-old newborn stage. Then the values slowly decreased and reached $(0.13 \pm 0.05) \times 10^{-8} cm^2/sec$ in a 6-month-oldstage. In older stages than 6-month-old the lateral diffusion coefficients scarcely changed with aging. As these changes of the membrane fluidity are accompanied by the decrease of regenerative capacities , it is thought that the membrane fluidity might change with aging in contact with changes of important cell functions.

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DOES INTRACELLULAR Ca INACTIVATE K-CURRENT IN BULLFROG SYMPATHETIC NEURONS? ARIYOSHI. M., TOKIMASA, T. AND KOKETSU, K., Department of Physiology, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830, Japan

Bullfrog symapthetic neurons were voltage clamped by means of a sample-and-hold/voltage-clamp device in Na-free, Ca-rich solution which contained tetraethylammonium (TEA). A brief inward Ca-current was followed by a long-lasting (up to 60 s at -40 mV) inward current. The long-lasting inward current corresponded to an after-depolarization which followed a Ca-spike under current clamp conditions. The long-lasting inward current was, therefore, called the inward after-current. It was largely due to an inactivation of a K-current which had many of the same characteristics as that fully described elsewhere (Adams et al., J. Physiol., 330: 537, 1982) and known as the M-current. The present study indicates that massive Ca entry through voltage-operated channels would lead to an inactivation of K-conductance in vertebrate nerve cells.

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ALTERED REACTION TO VERATRIDINE IN CULTURED NEURONS FROM TEMPERATURE-SENSITIVE PARALYTIC MUTANTS OF DROSOPHILA. SUZUKI, N AND WU, C.-F.* Dept. Physiol., Sch. Med., Kitasato Univ., 1-15-1, Kitasato, Sagamihara, Kanagawa 228, *Dept. Biology., Univ. of Iowa, Iowa City, Iowa 52242.

In vitro culture of central nervous system neurons from Drosophila larvae enables direct examination of effects of neurological mutations at a single-cell level not readily amenable to in vivo experimentation. Using this system, we examined the cytotoxic effect of veratridine, which selectively causes persistent activations of sodium channels, on the mutants $para^{ts1}$ and nap^{ts} known to have a temperature-dependent block in propagation of nerve action potentials. Even at a permissive temperature (22° C) for the mutant flies, the veratridine-induced neuronal lethality was significant lower in both $para^{ts1}$ and nap^{ts} cultures than in normal cultures. At a temperature (35° C) causing paralysis of mutant flies, nap^{ts} neurons showed the same high degree of resistance to a veratridine; while $para^{ts1}$ neurons showed an increased resistance to a level similar to that of nap^{ts} neurons. A similar reduction in the veratridine-induced neuronal death was also observed in normal cultures that were pretreated with sodium channel blocker TTX. These results suggest that both $para^{ts1}$ and nap^{ts} affect sodium channel functions at a level of isolated single neurons.

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RECTIFICATION PROPERTY OF THE DORSAL ROOT OF THE BULLFROG SPINAL CORD. HASHIGUCHI,T., Dept. of Physiol., Tokyo Medical College, Tokyo 160

The rectification property of the dorsal root of the hemisected spinal cord, isolated from the bullfrog anesthetized by cooling, was studied by means of a single sucrose-gap method with current injecting capability. An inward or outward going rectification was activated in response to hyperpolarizing or depolarizing current injection, respectively. The reduced effective resistance on polarization implied that the injected current would polarize only the membrane near the driving point. Moreover, the less uniform potential distribution along the root would distort potential recording from the remotely located primary afferent terminals. These rectifications were not affected by removing Na^+, Ca^{2+} or Cl^- ion. On the other hand, cesium (Cs) blocked an inward rectifier. Tetraethylammonium (TEA) antagonized an outward one, while 4-aminopyridine (4-AP) increased the effective resistance only at the depolarized potential range. These potassium channel blockers may be used as a pharmacological tool to increase the effective resistance and achieve more uniform potential distribution. In fact, the effective resistance increased over twofold in the TEA (110 mM), 4-AP (2 mM) and Cs (5 mM) containing solution compared with that in the normal solution.

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THE ANESTHETIC ENHANCEMENT BY MONO-CARBOXYLIC ACIDS INVESTIGATED WITH THE ADSORPTION MODEL. ICHIKAWA, O., Dept. of Physiology, Tottori Univ. Sch. Med., Yonago 683

The anesthetic adsorption model of a simple Langmuir type was successfully introduced to explain the dependence of block onset time on procaine concentrations. Parameters such as adsorption rate (a), desorption rate (β), critical adsorption ratio (n) and maximum adsorbable dose of procaine (D_m) were obtained by observing the time dependence of procaine desorption from the nerve which was preincubated in the procaine solution, using a U-V absorption technique. Those parameters given in the solutions of mono-carboxylic acids were compared among each other. The value β/a decreased with increasing lipophilicity of the added carboxylates, among aliphatic anions. Benzoate further lowered the value n, i.e. made the nerve be blocked with lower adsorption of procaine. The value of D_m, on the other hand, did not change in those additive nor additive-free solutions. As a result, the strength of anesthetic enhancement by those carboxylates estimated from the magnitude of β/a and n showed a good agreement with the observed ability of enhancement in the electrophysiological experiment.

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THE EFFECTS OF HALOTHANE ON THE GUINEA-PIG THALAMIC PARAFASCICULAR NUCLEUS NEURONS IN VITRO. SUGIYAMA, K. AND NISHI, S. Dept. Physiol., Kurume Univ. Sch. Med., Kurume

We have investigated the synaptic mechanism of IPSP which regulates the phasic pattern of firing and also studied the effects of halothane on the tonic and the phasic firing patterns, using slice preparations of guinea-pig parafascicular nucleus. IPSP and GABA-induced hyperpolarization were resistant to bicuculline, while IPSP was completely abolished during baclofen-induced hyperpolarization. IPSP was augumented by nipecotic acid, an inhibitor of GABA uptake system. IPSP and GABA hyperpolarization were decreased in amplitude by membrane hyperpolarization and were nullified at about -90 mV. The amplitude of either response was dependent on external K concentration. Superfusion of halothane (0.4 mM) inhibited IPSP more significantly than EPSP. Halothane also reduced the IPSP-rebound to being subliminal for triggering the phasic firing. The same concentration of halothane as above produced a hyperpolarization which was associated with a decreased input resistance. Its amplitude was dependent on external K concentration. The tonic firing that appeared when the membrane was deporalized disappeared during halothane hyperpolarization. These results suggest that IPSP is probably produced by an increase in K conductance of the GABA(B) receptor membrane and that both the halothane-induced reduction of IPSP-rebound and the halothane-hyperpolarization are responsible for suppression of the tonic and the phasic firing of parafascicular neurons.

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NORADRENALINE-INDUCED DEPOLARIZING AFTERPOTENTIAL IN SYMPATHETIC PREGANGLIONIC NEURONS IN VITRO. YOSHIMURA, M., *POLOSA, C. & NISHI, S. Dept. Physiol. Kurume Univ. Sch. Med., Kurume and *Dept. Physiol. McGill Univ., Montreal, Quebec, Canada.

Intracellular recordings were made from the intermedio-lateral nucleus in the upper thoracic spinal cord cut from adult cats and maintained in vitro. The neurons were identified by their antidromic responses to stimulation of various sites which included the white ramus, the ventral root and the lateral edge of the ventral horn. Noradrenaline (NA, 10-50 μM) applied by superfusion caused a decrease in amplitude of slow after-hyperpolarization (S-AHP) and an appearance of depolarizing afterpotential (DAP) which was associated with a decrease of membrane resistance. The DAP was depressed markedly in a solution contained cobalt or low calcium but not by tetrodotoxin. Substitution of choline or Tris for Na caused suppression of the DAP amplitude suggesting that underlying ions for producing the DAP are both Na and Ca. Another prominent effect of NA was abolition of the spike shoulder. Together with these data, it is suggested that NA caused the depression of S-AHP by decreasing the Ca influx during a spike and resulted in unmasking of DAP.

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INTRACELLULAR Ca OSCILLATION OF L CELLS IN WHOLE CELL RECORDINGS. OIKI, S., OHNO-SHOSAKU, T. and OKADA, Y. Dept. of Physiol., Fac. of Med., Kyoto Univ., Kyoto 606

A fast transient peak potential was observed immediately after penetration of a microelectrode into a fused giant L cell. Whole cell recordings by a giga-seal microelectrode gave more negative resting potentials and higher input resistances than usual intracellular recordings. During whole cell recordings, significant decreases in the membrane resistance and potential were observed upon impalement of a microelectrode. Thus, it appears that the impalement of a microelectrode produces leakage artifacts even in giant L cells. The question therefore arose as to whether the leakage artifacts are involved in the generation mechanism of membrane potential oscillations due to cyclic changes in the intracellular Ca^{2+} concentration. However, the oscillations were also observed by the whole cell recording technique under appropriate conditions. The oscillations were less frequently observed at low temperature (below 30°C) and were suppressed by the strong Ca^{2+} buffer capacity of the solution filled in the microelectrodes. Extracellular recordings with giga-seal electrodes also provided evidence that L cells exhibit oscillations without any leakage artifacts. Therefore, it is concluded that Ca oscillations are independent of microelectrode penetration but are endogenous under the experimental conditions.

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ELECTROPHYSIOLOGICAL AND BIOCHEMICAL STUDIES OF THE Na CHANNELS BY USING PHOTOACTIVABLE AZIDE DERIVATIVES OF TETRODOTOXIN. SEIJI UEHARA, KEIICHI UYEMURA, *RYOHEI SATO and MASAAKIRA KANO. Dept. of Physiol., Saitama Med. Sch., Moroyamacho, Irumagun, Saitama, *Dept. of Physiol., Fac. Med., Kitazato Univ., Kitazato, Sagamihara, Kanagawa.

Azide derivatives of tetrodotoxin (TTX), D1, D2 and D3 were synthesized by a reaction of 2-nitro-4-azidephenyl-β alanine and TTX for the studies of the Na channels. In the dark, all the derivatives reduced reversibly amplitude of action potential of a frog sciatic nerve. Upon photoirradiation of the nerve exposured to D1 and D3, irreversible reduction of the amlitude of action potential was observed, while D2 exhibited no irreversible reduction. This showed that photoirradiation resulted in covalently binding of D1 and D3 to the TTX binding sites. Rat synaptosomal membranes were photolabeled with tritiated D3 and extracted with 1.0% lubrol PX, and the lubrol extract was analyzed on Sephadex G 25 column equilibrated with 0.1% lubrol-50 mM ammonium acetate. A D3 binding component was eluated at the position of Kav=2.1. The purified component showd the specific binding activity of 41 pmol/mg protein, which was more than 240 times higher than that of the original membranes. Scatchard plot of the component yielded Bmax=0.24 pmol/mg membrane protein and Kd=44 nM.

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NERVE GROWTH FACTOR-INDUCED NEURONAL DIFFERENTIATION AND Na-CHANNELS IN PHEOCHROMOCYTOMA CELLS. Miyake, M. Kamei, K. and Hatanaka, H. Fac. of Pharmaceu. Sci., Hokkaido Univ., Sapporo 060 and Mitsubishi-Kasei Inst. of Life Sci., Tokyo 194

PC12 cells, a clonal cell line derived from rat pheochromocytoma, show a variety of neuronal properties resembling sympathetic neurons in response to nerve growth factor (NGF). Here we examined the developmental changes of Na-channel during the NGF-induced neuronal differentiation in PC12h-AG8 cells (one of the subclones of PC12) by measuring ^{22}Na influx in the presence of veratridine (Ver; an opener of the activation gate) and scorpion toxin (ScTX; an opener of the inactivation gate).

TTX-sensitive ^{22}Na-influx into NGF-untreated AG8 cells was appliciably stimulated (30-40 nmol/min/mg) by the application of both Ver (20 μM) and ScTX (500 ng/ml), but no or little stimulation was observed by Ver(up to 200 μM) alone. After 9-12 days in culture with NGF(50 ng/ml), the Ver and ScTX induced ^{22}Na-influx was enhanced 2- to 4-fold compared to the influx into the NGF-untreated cells. In these differentiated AG8 cells, the treatment with Ver alone leaded to a significant increase in ^{22}Na-influx (25-30 nmol/min/mg). The above findings are consistent with the result for mouse neuroblastoma cells that the voltage-dependece of the inactivation gate of Na-channel changes significantly in association with the neuronal differentiation, in additon to the developmental increase in the density of the channel (see No. 091 in this colume).

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A SPECIFIC INHIBITORY EFFECT OF CATECHOL ON THE FAST K CURRENT. ITO, I. AND MAENO, T. Dept. of Physiol., Shimane Med. Univ., Izumo, Shimane 693

The effect of catechol on various ionic channels of isolated primary afferent neurons of the bull frog were examined by the single suction electrode clamp system, which conbines internal perfusion and current or voltage clamp using an electronic switching circuit. Catechol was found to inhibit specifically the fast K current as does 4-aminopyridine (4-AP). It is merely about three times less potent than 4-AP. Ca, Na and delayed K currents were not affected. Hill plots of the dose-inhibition data of catechol yield a linear relation with a slope of almost unity (n_H=1.1). Althouth both 4-AP and catechol were specific inhibitors of the fast K channels, their sites of action were quite different each other. Catechol was effective when applied on the external surface of cell membrane whereas 4-AP acted internally. Furthermore, a positive allosteric interaction was observed between catechol and 4-AP. ED_{50} for the prolongation of action potential was about ten times greater than that for the facilitation of neuromuscular transmission.

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PRESSURE INJECTION OF CYCLIC AMP INTO AN IDENTIFIED NEURON IN THE ABDOMINAL GANGLION OF APLYSIA KURODAI. HARA, N.[1,*] ICHINOSE, M., SAWADA, M. AND MAENO, T. Dept. of Physiol. and Central Res. Lab.[1,*] Shimane Med. Univ., Izumo, Shimane 693

The ionic mechanism of membrane effect of pressure-injected adenosine 3',5'-cyclic monophosphate (cAMP) on an identified neuron of Aplysia was investigated with conventional voltage-clamp and ionic substitution techniques. The intracellular elevation of cAMP caused an inward current (I_{cAMP}) associating with a conductance decrease. The I_{cAMP} increased over the voltage range (-50 mV to -30 mV) and decreased at potentials more hyperpolarized than -60 mV. Duration of the I_{cAMP} was greatly prolonged by bath-applied isobutylmethylxanthine (50 μM), and imidazole (10 mM) had an opposite effect on the current. Tolbutamide (5 mM), an inhibitor of protein kinase reaction largely reduced the I_{cAMP}, however, the current was evoked in the presence of bath-applied TTX (50 μM), ouabain (50 μM), or triaminopyrimidine (5 mM). Reduction of $[Na^+]_o$ caused a reversible decrease of the I_{cAMP}. Alterations of $[K^+]_o$, as well as bath-applied 4-AP (5 mM) and TEA (30 mM) did not affect the I_{cAMP}. In the presence of Na^+, divalent cations such as Ca^{2+}, Ba^{2+}, and also Cl^- exerted an inhibitory influence on the I_{cAMP}. These results suggest that fast elevation of intracellular cAMP induces a slow sodium inward current and the current might be due to activation of cAMP-dependent protein kinase.

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EFFECT OF ENKEPHALIN ON CHOLINERGIC TRANSMISSION DEVELOPED AMONG TRANSDIFFERENTIATED ADRENAL CHROMAFFIN CELLS IN CULTURE. OGAWA, M., ISHIKAWA, T.* and OHTA, H.** Dept. of Physiol., Dept. of Anat.* and Dept. of Neuropsychiatry**, Kochi Medical School., Nankoku, Kochi 781-51

We have previously reported that the isolated adrenal chromaffin cells can transdifferentiate into neuronal cells and develop cholinergic transmission among them in culture. In the chromaffin cells enkephalin-like peptides coexist in association with other neurotransmitters as well as in many axons. And it is hypothesized that these peptides act as neurotransmitters or as neuromodulators of adrenal medullary function like those in the ganglionic transmission. The present study examined the effects of enkephalin on the cholinergic transmission which developed among the transdifferentiated chromaffin cells in culture. Met-enkephalin in concentration of 10 μM reduced the amplitude and the duration of the excitatory postsynaptic potentials evoked by presynaptic spike. The opioid also decreased the duration of the Na-Ca action potential and Ca-dependent afterhyperpolarization following the spike.

Based on these findings, it is suggested that the depression of synaptic transmission by enkephalin is caused by the reduction in Ca entry in both the pre- and postsynaptic sites.

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EFFECTS OF SOME ANTAGONISTS ON THE ACETYLCHOLINE INDUCED CURRENTS OF MOLLUSCAN NERVE CELLS. *FURUKAWA, Y. and KOBAYASHI, M.
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A few identified neurons in the right-parietal ganglion of the African giant snail, Achatina fulica Férussac, showed three types of hyperpolarizations in response to the administration of acetylcholine (ACh). In order to clarify the ionic mechanisms of the ACh responses, the neurons were voltage-clamped using two microelectrodes. Experiments revealed the existence of two different ACh-induced currents (Cl^- and K^+). It was concluded that three types of hyperpolarizing responses were due to the relative sizes of the two currents in a neuron.

Effects of some antagonists of ACh on those currents were studied. Benzoquinonium blocked both currents at nearly the same concentration. Curare, decamethonium and atropine blocked Cl^- current but not K^+ current. Tetraethylammonium blocked ACh-induced K^+ current at less concentration than needed to depress the other K^+ currents, but it had little effect on Cl^- current. From these results, it was considered that two currents might be mediated by the different ACh-receptors.

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DESENSITIZATION AND SEPARATION OF AGONIST FROM THE BOUND RECEPTORS IN SNAIL NEURONS. MARUHASHI, J. AND TANABE, N, Dept. of Biology, Daiichi College of Pharmaceutical Sciences, Fukuoka 815.

Glutamate receptors in Onchidium and Helix neurons are known their Glu-induced responses are practically no densitized during Glu application. But, after wash-out of applied Glu, boud Glu with the receptors remained long period. In Glu-weak response type neurons in Helix aspersa, after disappearance of N-acetylimidazole (NAI) activated Glu-induced responses , desensitization to Glu in the receptors remained more than 30 min. At the voltage clamped condition, NAI activated Glu-induced responses were very brief and followed desensitization recovered in short period. This means desensitization of the receptors start with separation of Glu from the bound receptors.

ACh-induced depolarizing reasonses were brief compare with Glu-induced responses in the same type cells. The rising phase of ACh-induced responses were slow before NAI-treatment. During perfusing NAI, ACh-induced responses became large and their slope became sharp, but their duration did not increase. This is due to removal of ACh from receptor.

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DEVELOPMENTAL CHANGES IN SINGLE GLUTAMATE CURRENTS AT THE INSECT MUSCLE. SAITO, M., MIWA, A. and KAWAI, N., Dept. of Neurobiol., Tokyo Metropolitan Inst. for Neurosci., 2-6 Musashidai, Fuchu-City, Tokyo, Japan 183

Developmental changes in glutamate receptor of insect (Tenebrio molitar) muscle have been studies by single channel current recordings. Using patch pipette containing 200 to 500 μM glutamate, three different classes of the glutamate activated channels were identified, one was in larva, the other two were in imagines muscles. In larval muscle, the conductance for single channel was 31.1 pS and mean open time was 2.34 ms. In imagines muscle, one type of the channel current which reversed current direction around +10 mV has the conductance of 29.4 pS but with shorter life time (1.14ms) than that of larva muscle. The other type of channel current was recorded as outward current at the resting membrane potential and converted to inward current around -100 mV. This type of channel seems to activate the hyperpolarizing type (H type) of the glutamate receptor found by the intracellular recording. Effects of glutamate agonists were studied on D type response of imagines. Quisqualate (200 μM) opens the channels longer than glutamate in accordance with the results reported in the locust muscle. On the other hand, mean open time of kainate activated channels was much shorter than that of glutamate.

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EFFECTS OF NERVE CRUSH AND NERVE GROWTH FACTOR ON MUSCLE SPINDLES IN NEWBORN RATS.

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Following crush of a muscle nerve in newborn rats, monosynaptic EPSPs produced in spinal motoneurons by stimulation of the muscle nerve are significantly depressed within one month. However, this EPSP depression can be partly prevented if nerve growth factor (NGF) is daily injected into the animal after nerve crush. We have examined whether these changes in central synaptic function of the sensory fibers are related to morphological alterations of the muscle spindle. For this purpose, the medial gastrocnemius nerve of the rat was crushed near the muscle on the day after birth and morphological observations on the muscle spindles were made 4 weeks later. The number of spindles remained unchanged, whereas their diameter and length were reduced by about 50%. Daily injection of NGF following the nerve crush significantly increased the number of spindles, although the spindles were still atrophic. It is suggested that NGF has trophic effects on the muscle spindles.

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ACETYLCHOLINESTERASE ACTIVITY IN MUSCLES OF THE AGED MOUSE.

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The cholinesterase activity at the motor end-plate of aged mice was investigated histochemically by the Karnovsky direct colouring technique and on using the homogenized preparation assayed with [^{3}H] Ach as a substrate. In histochemical work, a relative quantitative method was applied for studying the reaction by utilizing varying concentrations of eserine. The muscle tissue (diaphragm, extensor digitorium longus (EDL), and soleus) was obtained from aged mice (30-37 month old, C57BL) and young mice controls (2-5 month old, C57BL). The result showed that at the concentration of 10^{-5} M eserine the number of endplates reacting was significantly reduced in old diaphragm in comparison with control muscles, and the number was slightly but significantly increased in old soleus. In the work of radioactive assay, the muscle was homogenized in the buffer solution. Incubations were at 37°C and lasted usually 30 min. Eserine inhibited AchE activity, the I_{50} values (i,e, the concentrations of the inhibitor which caused a 50% inhibition) were almost the same in young and old diaphragms but were higher in old EDL than in young EDL. These results suggest that there might be a correlation between cholinesterase activity and type of muscles in the aged animal.

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DIFFERENCES IN MEMBRANE IONIC CONDUCTANCES OF SOMATA, AXONS AND TERMINALS OF CRUSTACEAN NEUROSECRETORY CELLS.

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The X-organ-sinus gland neurosecretory system was isolated from the eyestalk of crabs (Cardisoma carnifex and Podophthalmus vigil) to a chamber permitting continuous perfusion and changes of perfusate composition during continuous intracellular recording. Action potentials differed in their form as recorded from terminal dilatations, axons or somata. The inclusion of 50 mM TEA in the perfusate led to an increase in the duration of action potentials of terminals and somata by 50x and 10x, respectively, but not more than a 2X increase in that of axons. The TEA-induced plateau of terminals persisted in nominally Na-free saline or TTX (3×10^{-7} M) saline, but was clearly decreased by removal of Ca (Na normal) or addition of Cd (0.2 mM) to Na-free saline. Thus terminals are capable of sustained Ca current. Quinine (0.3 mM), which selectively inhibits Ca-mediated K conductance in some neurons, reduced the afterhyperpolarization and lengthened the duration of soma action potentials whereas it did not alter terminal action potential form. These observations show regional specialization of ionic conductances in the membrane of neurosecretory cells. This study was done at the Bekesy Laboratory of Neurobiology, Univ. of Hawaii. Supported by NIH grant NS 15453 to I. M. Cooke.

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PRESYNAPTIC ACTION OF DIVALENT CATIONS ON SNAIL NEURONS. YAI, H. Dept. of Physiol., Saitama Med. Sch., Moroyama, Iruma-gun, Saitama 350-04.

Chloride dependent hyperpolarization was often observed in high K medium or with ACh application in the same snail ganglion cells. The former is induced by probably presynaptic effect of K, whereas the latter is a direct action of ACh on the cell membrane. When Co replaced Ca or was merely added in the perfusing solution, the high K-induced hyperpolarization was significantly depressed and became depolarization in the most cases, in spite of depolarizing effect of Co itself on the resting potential. On the other hand, ACh-induced hyperpolarization was rarely affected by Co. High Ca, or high Mg with or without Ca in the external solution somewhat repressed the high K-induced hyperpolarization. In the medium omitted both Ca and Mg, high K-induced hyperpolarization was markedly decreased and changed to depolarization in almost all the cells, while it was hardly influenced by simple removal of either Ca or Mg. On the basis of these observations it is assumed that proper amount of Mg may substitute for Ca in presynaptic action, rather than antagonize it like Co, in this particular case.

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EFFECT OF SEA URCHIN TOXIN ON TRANSMITTER RELEASE AT FROG NEUROMUSCULAR JUNCTIONS. ANRAKU,M., KIHARA,H. and HASHIMURA,S. Dept.of Physiol., Kagoshima Univ. Fac. of Med., Kagoshima 890

The effect of a toxin from sea urchin on the frequency of miniature endplate potentials (MEPPs) and endplate potential (EPP) were studied. In normal K (2.5 mM) Ringer, the toxin increased MEPP frequency significantly depending on the concentration of Ca or Mg. In high K (7.5 mM) Ringer, MEPP frequency decreased by Mg depending on its concentration. The toxin counteracted the depressing effect of Mg on MEPP frequency in high K Ringer dose-dependently (0.1, 0.2 and 0.4 mg/ml). The counteracting effect of the toxin was complete at 0.4 mg/ml, and MEPP frequency gave rise to increase in proportion to the concentration of Mg. When Na concentration was changed from 0 to 115 mM in four steps in methylamine-HCl Ringer, MEPP frequency increased logarithmically against Na concentration. The toxin also increased EPP amplitude and quantal content of EPP (m). The toxin shifted the log m-stimulation frequency (0.33-6.25 Hz) relation without affecting the slope. These results were considered to show that the toxin increased the permeability of the nerve terminal to Ca, Mg and Na, and also increased the release probability of ACh quanta.

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CALCIUM CONCENTRATION CHANGE AND TRANSMITTER RELEASE AT MOTOR NERVE TERMINAL. TAKEUCHI, N., TAKIKAWA, Y. AND *YANA, K. Dept. of Physiol., Sch. of Med., Juntendo Univ., Hongo, Tokyo 113 and *Dept. of Electrical Eng., Coll. of Eng., Housei Univ., Koganei, Tokyo 184

Spontaneous miniature end-plate potentials (m.e.p.p.), end-plate potentials (e.p.p.) or end-plate currents were recorded at the frog neuromuscular junction. Mean quantal content ($\bar{m}$) of the e.p.p. and mean frequency ($\bar{f}$) of the m.e.p.p. in high K^+solution are highly sensitive to $[Ca^{++}]_0$ and both are depressed by Mg^{++}. On the other hand, Mg^{++} acted to increase $\bar{f}$ in lower $[Ca^{++}]_0$ solutions with normal level of $[K^+]_0$, even in 1 mM EGTA solution. 1 mM theophylline also increased the $\bar{f}$ in EGTA solution, while $\bar{m}$ of e.p.p. showed no marked change by theophylline. In statistical analysis of the m.e.p.p. occurrence time sequences obtained in these solutions, some significant deviations from expected range from Poisson process were observed. In high K^+solution a transient decrease and in the theophylline solution a transient increase in release probability of very short interval after the individual m.e.p.p. release were represented. These results suggest that there may be some difference between release properties triggered mainly by influx of external Ca^{++} through Ca-channel of surface membrane and triggered by a release of $Ca^{++}$from storage sites inside the nerve ending.

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DIVERSE MUSCARINIC RESPONSES IN CULTURED MAMMALIAN SYMPATHETIC NEURONS EVOKED BY DIFFERENT CONCENTRATIONS OF ACETYLCHOLINE. MOCHIDA, S., DEIE, M.* and KOBAYASHI, H. Department of Physiology, Tokyo Medical College, Shinjuku-ku, Tokyo 160.

Superior cervical ganglia (SCG) excised from male adult rabbits were enzymatically dissociated and neurons were cultured for 4-6 days. Conventional intracellular recordings were made to study the nature of their muscarinic responses. Single SCG neuron was shown to directly respond to exogenously applied acetylcholine (ACh), in the presence of sufficient concentration of D-tubocurarine, with four different types of electrical changes: 1) acceleration of the repolarizing phase of action potential with remarkable shortening of after-spike hyperpolarization, 2) slow depolarization (DP) with increase in membrane conductance (Gm) independent of membrane potential (Vm), 3) DP with decrease in Gm at Vm positive to about -65 mV and 4) decrease in Gm (often associated to hyperpolarization) possibly independent of Vm. The lowest concentrations of ACh for eliciting each of these types of response were about 10 nM for responses 1 and 2, 100 μM for 3 and 250 μM for 4, respectively. Response 1, in combination with response 3 which is presumably due to the suppression of M-current, forms a condition for the cell to maintain higher frequency of repetitive firing. Response 2 may operate to initiate the muscarinic DP when the cell is at the resting Vm (usually in the range of -65 mV or more). Functional significance of response 4 is to be further investigated.

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Inhibitory effects of aromatic amino acid derivatives on the excitability of identifiable neurones of an African giant snail (Achatina fulica Férussac). II. FUNASE, K., TAKEUCHI, H. and *ARIYOSHI, Y. Dept, Physiol., Sch. Med., Gifu Univ., Tsukasa-machi 40, Gifu. *Cent. Res. Lab., Ajinomoto Co., Kawasaki-ku, Suzuki-cho, Kawasaki.

It has been reported previously that the two derivatives of aromatic dipeptides, N-β-phenylpropionyl-L-Tyr (P-L-Tyr, L-Phe-L-Tyr lacking an amino group) and P-L-Trp (L-Phe-L-Trp lacking an amino group), showed the marked inhibitory effects on an identifiable giant neurone in the ganglia of an African giant snail. In the present study, as the screening tests, effects of P-L-Try on the thirteen giant neurones in the ganglia were examined. Of the neurones tested, the seven neurones were inhibited markedly by P-L-Tyr, while the others were not. Then, structure-activity relationships of the two substances mentioned and their related substances described as follows, on the four giant neurones inhibited by P-L-Tyr were tested: P-L-Phe, N-β-cyclohexylpropionyl-L-Tyr (C-L-Tyr), C-L-Trp, L-Phe-L-Tyr. The structure-activity relationships of these substances on the four neurones examined were observed to be comparable.

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EFFECTS OF SEROTONIN ON THE THREE TYPES OF DOPAMINE RECEPTORS IN APLYSIA GANGLION CELLS. MASANORI SHOZUSHIMA, MITSUHIKO MATSUMOTO, KAZUHIKO SASAKI, AND MAKOTO SATO Dept. Physiol., Sch. Med., Iwate Med. Univ., Morioka, Iwate 020

The ganglion cells of Aplysia include three types of DA receptors. The activation of one type produces K-dependent hyperpolarization (H_K-type). The other receptors are D_K- and D_{Na}-types, the activation of each produces K- and Na-dependent depolarization, respectively.

DA-induced responses were evaluated by the change in membrane conductance (ΔG) using the modulated resting clamp method. A 2 minute exposure to 10 μM Serotonin (5-HT) significantly depressed all types of responses to 10 μM DA. The relative blocking potency of 5-HT were measured on the number of different cells with the each type and found to be approximately $H_K > D_K \fallingdotseq D_{Na}$. The mode of blockade was studied by plotting dose-inhibition curve or Lineweaver-Burks type for each type of receptor activity. The systematic shift of these curves indicated the blockade was all "competitive". It was concluded that 5-HT competes with DA for a common binding site at the DA receptors of all types. However at low concentration (1 μM) 5-HT augmented only the D_K-type of response to 10 μM DA, which appeared to be cyclic AMP dependent.

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THE ROLE OF INHIBITORY INTERNEURON ON FIRING PATTERN GENERATION OF FEEDING MOTONEURONS IN APLYSIA. NAGAHAMA, T. AND TAKATA, M. Dept. of Physiol., Sch. of Dent., Tokushima Univ., Kuramoto-cho, Tokushima 770

In Aplysia buccal ganglia we have analyzed a neural mechanism of the firing pattern generation of motoneurons during the feeding cycle. Repetitive stimuli of cerebral-buccal connective (C-B conn.) produced synchronous membrane potential depolarization in both the mouth-opening (n_2(O)-MN) and -closing motoneuron (n_2(C)-MN), however, bursts of action potentials were evoked earlier in the n_2(O)-MN than the n_2(C)-MN. On the other hand, the membrane potential depolarization of the n_1-MN concerning swallowing occured in antiphase with those of the n_2(O)- and the n_2(C)-MN. Repetitive stimuli of C-B conn. produced patterned bursts of the motoneuron after cutting the nerves innervating buccal muscles, indicating that the rhythmic patterned burst is preserved in the absense of sensory inputs. During the feeding cycle, the membrane potential of both the identified interneuron and the n_2(C)-MN underwent synchronous membrane potential depolarization, however, when bursts of action potentials in the interneuron was at its maximum, the spike generation of the n_2(C)-MN was blocked. The removal of inhibitory inputs to the n_2(C)-MN from the interneuron by d-tubocurarine advanced the firing phase of the n_2(C)-MN, suggesting that onset of the firing in the n_2(C)-MN is governed by a burst activity of the interneuron.

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MECHANISM OF ACTION OF MEMANTIN ON FROG NEUROMUSCULAR TRANSMISSION. MASUO, K. AND MAENO, T. Dept. of Physiol., Shimane Med. Univ., Izumo, Shimane 693

Experiments are reported which show that Memantin (an adamantane derivative, 1,3-dimethyl-5-aminoamantane), in concentrations ranging from 0.5 to 50 μM, blocked transmission postsynaptically, but had negligible presynaptic action. Memantin had an effect on the mEPP frequency. However, this seems to have no influence on the depolarization-release coupling in the nerve terminal. It didn't affect the mean quantal content of EPP and kinetic P, statistical and kinetic parameters for the ACh release. The drug had a marked suppressing effect on the postsynaptic process as does Amantadine. It diminished and shortend the EPC, but its time course of decay remained approximately a single exponential curve. Also the EPC-membrane potential relation became hightly non-linear with hyperpolarization. An analysis of the ACh-induced noise showed that the drug shortens the mean channel lifetime. These suggest that the drug acts as so-called endplate channel blocker. We tentatively conclude that Memantin acts on the ACh-receptor as well as on the endplate channel. The observation that Memantin is less effective in the presence of dTC would support this idea.

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Pharmacological characteristics of molluscan identifiable giant neurones sensitive to β-hydroxyglutamic acid. WATANABE, K., SHIRATORI, Y., TAKEUCHI, H., *NOMOTO, K., *OHFUNE, Y., *TAKEMOTO, T. and **NAKAJIMA, T. Dept. Physiol., Sch. Med., Gifu Univ., Tsukasa-machi, Gifu 500; *Suntory Inst. for Bioorgan. Res., Shimamoto-cho, Osaka 618; **Biol. Div., Res. Lab., C. Koshiro Co., Ltd., Minoo, Osaka 562.

The six identifiable giant neuroes sensitive to β-hydroxy-L-glutamic acid (L-BHGA) of Achatina fulica Férussac have been previously classified into the following three types by their sensitivities to the two (erythro- and threo-) stereoisomers; erythro-type, combined type and threo-type neurones. In the present study, effects of L-glutamic acid analogues, such as ibotenic acid, quisqualic acid, α-kainic acid and domoic acid, were tested on the six neurones. The former two substances showed the same effects as those of L-BHGAs, on all of the neurones, suggesting that these substances acted as agonists of L-BHGAs. Ibotenic acid was markedly effective mostly on erythro-type and combined type neurones; while L-quisqalic acid on combined type and threo-type neurones. But α-kainic acid and domoic acid had the agonistic effects on the neurones excited by L-BHGAs and classified in whatever types.

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SLOW SYNAPTIC POTENTIALS EVOKED BY DESCENDING VOLLEYS IN SPINAL MOTONEURONS OF THE RAT.
TAKAHASHI, T. Dept. Physiol., Kyoto Univ., Fac. Med., Sakyo-ku, Kyoto 606

Intracellular recordings were made from motoneurons of neonatal rats in the lumbar spinal cord *in vitro*. Stimulation of the ventrolateral part at the lower thoracic segments evoked a slow excitatory postsynaptic potential (e.p.s.p.) lasting tens of second. The slow e.p.s.p. was accompanied by a decrease in input conductance. The amplitude of the slow e.p.s.p. was increased as the motoneuron was depolarized, whereas it was decreased by hyperpolarization. During the slow e.p.s.p., the afterhyperpolarization following an action potential was markedly diminished in amplitude as well as in duration. Thus, the ionic basis underlying the slow e.p.s.p. is attributed to suppression of the K^+ conductance. One week after cordotomy at the upper segment, the slow e.p.s.p. was no longer elicited. It is suggested that the e.p.s.p. is mediated by descending fibers.

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VOLTAGE-DEPENDENT SLOW MEMBRANE CURRENTS IN RAT SPINAL DORSAL HORN NEURONS IN VITRO AND THE ACTIONS OF SUBSTANCE P.
Kazuyuki MURASE, Joyce WILLETTS*, and Mirjana RANDIC*. Toyohashi Univ. of Technology, Tempaku, Toyohashi 440 Japan, and *Vet. Physiol. & Pharmacol., Iowa State Univ., Ames, Iowa 50011 USA.

Spinal dorsal horn neurons of 10-26 day-old rats in slice preparations were studied using a single micro-electrode voltage-clamp technique, at 29°C. Inwardly relaxing currents initiated by one-second negative steps from holding potentials of -70 to -25mV were examined in 38 neurons. Inward relaxations in 22 neurons for steps between -25 and -70mV resembled the M-current of sympathetic ganglion cells: Positive shifts of the inversion potential at raised external K^+ concentrations suggested its being K current; it was reduced by 0.5-4mM barium but not by 1mM cesium; muscarinic agonists, however, did not block it except in a few neurons. In contrast, inward relaxations triggered by steps negative to -70mV in 24 neurons appeared to reflect another current resembling an anomalous rectifier, I_Q, of Hippocampal neurons: It was blocked by cesium, but not by barium or muscarinic agonists. Substance P (SP: 10^{-7}-10^{-5}M) did reduce the M-like current irrespective to the mascarine sensitivity. In addition, SP induced novel inward relaxations for one-second positive steps from a holding potential of -60mV in a few neurons. The coexistence of both SP effects might account for the SP-induced depolarizations without significant conductance change observed in the neurons. Support by NIH.

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EFFECTS OF GLUCOSE DEPRIVATION ON THE NEUROTRANSMISSION AND THE LEVEL OF HIGH-ENERGY PHOSPHATES IN HIPPOCAMPAL SLICES IN VITRO.
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Using a thin hippocampal slice from guinea pig, neuronal activity of pyramidal neurons in CA_3 and CA_4 region was recorded after stimulation to granular cell layer of dentate gyrus and the level of high-energy phosphates such as ATP and creatine-P of each slice were studied during the deprivation of glucose(Glu) from standard medium(Krebs-Ringer containing 10 mM Glu). Neurotransmission was blocked in 30-40 min with initial slight increase in the amplitude. ATP(13.2) and Cr-P(27.3 mmol/kg protein) decreased slowly with a half time of 60 min. Glu in the medium was replaced with fructose, mannose, galactose, deoxy-Glu, sucrose, maltose, sorbitol, lactate, pyruvate and citrate. Fructose, galactose, mannose, lactate and pyruvate could be the substrate for the production of ATP and Cr-P but had no effect on the preservation of neural activity. Mannose could only prolong the onset of the loss of neural activity during the deprivation of Glu. Thus Glu is to be essential for the preservation of neural activity in addition to its role as the substrate for energy production.

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EFFECTS OF GLUTAMATE AND ASPARTATE ON CULTURED CEREBELLAR NEURONS. MORI-OKAMOTO,J. AND TATSUNO,J. Department of Physiology, National Defense Medical College, Tokorozawa, Saitama 359

Iontophoretically applied glutamate(GLU) and aspartate(ASP) induced depolarizations associated with decreases, increases or biphasic changes in input resistance in immature (6-13 days in culture) and mature (25-50 days) neurons, though immature neurons were less sensitive. Both amino acids showed similar reversal potentials in either immature or mature neurons. Amino acid sensitive neurons increased in number with maturation. In immature neurons, the amplitudes of GLU- or ASP-induced depolarizations were drastically increased when 10mM Ca was added to normal Tyrode's solution, and input resistances were always decreased by both amino acids. In mature neurons, on the other hand, 10mM Ca rather decreased the amplitudes of GLU- or ASP-induced depolarizations with greatr decreases in ASP responses. In 2% Na solution, however, high-Ca enhanced amino acid-induced depolarizations as remarkably as in immature neurons. These enhancing actions of Ca were abolished by Mn but only partially by 10mM glutamic acid diethylester or 1mM D-α-aminoadipate, though responses to both amino acids in normal solution were blocked by these antagonists at the same concentrations. These results suggest that GLU or ASP activates a receptor-uncoupled Ca conductance in addition to receptor-coupled Na and/or Ca channel activation.

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EFFECTS OF QUININE AND QUINIDINE ON THE EXCITABILITY OF PYRAMIDAL CELLS IN GUINEA-PIG HIPPOCAMPAL SLICES. FUJIMURA, K., YOSHIDA, S., MATSUDA, Y.
Dept. of Physiol., Nagasaki Univ. Sch. Med., Nagasaki 852

Effects of quinine (1-5 mM) and quinidine (25-1000 μM) on the excitability of CA3 pyramidal neurons were studied in guinea-pig hippocampal slices using intracellular recording techniques. The duration of action potentials was gradually prolonged, and finally neurons became inexcitable. The spike adaptation and the late component of the afterhyperpolarization were simultaneously inhibited by the drugs. The resting potential shifted to the depolarizing direction associated with an increase of the input resistance. Different from other reports, it took so much time to inhibit the Ca^{2+}-mediated K conductance underlying the late component of the afterhyperpolarization, even by applying high concentrations of the drugs (e.g., 60-80 min for the complete blockade by 500 uM quinidine), indicating that the Ca^{2+}-mediated K conductance is much less sensitive to the drugs in guinea-pig hippocampal pyramidal neurons. It is suggested that the blocking action of quinidine and its stereoisomer, quinine, is not specific to the Ca^{2+}-mediated K conductance, and that the drugs do not act on such conductance from the outside of the neuron. Rather, the drugs may affect some mechanism which controls such conductance after penetrating into the neurons.

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SYNAPTIC CURRENT RECORDED IN RED NUCLEUS NEURONS USING NEW VOLTAGE CLAMP TECHNIQUE BY ITERATION. TSUKAHARA,N., MURAKAMI, F., KAWATO,M., ODA, Y. ETOH, M. Dept. Biophys. Eng. Fac. Eng. Sci., Osaka Univ. Toyonaka, Osaka

We have devised a new algorithm of single electrode voltage clamp using iteration method. Using this method, we investigated the time course of postsynaptic current of interposito-rubral synaptic potentials which were produced by somatic synapses. We also tried to measure synaptic current relating to the corticorubral excitatory postsynaptic potentials. In this case, however, EPSPs were produced by synapses located on the distal dendrites in normal red nucleus neurons. After chronic lesions of the nucleus interpositus of the cerebellum, sprouting of corticorubral fibers occurred on the proximal dendrites and somata of the red nucleus neurons. We could successfully point clamp the corticorub ral synaptic current by taking advantage of this sprouting phenomenon. The shortest time to peak of the synaptic current of corticorubral EPSP was 0.35 msec, which was very close to that of the interposito-rubral EPSPs, 0.2-0.4 msec.

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ANALYSIS OF NEURONAL INPUT-OUTPUT RELATIONS BASED ON MEASUREMENT OF THREE DIMENSIONAL DENDRITIC STRUCTURES. Murakami, F., Kawato, M*., Kometani, M*., Hamaguchi, M*. and Tsukahara, N. Dept. Biophys. Eng., Fac. Eng. Sci., Osaka University, Toyonaka, Osaka 560

HRP was injected into red nucleus neurons after the recording of the voltage transients in response to a current step applied to the soma. Three dimensional morphology of the HRP-filled neurons was reconstructed from serial sections with the aid of camera lucida attached to the light microscope. Computer simulations of the voltage transients in response to a current step appllied to the soma were performed using a modified algorithm to obtain the solutions of Butz & Cowans' formula (Butz & Cowan 1974). The specific membrane resistance and the resistivity of the cytoplasm were determined so as to reproduce faithfully the voltage transients recorded in the HRP-filled neuron. Then the synaptic current, which was obtained in the voltage-clamp experiments (see the accompanying paper), was applied to various loci on the soma-dendritic membrane and the voltage response at the soma was calculated. This calculation made it possible to obtain the relation between the shape of the EPSPs and the actual distance of the synaptic sites from the soma.

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RELEASE OF NEUROTRANSMITTER FROM MOSSY FIBERS IN GUINEA PIG HIPPOCAMPUS: QUANTAL ANALYSIS. HIGASHIMA, M., SAWADA, S. AND YAMAMOTO, C. Department of Physiology, Faculty of Medicine, Kanazawa University, Kanazawa 920

Short pulses of glutamate administered iontophoretically to granule cells elicited trains of unitary excitatory postsynaptic potentials (uEPSP) in CA3 neurons in hippocampal slices. The values of quantal content (m) and quantal size (q) were calculated from the mean and variance of the amplitudes of uEPSPs. In a standard solution, the values of m and q were 4.4 ± 1.9 and 0.61 ± 0.17 mV, respectively. In a high calcium solution, these values were 16.0 ± 9.1 and 0.57 ± 0.26 mV, respectively. Results of a few preliminary experiments suggest that the value of m is reduced with no significant changes in the value of q during administration of 50 μM adenosine monophosphate (AMP). These results suggest that the number of quanta of transmitters released from mossy fibers is increased in high calcium concentrations and is decreased at the presence of AMP.

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RELEASE OF ENDOGENOUS GLUTAMATE FROM THE NEWBORNE RAT SPINAL CORD INDUCED BY DORSAL ROOT STIMULATION. TAKEUCHI, A., ONODERA, K. AND KAWAGOE, R. Dept. of Physiol., Sch. of Med., Juntendo Univ., Hongo 113

Hemisected spinal cord of newborne rats (7-8 days old) was fixed in the small bath filled with 150 μl oxygenated saline solution at 22°C and the concentrations of glutamate and aspartate in the perfusate were measured with a gass chromatograph-mass spectrometer. The stimulation was applied to L3-L5 dorsal roots and the ventral root potential (VRP) was recorded from L5 ventral root. The dorsal root stimulation at 10/sec induced a significant increase of glutamate release in a Ca dependent manner but the increase in the aspartate release was small and not significant. Application of substance P (SP) caused irregular potential changes in VRP and increased the release of glutamate and aspartate significantly. The glutamate release induced by SP was suppressed by TTX treatment. These results suggest that glutamate acts as the excitatory transmitter of primary afferents and of interneurones and SP increases the glutamate release pre- or transsynaptically.

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THE AFFERENT NEUROTRANSMITTER IN THE ACOUSTICOLATERALIS RECEPTORS: HPLC ANALYSIS OF PLASMA ACTIVE FACTOR. UMEKITA, S., OKANO, K. and OBARA, S., Dept. of Physiol., Teikyo Univ. Sch. of Med., Tokyo 173

On the basis of bioassay with Plotosus electroreceptors, several biogenic active factors have been reported to induce afferent excitation. The active factors of low M.W. (< 1000) in blood plasma were obtained from Japanese catfish Parasilurus, by a series of ultrafiltration and column chromatography (Sephadex G-10). On the basis of preliminary findings, the active samples were tentatively fractionated into Frs. 1-4. Of these, Fr. 4 showed high specific activity on bioassay with the Plotosus electroreceptors. The activity as well as the effect of natural transmitter were unaffected by JSTX, which blocks the afferent excitation induced by glutamate and its agonists. Fr. 4 also induced afferent excitation in isolated semicircular canal organs. Amino acid analysis of these fractions was performed by aqueous cation exchange HPLC with fluorescence detection of OPA. Fr. 4 contained prominent peaks of Phe (300 μM) and Met (3.3 μM), but not other amino acids such as glutamate or GABA which have been suggested as possible candidates in this system. In addition, however, some unidentified small peaks were observed. A few of them seemed to be identical to those revealed in HPLC analysis of the perfusate samples obtained in the stimulus-induced release experiments on the Plotosus electroreceptors.

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A WAVE IN A NEURON WITH MANY SYNAPSES. OONUKI, M., Dept. Physiol., Sch. Med., Kitasato Univ., Sagamihara 228, Kanagawa

There exists a statistical wave, Ψ exp i (ν t-x/λ), caused by the activation of synapses; seems to be ralated to the current between the synapses and the axonal hillock. Each synapse generates each wave, of frequency $\nu_j = r_j$, wave-length $= (1+\gamma_j h_j)/r_j \gamma_j$. The wave arrives at an axonal hillock after a period of $\tau_j = \gamma_j /(1+\gamma_j h_j)$ and generates the firing of a neuron with frequency of $\Sigma \nu_j / b\theta - \eta/b$. The existence of wave will give the expectation that synapses interfere each other in terms of Ψ, affecting the development.

NOTATIONS

γ_j : time constant of the j-th synapse

a_j : amplitude of a postsynaptic potential due to an activation of the j-th synapse

b_j : $= \exp -1/\gamma_j$

η_j : $= 1 - b_j$

ν_j^x : activation frequency of the j-th synapse

r_j : $= \gamma_j \eta_j a_j \nu_j^x$

R : $= \Sigma r_j$

θ : threshold

p_j : $= r_j / R$

θ_j : $= p_j \theta$

h_j : $= (r_j - \eta_j \theta_j)/b_j \theta_j$

b : $= \Sigma p_j b_j$

η : $= \Sigma p_j \eta_j$

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SYNAPTIC POTENTIALS AND CHEMOSENSITIVITIES OF THE NUCLEUS ACCUMBENS NEURONS OF THE GUINEA-PIG IN VITRO. UCHIMURA, N., HIGASHI, H., YOSHIMURA, M. & NISHI, S., Dept. Physiol. Kurume Univ. Sch. Med., Kurume 830

Intracellular recordings were made from neurons in the nucleus accumbens in tissue slices cut from forebrain of the guinea-pig. Focal stimulation by a monopolar electrode elicited fast e.p.s.p.s and depolarizing i.p.s.p.s which were reversed at about +10 mV and -40 mV, respectively. Glutamate and GABA applied by superfusion caused depolarizations associated with a decrease in membrane resistance. Glutamate-induced depolarization had a reversal potential of about +5 mV and GABA induced depolarization reversed at about -35 mV. During GABA depolarization spontaneous firings were markedly reduced. Both the fast e.p.s.p. and glutamate-induced depolarization were abolished by D-amino adipic acid, a glutamate antagonist. The depolarizing i.p.s.p. was suppressed by bicuculline. These results suggest that the e.p.s.p. and the i.p.s.p. were mediated by glutalate and GABA, respectively. Dopamine caused hyperpolarization in 20 % of the neurons tested, depolarization in 20 % and hyperpolarization followed by depolarization in 40 %. The depolarization was inhibited by haloperidol and sulpiride, while the hyperpolarization was suppressed by haloperidol but not by sulpiride. These data are indicative of that the dopamine-induced hyperpolarization and depolarization were mediated by D1 and D2 receptors, respectively.

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PROPERTIES OF MINIATURE INHIBITORY POSTSYNAPTIC POTENTIALS IN RAT MOTONEURONS.
KOJIMA, H. & TAKAHASHI, T. Dept. Physiol., Kyoto Univ. Fac. Med., Sakyo-ku, Kyoto 606

Spontaneous miniature inhibitory postsynaptic potentials (i.p.s.p.s) were recorded from motoneurons in the isolated spinal cord of the rat in the presence of tetrodotoxin (TTX, 10^{-6}g/ml). The mean amplitude of miniature i.p.s.p.s was little affected by a reduction in external Ca^{2+} concentration. The frequency of miniature i.p.s.p.s was markedly increased in high Ca^{2+} saline, but slightly decreased in Ca^{2+} free (Mg^{2+}) saline. An increase of external K^{+} concentration caused an increase of the miniature i.p.s.p. frequency only when external Ca^{2+} was present. The relation between the K^{+} concentration and the frequency of miniature i.p.s.p.s was highly nonlinear with a maximum slope of about 4.6. An increase of the osmotic pressure by sucrose caused an increase of the frequency, but its effect was apparently less marked compared with that reported for the miniature e.p.p. in the frog. As the temperature was raised, the frequency of miniature i.p.s.p.s increased linearly with a Q_{10} of about 3.0 between 25 and 35°C. These characteristics of the miniature i.p.s.p. are basically the same as those of the miniature e.p.p. It is thus concluded that the miniature i.p.s.p. in motoneurons is produced by the spontaneous release of quantal packages of the inhibitory transmitter.

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CHARACTERIZATION OF MUSCARINIC RESPONSES INDUCED IN *XENOPUS* OOCYTES BY RAT BRAIN mRNA.
SUGIYAMA, H. and HIRONO, C. Lab. Membrane Biol., Natl. Inst. Physiol. Sci., Okazaki 444

Microinjection of mRNA from adult rat brains into *Xenopus* oocytes induced an electrophysiological response to acetylcholine in the cells. The cells responded to acetylcholine through muscarinic receptors which appeared on the cell surface after microinjection of the mRNA and showed the depolarization, mainly due to an increase in membrane permeability to Cl ions. The acetylcholine reaction did not require extracellular Ca ions and was suppressed by pertussis toxin, suggesting the involvement of the inhibitory guanine nucleotide-binding regulatory protein.

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Effects of the agonists of the amines on the identifiable giant neurones of an African giant snail (Achatina fulica Ferussac).
KU, B. S. and TAKEUCHI, H. Dept. Physiol., Sch. Med., Gifu Univ., Tsukasa-machi 40, Gifu.

The effects of twenty-three amine agonists, including mescaline, neosynephrine, ergometrine, methylergometrine, ethylnorepinephrine and tolazoline, on the following eight identifiable giant neurones of Achatina fulica were examined: (1) the five catecholamine-sensitive neurones (CA-s Ns) (PON, TAN, l-VMN, d-RPeAN and VIN); and (2) the three monophenolamine-sensitive neurones (MA-s Ns) (FAN, d-RPeLN and d-LCDN). Mescaline had the marked agonistic effects on the two CA-s Ns (PON and VIN). Neosynephrine showed the marked agonistic effects on the three CA-s Ns (l-VMN, d-RPeAN and VIN) and a MA-s N (d-LCDN). Ergometrine and methylergometrine had the marked effects on the four CA-s Ns (PON, TAN, l-VMN and VIN), but no effect on the MA-s Ns. Ethylnorepinephrine presented the marked effects on the two MA-s Ns (d-RPeLN and d-LCDN), but no effect on the CA-s Ns. Tolazoline showed the marked effects on a MA-s N (d-LCDN).

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Effects of putative neurotransmitters on the giant neurones identified in the pedal ganglia of an African giant snail (Achatina fulica Férussac). BOYLES, H. P. and TAKEUCHI, H. Dept. Physiol., Sch. Med., Gifu Univ., Tsukasa-machi 40, Gifu,

The three giant neurones, d-LPeLN, d-LPeCN and d-RPeAN, were identified on the dorsal surface of the pedal ganglia, and the sensitivities of these neurones to the main putative neurotransmitters and their derivatives were examined. d-LPeLN (dorsal-left pedal large neurone), about 250 um in diameter, was found near the left cerebro-pedal connective. The neurone was excited by octopamine (OA), 5-hydroxytryptamine (5-HT), histamine (HA) and inhibited by dopamine (DA), GABA, '-hydroxy-L-glutamic acid (L-BHGA) and acetylcholine (Ach). d-LPeCN (dorsal-left pedal constantly firing neurone), 150 um in diameter, was situated near the midline. The neurone was excited by DA, 5-HT, and inhibited by GABA and Ach. d-RPeAN (dorsal-right pedal autoactive neurone), 150 um in diameter, was located near the right cerebro-pedal connective. The neurone was excited by 5-HT and GABA, and inhibited by DA, OA, L-BHGA and Ach.

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Putative neurotransmitters of giant neurones of an African giant snail (Achatina fulica Férussac). TAKEUCHI, H., *KU, B. S., WATANABE, K., *MATSUOKA, T., *ISOBE, K., *BOYLES, H. P., FUNASE, K. and YAMAMOTO, N. Dept. Physiol., Sch. Med., Gifu Univ., Tsukasa-machi 40, Gifu.

The twenty-two giant neurones have been identified in the ganglia of an African giant snail (Achatina fulica Férussac) up to the present time in our laboratory. The sensitivities of these neurones to the main putative neurotransmitters, such as dopamine (DA), L-octopamine (L-OA), 5-hydroxytryptamine (5-HT), GABA, '-hydroxy-L-glutamic acid (L-BHGA), histamine (HA) and acetylcholine (Ach), were also tested. Among the neurones examined, the five ones were excited and the twelve were inhibited by DA; the five were excited and the other five were inhibited by L-OA; the sixteen were excited and only one was inhibited by 5-HT; the five were excited and the seven were inhibited by GABA; the six were excited and the seven were inhibited by L-BHGA; the seven were excited and the three were inhibited by HA; and the seven were excited and the three were inhibited by Ach.

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Pharmachological characteristics of newly identified giant neurones in the suboesophageal ganglia of an African giant snail (Achatina fulica Férussac). MATSUOKA, T., GOTO, T. and TAKEUCHI, H. Dept. Physiol., Sch. Med., Gifu Univ., Tsukasa-machi 40, Gifu 500.

Twenty-two neuroes have been previously identified in the ganglia of Achatina fulica, and their pharmacological characteristics have been also examined. In the present study, the two more neurones, which were located near TAN (tonically autoactive neurone), were newly identified. They were called TAN-2 and TAN-3, since their features were similar to those of TAN. The size and firing pattern of the three neurones, TAN, TAN-2 and TAN-3, were identical; their sensitivities to the putative neurotransmitters were identical too, showing that 5-hydroxytryptamine and histamine produced their excitations, and dopamine, L-epinephrine, GABA and acetylcholine caused the inhibitions. The axonal pathways of the three neurones, studied by the intracellular injection of Lucifer yellow, were also identical, together projecting the branches into the right and the left anterior pallial, the right and the left posterior pallial, the intestinal and the anal nerves.

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EFFECTS OF TONICITY ON THE DECLINE IN MINIATURE END-PLATE POTENTIAL FREQUENCY FOLLOWING A TETANUS. NARITA, K. AND KITA, H. Dept. of Physiol., Kawasaki Med. Sch., Kurashiki, Okayama 701-01

Miniature end-plate potentials (MEPPs) were recorded intracellularly from a sciatic nerve-sartorius muscle preparation of the frog before, during and after tetanic stimulation (50 Hz, 2-3 min) of the nerve. The time course for the decline in MEPP frequency, F, after a tetanus can be expressed by the equation, $F = F_0 + F_1 \cdot \exp(-t/\tau_1) + F_2 \cdot \exp(-t/\tau_2)$, where t is the time, τ_1 and τ_2 are the time constants, F_1 and F_2 are the frequencies at t=0 associated with the first and second components of the decay and F_0 is the frequency before the tetanus. Since F_1 was about 100 times larger than F_2 in most cases and, therefore, F was determined mainly by τ_1, the value for τ_1 was examined in the following solutions. (1) In solutions made with no added Ca^{2+} but containing 1 mM MgEGTA and 25-100 mM sucrose, there were no major changes in the value for τ_1 with increases in tonicity. (2) In the same solutions containing 2-3 mM caffeine in place of sucrose, τ_1 increased as the concentration of caffeine was raised, showing that the recovery was delayed. (3) In solutions made without EGTA but containing 4-10 mM Mg^{2+} and 0.05-0.2 mM Ca^{2+}, the values for τ_1 were smaller at higher concentrations of Ca^{2+}, indicating that extracellular Ca^{2+} accelerated the decay.

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ACTIVATION OF THE ELECTROGENIC Na-PUMP IN BULLFROG ATRIUM MUSCLE FIBRES BY ACh. HASUO,H., MINOTA,S.# and KOKETSU,K. Dept. Physiol., Kurume Univ. Sch. Med., Kurume and Dept. Physiol., Saga Med. Sch., Saga#.

The membrane of cardiac muscle fibre is hyperpolarized by the action of acetylcholine (ACh). We had previously reported that the ACh-induced current (ACh current) recorded in the K-free solution was greatly reduced by ouabain. In the present experiment, the nature of ACh current was further studied and compared with that of the K-activated current which was induced by an activation of the electrogenic Na-pump. The membrane conductance increased during the generation of ACh current. The ACh current was completely blocked by either atropine or Ba^{2+} while the K-activated current was not. The amplitude of ACh current decreased when the membrane was hyperpolarized. The K-activated current was suppressed during an initiation of the ACh current. These results suggest that an increase in K-permeability by ACh increases a concentration of K immediately outside of the membrane and this increase of K stimulates the Na-pump. The contribution of the electrogenic Na-pump to ACh current was next examined in the K-containing solution. The Na-pump component of the ACh current was decreased by an increase in the external K^+; the proportions of the pump current to the ACh current in the 0.5, 1 and 2 mM K solutions were 54, 34 and 14 %, respectively. This suggests that ACh can activate the electrogenic Na-pump even under the physiological condition.

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BARIUM INACTIVATES K-CURRENT IN GUINEA-PIG MYENTERIC NEURONS. TOKIMASA, T., ARIYOSHI, M. AND KOKETSU, K., Department of Physiology, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830, Japan

The sample-and-hold/voltage-clamp techniques were applied for myenteric plexus neurons of the guinea-pig ileum. Barium (1 - 10 mM) caused an inward shift of the holding current at -60 mV which was not blocked by tetrodotoxin (300 nM), hexamethonium (200 μM) or hyoscine (1 μM). Barium mimicked the muscarinic slow synaptic current (North & Tokimasa, J. Physiol., 333: 151, 1982) when it was applied directly onto the cell membrane by brief (30 ms - 1 s) pressure ejection from the pipettes which contained $BaCl_2$ (4 mM). Barium induced inward current was associated with a marked reduction in membrane conductance and the estimated reversal potential was about -90 mV. It was largely due to an inactivation of a K-current which does not have any of the same characteristics as that fully described elsewhere (Adams et al., J. Physiol., 330: 537, 1982) and known as the M-current. Barium induced inward current was blocked by Co (1 - 3 mM) suggesting that barium penetrates the cell membrane through Ca-channels and thereby inactivates K-channels from the inner surface of the membrane. Barium also depressed Ca-activated K-current in a dose-dependent fashion.

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SLOW HYPERPOLARIZING SYNAPTIC POTENTIAL IN NEURONES OF CAT VESICAL PARASYMPATHETIC GANGLIA. AKASU, T. & SHINNICK-GALLAGHER, P.*, Dept. Physiol. Kurume Univ. Sch. Med., Kurume 830, Japan, *Dept. Pharmacol, & Toxicol., Univ. Texas Med. Branch, at Galveston, Galveston, Texas 77550, U.S.A.

It has been suggested that purines, adenosine and ATP function as neurotransmitters in the central and peripheral nervous system. Burnstock (1978) has proposed that purinergic receptors be classified into two agonist prototypes, P_1 and P_2. The presence of a non-cholinergic, non-adrenergic nerve supply to the urinary bladder has been postulated and termed purinergic. We report the presence of a slow hyperpolarizing synaptic potential (slow h.s.p.) in neurones of cat vesical parasympathetic ganglia, produced by stimulating the preganglionic nerves, and provide evidence that the slow h.s.p. is mediated by adenosine. Caffeine blocked the slow h.s.p. and adenosine-induced hyperpolarization. Adenosine deaminase depressed the amplitude of slow h.s.p. The slow h.s.p. was blocked after desensitization of P_1 purinoceptor produced prolonged application of adenosine. The ionic mechanism of the slow h.s.p. was similar to that of adenosine hyperpolarization. The action potentials observed in spontaneously firing neurones were blocked during slow h.s.p. It was suggested that adenosine may mediate inhibitory postsynaptic potential, slow h.s.p., and control the function of urinary bladder.

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EFFECT OF TAURINE ON THE SYNAPTIC TRANSMISSION OF THE BULLFROG LUMBAR SYMPATHETIC GANGLIA. TOSAKA, T., HASHIGUCHI, T., SAKAI, S. and TASAKA, J. Dept. of Physiol. Tokyo Med. Coll., Shinjuku-ku, Tokyo 160

Taurine (2-aminoethanesulfonic acid), which has been presumed as an inhibitory transmitter or modulator in the vertebrate central nervous system, was found to exist in the bullfrog sympathetic chain (12.35±3.80 SD, nmoles/mg protein, n=5). Both pre- and postganglionic stimulation and high K^+ solution enhanced ^3H-labelled taurine release from the chain. Preganglionic C-stimulation, however, was most effective even when the synaptic transmission was blocked. This suggests that the major part of released ^3H-taurine resulted from the excitation of preganglionic C fibers. Hypotaurine (5 mM) inhibited effectively ^3H-taurine uptake, not affecting the release mechanism. Although Ca^{2+} removal reduced the ^3H-taurine output to some extent, the release mechanism of ^3H-taurine was essentially Ca^{2+} independent. Taurine, at a concentration of 1 to 10 mM, depolarized the postganglionic membrane and inhibited the orthodromic transmission. Taurine may, thus, play a modulatory role in the synaptic transmission of the sympathetic ganglia.

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EFFECTS OF HISTRIONICOTOXIN DERIVATIVES ON ACTION POTENTIALS OF SYMPATHETIC GANGLION CELLS IN THE BULL FROG. OGURA, T. AND WARASHINA, A. Dept. of Biol., Fac. of Sci., Niigata Univ. and Dept. of Physiol., Niigata Univ. Sch. of Med., Niigata, 951

Four kinds of Histrionicotoxin (HTX) derivatives (H_8-, H_{12}-, C_4H_{10}-, C_5H_{10}-HTX; gifted by Dr. Y. Kishi) were applied to sympathetic ganglia in the bull frog to compare their toxin effects on action potentials. H_{12}-HTX (60 μM) and C_5H_{10}-HTX (100 μM) had an effect to reduce the amplitude of action potential accompanied by prolongation of its duration. This suggests that H_{12}-, C_5H_{10}-HTX block both Na and K channels. Furthermore, use-dependent Ca channel-blocking action was also observed with H_{12}-HTX at 100 μM. On the other hand, H_8-HTX (60 μM) showed an obvious inhibition of synaptic transmission. C_4H_{10}-HTX (60 μM) exhibited intermediate effects between H_8- and H_{12}-HTX. These results reveal that all four HTX derivatives inhibit synaptic transmission probably due to blocking Ach receptors, and that they also block Na and K channels of the post synaptic membrane. However, the toxin actions to these sites occur in different degrees with the respective derivatives. These toxin effects were reversed after long-termed washes of toxin-treated ganglia. During washing, partially reversed toxin actions strengthened again as the stimulus frequency was increased. From these, it is inferred that these derivatives have access to their sites from the cytoplasmic side or through lipid parts of the membrane.

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THE MEMBRANE PROPERTIES AND SYNAPTIC TRANSMISSION IN CARDIAC GANGLION CELLS OF THE GUINEA-PIG. IKEDA, K., NISHI, S. Dept. Physiol., Kurume Univ. Sch. Med., Kurume 830

Intracellular stainings and recordings were made from cardiac ganglion cells of the guinea-pig. Many cells appeared to be monopolar, protruding an axon, while a few cells showed several dendrites and an axon. The ganglion cells could be divided into 3 types according to the characteristics of their electrotonic potentials. The electrotonic potentials of type 1 and type 2 showed specific time courses due to generation of 'A' current and 'Q' current, respectively. Noradrenaline (NA) applied by superfusion (100 μM) caused no significant changes in the resting membrane potential, the input resistance and the spike action potential. NA (100 μM) reduced the afterhyperpolarization in both amplitude and duration. Reduction of the extracellular calcium to 1/10 or replacement of calcium with cobalt (2 mM) also reduced the amplitude as well as the duration of afterhyperpolarization. In contrast, 4-aminopyridine reduced only the duration of afterhyperpolarization. These results suggest that the reduction of afterhyperpolarization by NA is probably due to a decrease in both the calcium activated potassium current and 'A'current. The fast EPSP which was elicited by a focal stimulation of the interganglionic nerve fibers was reversibly blocked by cobalt (2 mM) or d-tubocurarine (10 μM). In some cells NA (100 μM) reversibly depressed the fast EPSP.

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THE RELEASE MECHANISM OF ACETYLCHOLINE AND AN LHRH-LIKE PEPTIDE FROM THE SAME PREGANGLIONIC NERVE TERMINALS. INOKUCHI, H., NISHI, S., Dept. Physiol., Kurume Univ. Sch. Med., Kurume 830

The terminals of preganglionic C fibers in bullfrog sympathetic ganglia contain acetylcholine (ACh) as well as a luteinizing hormone-releasing hormone (LHRH)-like peptide. When released by nerve impulses, the ACh mediates the fast (nicotinic) and the slow (muscarinic) EPSPs, and the LHRH-like peptide produces the late slow EPSP in ganglion cells. In an attempt to characterize the mechanism underlying the co-release of ACh and LHRH-like peptide, we have studied the behaviors of the fast and the late slow EPSPs in various experimental conditions. The results were as follows: The late slow EPSP decreased in parallel with the fast EPSP in low Ca/high Mg media. Hemicholinium-3, a typical choline uptake inhibitor, reduced not only the fast EPSP but also the late slow EPSP. Botulinum toxin A, a selective inhibitor of ACh liberation, also depressed both EPSPs, and the degrees of their depression were almost the same. Blockade of presynaptic muscarinic receptors by atropine increased the fast and the slow EPSPs to the same extent. Thus, none of these procedures could not discriminately affect the cholinergic and the peptidergic transmission. The results seem to suggest that the ACh and the LHRH-like peptide might co-exist in the same vesicles or the two substances might be liberated by an inseparable mechanism.

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MODE OF ACTIONS OF GABA ANTAGONISTS. INOUE, M., AKAIKE, N. AND OOMURA, Y. Dept. Physiol., Fac. Med., Kyushu Univ. 60, Fukuoka 812

The mode of actions of GABA antagonists was studied with three different kinds of techniques namely internal perfusion, rapid external solution exchange (concentration-clamp) and patch clamp.

Picrotoxin (PTX) suppressed the maximum GABA response without any change of affinity in a dose-dependent manner. Bicuculline (BIC) produced parallel shift of the GABA dose-response curve to the right. PTX did not affect the activation phase but selectively accelerated the rate of desensitization whereas BIC prolonged the desensitization onset. PTX shortened the open time of GABA-gated single Cl^- channel currents while BIC markedly reduced the number of events.

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ACTION OF A VARIETY OF ANTAGONISTS ON GABA-INDUCED Cl^- CURRENTS. YAKUSHIJI, T., AKAIKE, N. AND OOMURA, Y. Dept. Physiol., Fac. Med., Kyushu Univ. 60, Fukuoka 812

The Cl^- currents in GABA-sensitive neurons of the frog dorsal root ganglion were studied using the suction pipette technique which allows internal perfusion and whole-cell voltage clamp. The Cl^- currents were separated from Na^+, Ca^{2+} and K^+ currents. The mechanisms of action of a variety of antagonists have been analysed. Among the metal ions studied, the order of the blocking efficacy was Zn = Cd>Ni = Cu>Co>Cu^+>Mn. Of the organic antagonists studied, the order of potency was bicuculline>picrotoxin>strychnine>curare>SITS>DIDS>bemegride>penicillin>folic acid>atropine. For both bicuculline and the metal cations the GABA dose-response curves shifted to the right with increasing concentrations but the peak response was unchanged, indicating competitive inhibition. Penicilline action at low concentration appeared to be competitive. The penicillin block was highly voltage dependent. This depression decreased with increasing depolarization. Picrotoxin showed a non-competitive inhibition, as indicated by a shift to the right and a depression of peak current.

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ENHANCEMENT OF GABA-INDUCED Cl^- CURRENTS BY PENTOBARBITAL AND DIAZEPAM. TOKUTOMI, N., AKAIKE, N. AND OOMURA, Y. Dept. Physiol., Fac. Med., Kyushu Univ. 60, Fukuoka 812

Cl^- currents in GABA-sensitive frog sensory neurons were separated from Na^+, Ca^{2+} and K^+ currents by using internal perfusion technique which allows whole-cell voltage clamp. A "concentration-clamp" technique that permits rapid external solution changes within 0.5 msec was employed.

The presence of pentobarbital (PB) shifted the GABA dose-response curve to the left and increased slightly the maximum saturation current whereas diazepam (DZP) shifted the curve to the left without affecting the maximum saturation current. In addition, the facilitatory effect of GABA-induced Cl^- current by PB was several times greater than that of DZP. PB prolonged the fast time constant (τ_{fast}) of desensitization of GABA-induced Cl^- current while DZP shortened it. PB prolonged markedly the open time of GABA-gated single Cl^- channel current recorded with patch clamp technique and shortened the closed time. DZP prolonged both the open and the closed time.

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EFFECTS OF TEMPERATURE AND pH ON THE GABA INDUCED CURRENTS IN CAT SPINAL GANGLION CELLS IN VITRO. HIJIRIKAWA, K., HIGASHI, H., TANAKA, E., NISHI, S. Dept. Physiol., Kurume Univ. Sch. Med., Kurume 830

GABA induces in the ganglion cells an inward current associated with an increased chloride conductance. We found that the amplitude of the GABA-current was reduced by raising temperature from 26°C to 36°C or by changing pH from 7.4 to 8.0 or to 5.3 without affecting voltage dependency and reversal potentials of the current. The mechanisms of these actions on the gating kinetics of GABA-operated chloride channels were studied with the noise analysis methods which made it to estimate the mean life time (τ), the conductance (γ) and open probability (N) of activated single channels. Depression of the GABA-current at a high temperature was accompanied by reductions of the maximum value (I_{max}), the apparent dissociation constant (K_m) of the GABA-current, N and τ. The result suggests that a rise in temperature would increase desensitization of the GABA receptors and the backward rate constant (α) of conformational changes in the ionophore. When extracellular pH was lowered, I_{max} and N were reduced and τ was markedly prolonged. This might imply that hydrogen ions would non-competitively inhibit the GABA receptor-ionophore complex by decreasing the forward rate constant (β) of the comformational change, but enhance γ of the open channels.

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THE EFFECT OF VARIOUS CATIONS AND ANIONS ON GABA-INDUCED CURRENTS IN CAT PRIMARY AFFERENT NEURONS IN VITRO. TANAKA, E., HIJIRIKAWA, K., HIGASHI, H., NISHI, S., Dept. Physiol. Kurume Univ. Sch. Med., Kurume 830.

Fluctuation analysis of the GABA-induced current in neurons of the isolated cat spinal ganglion has been made of estimate the single channel conductance (γ), single channel mean life time (τ), and open channel probability (N) of the GABA-activated chloride channels. Replacement of external Na^+ with Li^+ or addition of nipecotic acid (an inhibitor of glial GABA uptake system) caused an enhancement of the GABA current, which was associated with a decrease in Michaelis constant (Km) and τ. The result suggests that Li^+ and nipecotic acid would increase the effective GABA concentration by blocking the uptake system. In contrast, replacement of Na^+ with choline or Tris decreased the GABA current and increased Km and τ, suggesting that extracellular Na^+ would be necessary for the binding of GABA with its receptors. When extracellular Cl^- was replaced with Br^-, the maximum velocity (Imax) of the GABA current, τ and N were increased and Km was unaffected. This probably means that Br^- would enhance the forward rate constant of conformational changes in the GABA-sensitive ionophore. Replacement of Cl^- with isethionate reduced Imax, γ and τ without affecting Km. This may imply that isethionate would noncompetitively inhibit the GABA receptor and enhance the backward rate constant of the gating process.

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ANALYSIS OF DIFFERENTIATION IN EXCITABLE TISSUES OF THE AMPHIBIAN LARVAE WITH MONOCLONAL ANTIBODIES. MITANI, S.* & OKAMOTO, H. Department of Neurobiology, Institute of Brain Research, Faculty of Medicine, University of Tokyo, Tokyo, Japan.

To study early differentiation of excitable tissues, it is useful to have specific markers for them. One of the best markers known is the monoclonal antibody (mAb) which, with its high degree of specificity and affinity, may allow both qualitative and quantitative analysis of the differentiation process.

We prepared neural and muscular tissue-enriched material from Xenopus laevis larvae, and raised the mAb's against crude particulate fraction from it. When examined on frozen sections of larvae with the aid of indirect immunofluorescent microscopy, three of them are specific for epidermis (E1, 2 and 3), one specific for muscle (Mu1), one specific for both neural and muscular tissues (NM1), one specific for neural crest-derived melanophores (NC1) and one specific for neural tissue (N1). We also determined the embryonic stages when the respective antigens recognized by these mAb's were first detected. They range from st.13 (E1) to st.33/34 (N1). It is suggested that the appearance of the antigens was regulated in tissue- and stage-specific manners.

It was also found, when further examined at various rostro-caudal levels, that the distribution of NM1 and N1 antigens differed at each level and that NM1 antigen existed on the neurites of early matured neurons.

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EFFECT OF THE AFFERENT INPUT ON EPAXIAL TRUNK MUSCLES IN LOCOMOTION OF THE VERTEBRATE. TOKURIKI, M., WADA, N.* Dept. of Vet. Physiol., Fac of Agr. Yamaguchi Univ., Yamaguchi 753, and Dept. of Physiol., Chiba Univ. Sch. Med.,* Chiba 280

During walking in the newt (Triturus pyrrhogaster), the epaxial muscle occurred in the intense activity in the stance phase of a ipsilateral hindlimb and weak one in that of a contralateral hindlimb. The former activity appeared when a epaxial muscle was stretched. In swimming the epaxial muscle showed concentric contraction and co-activation. After deafferentation of 6th to 13th spinal nerve on left side or both sides, the epaxial muscle occurred in the similar activity to that in intact newts. The muscle tone did not also disappear. The epaxial muscle in the lizard (Takydromus tachydromoides) showed the same pattern of activity as that in the newt during walking and swimming before and after deafferentation of 6th to 10th spinal nerve on left side or both sides. The myotome of multifidus, longissimus and iliocostalis in the cat and dog was investigated by the evoked electromyography in order to make clear the effect of deafferentation. The epaxial muscles in cats and dogs which appeared in the aft-stance phase of the ipsilateral hindlimb during intact locomotion decreased or disappeared the activity after ganglionectomy of L1 to L5 dorsal ganglion.

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On accuracy of stepping during locomotion of chronic cats with bilateral spinal hemisections at different levels. KATO,M., HIRAYAMA,H., HIKINO,K.* Dept. Physiol., Hokkaido Univ. Sch. Med., Sapporo 060

Accuracy of stepping during locomotion was investigated in chronic cats with bilateral hemisections at different levels of the spinal cord. Ten cats were hemisected first either at Th_{12} or C_2 on one side and studied for 7 to 90 days. Then the second hemisection was applied at about mid-thoracic level on the contralateral side. The double hemisected cats recovered to stand up and walk 2-3 weeks following the second hemisection. Three intact cats served as control. The cats were encouraged to stand with four legs on force plates in order to measure distribution of floor reaction force (FRF) during quiet standing. For about 7 days following the first hemisection at a lower thoracic level FRF of hindlimb of the operated side decreased to 30-50 % of the normal value, then recovered to control level. During these period the cats were also encouraged to walk on grid (80 cm x 190 cm, made of 50 mm x 50 mm mesh) in order to test accuracy of the stepping. The limb of operated side showed for about 2 weeks deteriorated accuracy of stepping to 10-70 % of the scores of normal cats which showed unfailed landing on the rung of the grid. These results indicate the importance of descending impulses for control of both standing and accurate stepping of the legs.

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REFLEX ACTIONS FROM THE FOOT PADS. HONGO, T., KUDO, N., OGUNI, E. AND YOSHIDA, K.
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Reflex actions evoked from the central and toe pads of the foot on motoneurones innervating various hindlimb muscles were investigated in Nembutal anesthetized cats with or without cord transection at Th_{13}-L_1. Responses to mechanical and electrical stimulation of pads were analysed by means of EMG and intracellular recording from motonruones. Stimulation of the central pad evoked excitatory effects on motoneurones of toe extensors and other muscles as previously described (Engberg, 1964). The excitatory effects on extensors of the II-Vth toes were similarly potent and enough tp evoke discharges. In contrast, reflex effects from the toe pads on toe extensor motoneurones were inhibitory or excitatory, and varied with the topographical relations between the stimulated pad and the digit which the motoneurone innervated. Thus, a motoneurone of a given toe received strongest inhibition from its own toe and weakest inhibition from the farthest toe. Excitatory effects, when present, were strongest from the most distant toe. Effects on most of more proximal muscles were of flexion reflex pattern, i.e. excitation of flexors and inhibition of extensors. It was concluded that stimulation of the central and the toe pads evokes reflexes of highly specific patterns. Functional significance of these skin reflexes is discussed.

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POSTSYNAPTIC POTENTIALS EVOKED BY STIMULATION OF THE VENTRAL ROOT AFFERENTS IN SPINAL MOTONEURONS. KAYANO, F., KANG, Y., ENDO, K. and ARAKI, T. Dept. of Physiol., Fac. of Med., Kyoto Univ., Sakyo-ku, Kyoto 606

The synaptic action of the ventral root afferents on hindlimb motoneurons of the cat anesthetized with sodium pentobarbital was analyzed by intracellular recording. In 69 (31 flexor and 38 extensor motoneurons) out of 78 motoneurons tested, postsynaptic potentials (PSPs) with long latencies (13.5-68.0 msec) were evoked by stimulation of the distal stump of the cut S_1 ventral root. In contrast, stimulation of the proximal stump of the cut S_1 ventral root evoked similar long latency PSPs but of smaller amplitude in only 8 out of 137 motoneurons tested. Stimulation of the distal stump of the cut ventral root produced predominantly EPSPs (22 out of 31 cases) and IPSPs (25 out of 38 cases) in flexor and extensor motoneurons, respectively. These patterns of PSPs in flexor and extensor motoneurons were similar to those observed in the nociceptive flexion reflex. Measurements of the latencies of the PSPs and the thresholds in the responsible afferent fibers showed that PSPs evoked by ventral root stimulation were produced presumably polysynaptically, mainly by the activity of the unmyelinated C-fibers in the ventral root that enter the spinal cord through the dorsal root. It may be postulated that appreciable number of the spinal ganglion cells send axons and/or axon collaterals into the ventral root, thus constituting the ventral root afferents.

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CHRONIC STIMULATION OF THE DORSAL ROOT ENHANCES DENDRITIC ACTIVITIES IN PIG AND RABBIT MOTONEURONS. FUJITA, Y., HARADA, H., KITAMURA, T.* AND MINAMI, S.* Dept. of Physiol., Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113.

Infant pigs (7-10 days old) and adult rabbits were implanted with silver or platinum electrodes in the vicinity of S1 (pigs) or L7 (rabbits) dorsal root. They were stimulated with a train of pulses lasting for 1-1.33 s at 500 or 50 Hz. The time stimulated, excluding the intervals between trains, was 3-4 h a day and 12-32 h in total per individual animal. The effects of the stimulation upon motoneurons were studied in acute experiments under Nembutal anesthesia in comparison with the activities of motoneurons in normal animals. Motoneurons of normal infant pigs exhibited dendritic spikes, while those of rabbits did not. The chronic stimulation enhanced dendritic spikes in pigs and produced dendritic spikes in rabbits. Furthermore, in both animals the chronic stimulation produced a slow wave of 6-12 mV in amplitude and 10-20 ms in duration. The wave had a refractoriness and was blocked by a hyperpolarizing current. The wave was therefore considered as summated dendritic spikes.

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INTRASPINAL TOPOGRAPHIC ANALYSIS OF SEGMENTAL SPINAL EVOKED POTENTIALS.
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This study was aimed to investigate generators of the early components of the segmental spinal evoked potentials elicited by peripheral nerve stimulation. Experiments were performed on anaesthetized cats. Field potentials were recorded from fourty-one locations with a tungsten microelectrode inserted into the spinal cord ipsilateral to the stimulation. Equi-potential topography was obtained, using interpolation between these field potentials at a given latency. N_2 wave of the cord dorsum potential, elicited by a weak intensity of stimulation of the medial gastrocnemius nerve, had negative foci in the ventral horn and in part around the intermediate zone. These topographies seemed to show that the N_2 wave reflects EPSP activities generated monosynaptically in motoneurons and interneurons. N_3 wave of the cord dorsum potential, elicited by a strong intensity of stimulation of the sural nerve, had negative foci around the lateral part of the dorsal horn and in the dorsal column. These results seemed to indicate that the N_3 wave reflects activities of dorsal horn cells and axons in the dorsal column.

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THE EFFECT OF PAG STIMULATION ON THE HETEROSEGMENTALLY EVOKED POSITIVE WAVES IN THE SPINAL CORD.
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The strong electrical shocks to the forepad and hindpad in the rat produce the slow positive waves in the lumbar and cervical spinal cords (heterosegmental slow positive waves), respectively, besides the slow negative (N) and positive (P) segmental cord dorsum potentials. The heterosegmental slow positive (HSP) wave has been shown in this laboratory to be very sensitive to anesthetics and to disappear by spinal cord transection at the level of C_{1-2}, suggesting that the HSP wave is produced in the spinal cord by a descending volley via supraspinal structures.

The effects of periaqueductal gray (PAG) stimulation on the HSP waves were studied in the rat. PAG stimulation suppressed the HSP more profoundly than the segmental N and P waves. The suppressive effect of PAG stimulation on the HSP and the segmental P waves was prolonged more than 150 ms, and, in some instances, more than 1000 ms. There were no significant differences in inhibitory effects of PAG stimulation on cervical and lumbar spinal cord potentials. The strongest inhibitory sites in terms of the spinal cord potentials were frequently found in the dorsal region of PAG. The inhibitory effect of PAG stimulation on the HSP as well as the segmental slow waves were blocked by an inhalation anesthetic, isoflurane.

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EFFECTS OF SOME DRUGS ON THE SELF-MUTILATION OF THE RAT INDUCED BY PERIFERAL NERVE SECTION.
YAMAGUCHI,Y.,YASUMO,W.,NISHIGIRI,A. and ASADA,H. Laboratory of Clinical Physiol.,College of Biomedical Technilogy, Osaka Univ. 1-1 Machikaneyama, Toyonaka, Osaka 560

Self-mutilation of the rat induced by peripheral nerve section is considered as an expression of phantom sensation including pain. In this experiment, forepaw main nerves (the radial,ulnar and median nerves) were sectioned unilaterally and effects of the respective naloxone, pargyline and bradykinin on the mutilation were examined. Daily intraperitoneal injection of each drug was done for three weeks after the section and observation continued for three weeks more. Naloxone(10mg/kg/day), antagonist of morphine, showed no significant effect on the mutilation. The involvement of endogenous morphine like substances was not clear in the mutilation. Pargyline(10 or 20mg/kg/day), monoamine oxydase inhibitor, facilitated the mutilation in the later period of the experiment. The facilitatory effects lasted after the cessation of the injection. The monoamine is suggested to play an important role in the mutilation. Bradykinin(10μg/day), a painful substance, tended to facilitate the mutilation in the early period of the injection.

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RE-EVALUATION OF 'VISCEROTOPIC ORGANIZATION' OF SACRAL SPINAL CORD IN CATS. TAMURA, K. and TAKAHIRA, H. Dept. of Physiol., Tokai Univ., School of Med., Isehara, 259-11

In our previous studies (Tamura et al.; 1983, Takahira et al.; 1984), we found that the sacral ventral roots provided a powerful inhibitory action upon internal anal sphincters and that the variation of anal canal pressure induced afferent discharges of sacral dorsal roots, originated from spindle secondaries in external anal sphincters. Recently, de Groat and his colleagues (1981) proposed a new hypothesis which indicated that the sacral cord was organized 'viscerotopically' in terms of neuron location, conduction velocity of preganglionic fiber, innervated organ and so on. We wished to know in the present experiments how the anal canal-regulating neuron might be related to those bladder- and colon-regulating neurons, described by de Groat et al. Experiments were done on decerebrate cats. The microelectrodes were used for intracellular and extracellular recordings from sacral neurons. We are now at the stage of neuron identification, however, several interesting findings, such as firing mechanism of sphincter motoneurons, afferent convergence upon Renshaw cells and others, were obtained.

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EFFECTS OF LOCAL COMPRESSION ON VARIOUS NEURAL ACTIVITIES OF THE SPINAL CORD. MIYAWAKI, Y., NAKATA, N., AND MATSUURA, S. Dept. of Physiol., Osaka City Univ. Medical School, Abeno-ku, Osaka 545

Local compression of the spinal cord disturbs various neural activities. We recorded monosynaptic reflex produced by stimulating Biceps-semitendinosus nerve or dorsal root (DR) and various kinds of inhibitions to compare effects of compression between the reflex and inhibitory actions in the cat anesthetized with Nembutal. Magnitude of inhibitory actions was estimated by measuring reflex amplitude decreased by antagonistic, recurrent and presynaptic inhibitions due to conditioning activation of Quadriceps nerve, VR and DR. Monosynaptic reflex and antagonistic inhibition were depressed by local compression, but preceded with an augmentation at the early stage. Depressing actions of compression were severer for the latter response than for the former. Recurrent inhibitory actions were more sensitive to compression than antagonistic ones. For presynaptic inhibitory actions and dorsal root reflex, depressing effects of compression were usually more severer than that for monosynaptic reflex and the other inhibition and shown no augmentation. Action potentials of dorsal column tract showed no change, when DR reflex was almost vanished. Inhibitory actions were more vulnerable to compression than the excitatory spinal reflex and conduction of the action potential.

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MONOSYNAPTIC CONVERGENCE PATTERN, POLYSYNAPTIC REFLEXES AND VENTRAL ROOT INPUT IN THE TURTLE HINDLIMB MOTONEURONES. YAMASHITA, M. Dept. Neurophysiol., Brain Res. Inst., Sch. Med., Univ. of Tokyo, Tokyo 113

Monosynaptic convergence pattern was investigated intracellularly from hindlimb motoneurones of the freshwater turtle anaesthetized with pentobarbital sodium. Besides monosynaptic connexion between low threshold muscle afferents and homonymous motoneurones, the monosynaptic connexion was found in any pair of heteronymous muscles in the dorsal muscle group (puboischiofemoralis, ambiens, femorotibialis, iliotibialis, ankle and toe dorsi-flexors) or in the ventral muscle group (flexor tibialis, ankle and toe plantar-flexors). No connexion was observed between antigravity muscles at different joints as revealed in the cat. Stimulation of low threshold muscle afferents evoked IPSPs in the antagonistic motoneurones and in the motoneurones to muscles at different joints. Polysynaptic EPSPs induced from cutaneous nerves were dominant in hip and knee flexors. After stimulation of the ventral roots, small depolarizing potentials were induced in neighbouring motoneurones. Their latencies were too short to be mediated by chemical transmission, suggesting electrical coupling between motoneurones. No recurrent inhibition was observed.

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WGA-HRP STUDY ON PINNA AFFERENT PROJECTIONS WITHIN THE CERVICAL CORD OF CAT

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We previously reported that spinal segmental levels of pinna afferents responsible for scratch reflex ranged from cervical C1 to C3 dorsal root determined by retrograde HRP study. Labeled cells were most numerous in C2 dorsal root ganglion.

In the present study, we further traced the intraspinal projection of the pinna afferents to the spinal dorsal horn by WGA-HRP method and electrophysiological analysis. Animals were anesthetized with Nembutal and WGA-HRP solution (5-10%, 1.5-2.5 μl) was injected to the C2 dorsal root ganglion. After 24-48 hrs survival time, cervical cord frozen sections of 40 μm thickness were cut transversely or longitudinally. Labeled afferent fibers were traced rostrally to the middle part of C1 segment and caudally to the lower end of C3 segment. Axon terminal enlargements were observed in the ipsilateral Rexed's I-III.

In electrophysiological analysis, tungsten microelectrodes were inserted to the C2 dorsal root and dorsal horn. Afferent impulses evoked by tactile stimuli applied to pinna receptive fields for scratching reflex were recorded in C1 spinalized cats.

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EXPERIMENTAL STUDY ON THE RELATIONSHIP BETWEEN CARDIAC ARRHYTHMIAS AND SLEEP STATES
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We previously reported in 5 rats that ambulatory ECG-EEG monitoring system was a good tool for clarifying the relationship between sleep states and occurrences of arrhythmias, and that bradyarrhythmias appeared predominantly during paradoxical sleep.

In this study, such arrhythmogenic properties of paradoxical sleep were certified by analyzing records for 100 days in 15 rats. During the observed period, alertness, slow wave sleep and paradoxical sleep appeared 14640, 15516 and 5194 times, respectively. Bradyarrhythmia incidences in the each sleep stage were 0.14, 0.10 and 14.1%, respectively. How the occurrence of arrhythmias was related to REM episodes : characteristic phasic events of the paradoxical sleep, was also investigated. Horizontal nystagmograms were registered as the EOG. The time constant for EOG recordings was 1.5 sec. As a result, 69.0% of the total episodes of bradyarrhythmias appeared related to REMs, and besides the arrhythmogenesis of REMs increased along with the increase in REM density, which was defined as the frequency of REM episodes during a 10-sec-long period of paradoxical sleep.

These results suggest that REMs are one of the important causes of arrhythmogenesis of paradoxical sleep.

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ROLE OF SLEEP APNEA EPISODES IN THE CIRCADIAN RHYTHM OF CARDIAC ARRHYTHMIAS IN RATS
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We have already reported that 88.1 % of bradyarrhythmia episodes observed in 5 rats were related to apneic episodes.

The purpose of this study is as follows: 1.whether or not, there exists a circadian rhythm in an apnea index (a ratio of the number of apneic episodes to the number of paradoxical sleep in each hour). 2.whether or not, there exist characteristic breathing patterns related to sleep states, which could be an index suggesting an arrhythmogenic sleep state. Records for 32 days in 7 rats were analyzed. As a result, 89.2 % of bradyarrhythmia episodes were observed related to disordered-breathing the same as we previously reported. Circadian periodicity in the apnea-indices was demonstrated. The highest peak of the apnea-index was 8:00 am, which coincided with the highest peak of the circadian rhythm in bradyarrhythmia incidences. Amplitude of respiration records during paradoxical sleep was irregular and, coefficient of variance of respiration intervals during paradoxical sleep was significantly higher compared to during alertness or slow wave sleep. There results suggest that disordered breathing is one of causes of arrhythmogenesis of paradoxical sleep.

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INHIBITORY EFFECTS OF MEDIAL RETICULAR NEURONS IN THE MEDULLA OBLONGATA UPON ALPHA-MOTONEURONS INNERVATING HINDLIMB EXTENSOR MUSCLES.
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In a decerebrate reflex standing cat, and in an immobilized cat, repetitive stimulation (duration; 0.2 ms, frequency; 50 pulses/s, period; 5-10 s) delivered to the dorsal part of the tegmental field in the pons evoked hyperpolarization of membrane potentials in alpha-motoneurons (MNs) innervating hindlimb extensor muscles. In the medulla oblongata, some medial reticular neurons were antidromically activated by the spinal cord stimulation, and were orthodromically activated by the dorsal stimulation. To test whether or not such neurons evoke membrane hyperpolarization, glutamate was applied iontophoretically to the reticular neurons so as to evoke tonic discharges of them. With each spike of the tonic discharges, membrane potentials of the alpha-MNs innervating the soleus muscle and the medial gastrocnemius muscle were averaged. In several of recorded cells (n = 7), hyperpolarizing potentials with various time courses were recorded with latencies in the range between 6.2 to 9.2 ms. Initial component of the membrane hyperpolarization was reversed in its polarity by injecting hyperpolarizing current intrasomatically. These results suggest that a part of the pontine-evoked inhibitory effects is mediated to the alpha-MNs by way of the medial reticular neurons in the medulla oblongata.

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NEURONAL MECHANISMS OF SNEEZE IN SPONTANEOUSLY RESPIRATING DECEREBRATE CATS. NONAKA, S., OHTA, Y., MORI, S. Dept. of Physiol., Asahikawa Med. Coll., Asahikawa 078-11

Sneeze can be evoked by interrupting normal respiratory rhythm. It is characterized by an abrupt increase in the subglottic pressure followed by forceful, active expiration. In decerebrate cats, sneeze can be evoked by natural stimulation delivered to the nasal mucosa, and by microstimulation of a specific area in the medulla oblongata, from where complex field potentials can be evoked by the nasal stimulation. Before and during "sneeze", respiratory and laryngeal muscles changed their activities in a fixed time sequence. In this study, we have attempted to answer a question as to how respiratory neurons change their activities with the evoked sneeze. To do this, respiratory neurons located ventro-medial to the nucleus ambiguus were extracellularly recorded, along with subglottic pressure and EMGs of the respiratory muscles. From the discharge phase of the respiratory neurons, they were identified as inspiratory an expiratory neurons. It was found that the inspiratory, and the expiratory neurons were actively recruited contributing to the abrupt inspiratory phase and to the explosive expiratory phase of the "sneeze". All these results suggest that the specific sneeze-evoking area in the medulla oblongata receive afferents from the nasal mucosa by way of the trigeminal nerve, and this area controls the activities of respiratory neurons so as to arrange their phases of discharges adequate for sneeze.

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SYNAPTIC INPUTS FROM THE BASAL FOREBRAIN AREAS TO THE FACIAL MOTONEURONS IN THE CAT. ASAHARA, T., NISHIMURA, Y. AND TANAKA, T. Dept. of Physiol., Mie Univ. Sch. Med., Tsu, Mie 514

Synaptic potentials in the facial motoneurons(FMNs) of cats, anaesthetized with pentobarbitone sodium(35 mg/kg, I.P.), were recorded on stimulation of the central nucleus of the amygdala(ACe) and the paraventricular nucleus of the hypothalamus(PVH). The EPSPs with latencies of 2.0-5.0 msec(mean 3.7±0.8 msec, 97 cells) were elicited in the FMNs by stimulation of the ipsilateral ACe. Single or double volleys in the contralateral ACe evoked the EPSPs with latencies of 2.0-4.6 msec(mean 3.0±0.7 msec, 16 cells) in the FMNs. Stimulation of the ipsi- and contralateral PVHs also produced exclusively the EPSPs with similar ranges of the latencies to those induced by stimulation of the ACe. These findings show the existence of a di- or polysynaptic excitatory projection from the basal forebrain(ACe and PVH) to the FMNs bilaterally. In order to define an afferent pathway involved in this projection, electrolytic lesions of the parabrachial nucleus(PBN) that is reported to receive afferent fibres from ACe and PVH were performed on several animals. The ACe- or PVH-evoked EPSPs elicited in the FMNs remained unchanged in animals with PBN lesions. Furthermore, additional electrolytic lesions of the central gray matter or the lateral lemniscal zone were ineffective on elimination of an excitatory effect of the forebrain upon the FMNs.

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AUDITORY BRAIN STEM RESPONSES IN SCHIZOPHRENIA. HAYASHIDA, Y., TOMITA, S., HOSOMI, H. Dept. of Systems Physiol., Univ. of Occupational and Environmental Health, Kitakyushu 807 & Dept. of Physiol. Kagawa Med. Sch., Kagawa 706-07

Auditory brain stem responses (ABSR) were examined both in the normal and schizophrenic subjects. The schizophrenics were classified into "non-deteriorated" and "deteriorated" groups through clinical symptoms and Kraepelin performance test. All ABSR waveforms were evoked by the click stimuli delivered binaurally through the headphone in the normal subjects and in the non-deteriorated group of the schizophrenics. Some ABSR waveforms failed and amplitudes of each ABSR waveform were low in the group of schizophrenics with decreased level of the personality. There were no significant differences in latencies of ABSR waveforms between the normal and schizophrenic groups. Characteristics of ABSR waveforms were well related to the clinical symptoms of the schizophrenic illness.

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EFFECT OF RHYTHMICAL SOUND ON EXCITABILITY OF THE BRAIN STEM RETICULAR FORMATION. NAITO, H., TAKEMURA, K., TAKEDA, N., YAMAMOTO, T., HIRAI, T. and YASUHARA, M. Dept. of Physiol., Kansai Med. Univ., Osaka 570

As a part of the study to elucidate the influence of rhythmical sound on the excitability of the brain stem reticular formation(RF), the effect on the recruiting response was investigated in the rabbit. The recruiting response in the cerebral cortex EEG, induced by 8 Hz stimulation of the nucleus ventralis anterior(VA), was facilitated by the rhythmical sound of 60/min beat frequency and 500 Hz pitch. The recruiting response was inhibited by the rhythmical sound of 450/min and 1000 Hz. Increase in the threshold of the arousal reaction and evoked muscular discharge due to stimulation of the RF following sonic stimulation of 60/min and 500 Hz, and decrease in the threshold following sonic stimulation of 450/min and 1000 Hz, were abolished by destruction of both the VA. These results suggest that the decrease in excitability of the RF by rhythmical sound of a low frequency and low pitch and the increase in excitability of the RF by rhythmical sound of a high frequency and high pitch can be attributed to a rise in indirect inhibition and a decrease in indirect inhibition of the RF, respectively, via the diffuse thalamocortical projection system.

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CIRCLING BEHAVIOR OF THE RAT WITH LESION OF NIGROSTRIATAL DOPAMINE PATHWAY AND RECOVERY BY TRANSPLANTATION OF FETAL DOPAMINE CELLS. NISHINO, H., ONO, T., TAKAHASHI, J., FUKUDA, M., KIMURA, M.*, SHIOSAKA, S.**, AND TOHYAMA, M.** Dept. of Physiol. and Anat.*, Toyama Med. Pharmaceu. Univ., Toyama 930-01 and Dept. of Neuroanat., Inst. Higher Nerv. Activ., Osaka Univ., Osaka 530**

Systemic injection of Met-amphetamine (3-5 mg/kg) induced ipsilateral circling behavior (10-20 turns/min) in the rat with unilateral lesion of nigrostriatal dopamine pathway. Caudate dopamine content in the lesioned side decreased to less than 10% of normal. Transplantation of fetal midbrain dopamine cells in the lesioned caudate nucleus improved the behavioral imbalance, and circling behavior disappeared in the fourth week after transplantation. In this state, the dopamine content recovered up to 20% of normal. Tyrosine hydroxylase positive cells stained by an immunohistochemical (PAP) method were distributed inside of the lesioned caudate nucleus. If the cells were located in far rostral or far caudal the behavioral improvement was poor, but if they were located in the head of the caudate nucleus, especially in the medial half (near the lateral ventricle) recovery was prominent and the animal often turned in the opposite direction. Data suggest that location of the transplantation is a critical factor in restoring motor imbalance.

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THE ROLE OF RHYTHMICAL INHIBITORY INPUTS TO JAW-CLOSER MOTONEURONS IN THE CORTICALLY INDUCED MASTICATORY RHYTHM IN CATS. ENOMOTO,S., SUNADA,T., HIROSE,Y.* AND NAKAMURA,Y. Depts. Physiol. & Dent. Anesth.*, Fac. Dent., Tokyo Med. Dent. Univ., Tokyo 113

The masticatory rhythm in the jaw-closer motoneurons (JCMN) induced by repetitive stimulation of the cortical masticatory area (CMA) consists of a rhythmical alternation of EPSP and IPSP phases. We studied the role of rhythmical inhibition of JCMNs in the CMA-induced masticatory rhythm by injection of tetanus toxin (TT) unilaterally into the cat's masseter muscle (MASS) that completely blocked the IPSPs in JCMNs evoked by stimulation of the inferior alveolar nerve. In chronic animals, the CMA-induced rhythmical jaw movement (RJM) persisted after TT injection with no apparent changes in the cycle time, though the jaw opening was reduced. There was no change in the rhythmical digastric EMG activity, but a tonic EMG activity was induced in the TT-injected MASS which was superimposed by a rhythmical increase coincident with the opening movement. In the acute encephale isole, the TT-injected MASS showed a rather steady activity accompanied by no rhythmical fluctuations during the CMA-induced RJM. The results have shown that the rhythmical IPSP of JCMNs plays a role to counteract the excitatory effects on JCMNs of spindle afferents from jaw closing muscles during the opening movement in mastication. It is also suggested that the rhythmical interruption of tonic excitation by IPSPs may be involved in the CMA-induced masticatory rhythm in JCMNs.

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POSTNATAL DEVELOPMENT OF SYNAPTIC TRANSMISSION OF TRIGEMINAL MOTONEURONS IN RATS.
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We reported that the nuclear delay in the trigeminal motor nucleus (TMN) from the spinal trigeminal nucleus (STN) neuron to the anterior digastric motoneuron (DMN) decreased in parallel with a remarkable reduction of the latency of jaw opening reflex during the 2nd postnatal week in rats (Iriki et al., Jap. J. Oral Biol., 25, Suppl.:43, 1983). To study the mechanisms underlying this decrease, we compared intracellular monosynaptic responses of DMNs to STN stimulation between 6 days-old infants (Inf) and adults (Adt). Between 10 and 17 DMNs (resting potential greater than -55 mV) recorded from Inf and Adt, respectively, (1) there was no difference ($p>0.05$) in duration of the terminal potential (TP) recorded in the DMN pool (0.25±0.02 ms, n=9 in Inf; 0.25±0.02 ms, n=8 in Adt), (2) the synaptic delay from the positive peak of TP to the onset of EPSP in DMNs was not different (0.42±0.04 ms in Inf; 0.37±0.03 ms in Adt), and (3) the latency of spikes from the EPSP onset was longer ($p<0.05$) in Inf (5.90±0.92 ms) than in Adt (2.16±0.40 ms). The synaptic transmission from the mesencephalic neuron to the masseter motoneuron showed essentially the same pattern of development as in DMNs. We concluded that the reduction of the TMN nuclear delay in jaw opening and closing reflexes is due to decrease in the spike latency from the EPSP onset in trigeminal motoneurons.

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SALIVARY REFLEX INDUCED BY MECHANICAL STIMULATION OF THE ORAL REGION IN RATS.
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The neural mechanisms of the reflex salivary secretion induced by taste, mechanical and thermal stimulation was analyzed in rats. The pinching with small forceps, the taste stimuli (1M NaCl) and the thermal stimuli (45-55°C) of the tongue vigorously induced submandibular salivary secretion. The salivary secretion by the pinching and the thermal stimuli was disappeared after the lesion of the trigeminal subnucleus caudalis, while the salivary secretion by the taste stimuli was not affected by this lesion. Through functional single fiber analysis, the preganglionic parasympathetic fibers (secretory fibers) innervating the submandibular and sublingual glands were classified into two types; the fibers activated by the taste stimuli (taste-related fibers) and the fibers activated by the pinching of the oral region (pinch-related fibers). The reflex discharges of the pinch-related fibers was impaired by the lesion of the trigeminal subnucleus caudalis. However, the reflex discharges of the taste-related fibers was not affected by the lesion. The taste afferents are conducted via chorda tympani, and the mechanical and thermal afferents are mainly conducted via lingual nerve proper. There is a possibility that the reflex arc of the salivation by the mechanical stimulation is independent of the gustatory-salivary reflex arc.

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THE ANALYSIS OF CENTRAL PATHWAYS ON TRIGEMINO-SALIVARY REFLEX IN THE CAT.
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This study was undertaken to define the central pathways of the trigemino-salivary reflex in urethane-chloralose anesthetized cat. A total of 26 superior salivatory neurons (SS neurons) was tested to the stimulation of trigeminal main sensory nucleus (MS), trigeminal subnucleus caudalis (CA) and three trigeminal sensory branches;lingual, infraorbital and inferior alveolar nerve. Nineteen neurons (73%) were activated by the stimulation of MS with latency range of 1.6-19.0 msec, and eighteen neurons by the stimulation of CA with latency range of 6.0-19.0 msec. Most of reflex responses due to MS and CA stimulation would be activated polysynaptically. Fourteen neurons (54%) received excitatory inputs from MS,CA and any of trigeminal branches. Eight neurons (30%) were activated from any of trigeminal branches and either MS or CA. Remaining neurons were excited by any of trgeminal branches or only MS. This would suggest that MS and CA play a important role as the relay center in trigemino-salivary reflex.

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THE AUDITORY 40Hz ERP(EVENT RELATED POTENTIAL): ELECTROPHYSIOLOGICAL STUDY IN RABBIT.
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The auditory 40Hz ERP (Galambos et al, 1981) is the summed responses of auditory middle latency response (MLR), which are induced by auditory stimulation at a rate of 40/sec. We studied rabbits to investigate the origin of the potential. The auditory stimuli consisted of 500 Hz tone burst, 3 msec duration, 2 msec rise and decay, 85 dB HL intensity, and were presented binaurally via headphones. Standard electrodes were placed on the bregma and lambda as active electrodes, and on an earlobe as a reference. Responses were filtered with bandpass of 15 - 100 Hz, and 1000 responses were averaged. Intravenous administration of barbiturate gradually decreased the amplitude of the potential, while both chlorpromazine and diazepam had little effect. Bilateral electric lesions in the inferior colliculus changed the wave form of this potential, but lesions in the medial geniculate had no effect. Brainstem transection at precollicular level only reduced the amplitude of the potential, though the transection at postcollicular level resulted in a change of wave form. Recordings with deep electrodes inserted in the brainstem suggested that the auditory 40Hz ERP had multiple origins, such as superior olive and inferior colliculus. Medial geniculate seemed to have no role, but some other higher centers might have connections with the potential.

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THE OTOLITH ORGANS AND THEIR INFLUENCE ON EYE MOVEMENTS IN THE CAT.
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In 25 adult cats the left utricular and saccular maculae were stimulated systematically by means of the micro-stimulation technique, using a glass micropipette of 20-25um in tip diameter, filled with 3M NaCl-agar, through which the electric pulse train at 100Hz was delivered monopolarly with a constant current isolator. Movements of the two eyes were photographed on a film as a Strobo photo superposed at a certain interval. The results were compared with those from the semicircular canals(SC) in each animal. Influences of the anesthesia(Ketalar or Nembutal) were compared with non-anesthetized preparations(spinalized between C1-C2). In light anesthesia as well as in spinalized, but asleep, condition, the SC provoked linear shifts in the direction proper to each nerve stimulated, the movements being coordinate. In contrast, the utricule provoked incoordinate movements, counterclockwise rotation in the right eye and linear shifts toward right side in the left eye. The utricule showed no regional difference in the induced eye movements. In awakefulness, the utricular stimulation also elicited coordinate eye movements and showed regional characteristics. The saccule provoked similar results as the utricule, mainly when stimulated in its anterior part or striola but its posterior part provoked no eye movements. It was suggested that the maculo-ocular reflex arc consists of more complex integration circuits than the SC system.

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RELATION BETWEEN SPONTANEOUS ACTIVITY OF MEDULLA OBLONGATA AND SYMPATHETIC RESPONSE INDUCED BY STIMULTION IN VESTIBULAR NERVE.
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Single neuronal activity were extracellulary recorded by glass microelectrodes in the medulla oblongata under chloralose anesthetized mioblock immobilaized cats and observed by stimulaton of vestibular nerve (V) and cutaneous superficial peroneal nerve (P). Vestibular nerve stimulation strength was used 2 times threshold values of nystagmus. When vestibular nerve-conditioning and cutaneous nerve-testing stimulation were used, the test response initially recovered at 250 ms intervals. Weak stimulation was applied at the unit activity recording point in the reticular formation (RF). Blood pressure was depressed, and reflex potential on the renal nerve was simultaneously recorded in a similar manner by cutaneous or vestibular stimulation. RF-conditioning and V-testing, RF-conditioning and P-testing stimulation were tested on the renal nerve potential, the recovery potential of the test respone appeared at 250 ms intervals of conditioning-testing stimlulation. And also pulsation related neurons were found in the reticular formation 1-5 mm rostral to the obex, and RF stimlulaiton in this region were usulally observed depressor response.

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PROPERTIES OF PHOTICALLY EVOKED LID POTENTIAL CHANGES IN RABBITS, ESPECIALLY RELATED TO THE INTENSITY OF FLASH LIGHT STIMULATIONS. TAKEO,T., OZAKI,T., SUGA,S., NIKARA,T.*, IGARASHI,K.**, NAKA,S.**, SASAKI,D.*** Dept. of Physiol., Hirosaki Univ. Sch. of Med., Lab. of Physiol., Sch. allied med. sci., Hirosaki Univ.*, Dept. of Pediat.** and Dept. of Internal Medicine,*** Hirosaki Univ. Sch. of Med., Hirosaki 036

The average photically evoked lid potential changes obtained in healthy adults are known to consist of electromyographic(EMG), electroretinographic(ERG) and electrooculographic (EOG) components. In the present study, the physiological properties of the average photically evoked lid potential changes were studied polygraphically from the standpoint of the stimulus strength in rabbits. The photically evoked lid potential changes were obtained with the summation technique. The intensity of a flash stimulus was changed by electric energy delivered to a Xenon gas stroboscope in several grades ranging from 0.05 to 10 or 20 Joule. In the low intensity of 0.05 J, the late slow components appeared evidently, while the EMG and ERG components were not observed. In the moderate and high intensity, EMG and ERG components appeared and increased according to the intensity strengthened, although the late slow components were not so increased and shortened in amplitude and peak latency, respectively. From the above results, it was suggested that each component of the photically evoked lid potential changes may be differentiated by changing the intensity of the stimulation.

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PHOTICALLY EVOKED LID POTENTIAL CHANGES IN RABBIT, ESPECIALLY RELATED TO THE POSITION OF THE SURFACE ELECTRODES PLACED ON THE EYELID. HOSHINA,Y., OZAKI,T., MIYASHIGE,M., YOZA,H., TANNO,T. Dept. of Physiol., Hirosaki Univ. Sch. of Med., Hirosaki 036

In the present report the photically evoked lid potential changes were studied polygraphically from the standpoint of the lead. The average photically evoked lid potential changes led off from the middle part of the superior lid were observed to be composed of the EMG, ERG and slow components in monopolar leads with indifferent electrode on the auricle, whereas the late component was decreased considerably or disappeared with indifferent electrode on the nose. In the lid potential changes led off unipolarly by placing one indifferent electrode on the auricle and the other exploring electrodes on different parts of the eyelid, each component of lid potential changes showed almost the same pattern in every position of the lead. In the bipolar leads with both exploring electrodes placed over different parts of the superior and inferior lids, the late slow components disappeared and the ERG with fast components were decreased considerably in amplitude as compared with those obtained by unipolar leads. The component with peak latency of about 70 msec following the ERG components was decreased or disappeared in the atropinized or occluded eyes. The above results suggest that photically evoked lid potential changes as the indicator of blinking should be led off polygraphically in rabbits.

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PROJECTION OF EXPERIMENTALLY PRODUCED INFRAORBITAL NEUROMA TO CAUDAL MEDULLA OBLONGATA. KOYAMA, N. and YOKOTA, T., Dept. of Physiol., Med. Coll. of Shiga, Otsu 520-21

In the urethane-chloralose anesthetized cat, the medulla oblongata caudal to the obex was explored for units responsive to mechanical distortion of an experimentally produced neuroma in the infraorbital nerve. Two weeks after the preliminary surgery to produce the neuroma, units responisve to tapping over the neuroma were found in the superficial layers of the trigeminal subnucleus caudalis, the lateral part of the subnucleus reticularis dorsalis and the subnucleus reticularis ventralis. In the normal cat, these areas contain trigeminal nociceptive units. Seven weeks after the preliminary surgery, units responsive to mechanical distortion of the neuroma were also found in the magnocellular layer of the trigeminal subnucleus caudalis. In the normal cat, this layer contains trigeminal low threshold mechanoreceptive units. These results suggest that 2 week after the preliminary surgery, nerve impulses from the neuroma are primarily carried by small nerve fibers including nociceptive fibers, whereas 7 weeks after the preliminary surgery, nerve impulses are also carried by large nerve fibers.

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DIRECT EXCITATORY PROJECTION OF NEURONS IN THE MEDIAL MIDBRAIN TEGMENTUM TO THE MEDIAL RECTUS SUBDIVISION OF THE CAT OCULOMOTOR NUCLEUS.
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Neurons activated antidromically by microstimulation (less than 30 μA) within the medial rectus (MR) subdivision of the oculomotor nucleus were explored in the medial part of the midbrain tegmentum of anesthetized cats. Most of these neurons were found histologically in the reticular formation ventrolateral or caudal to the interstitial nucleus of Cajal, in the periaqueductal gray close to the Edinger-Westphal nucleus and in the area ventral to the MLF. Systematic tracking with the stimulating microelectrode, performed on some of the neurons in the periaqueductal gray, revealed that lower threshold sites for antidromic activation were scattered within the MR subdivision, in the ventralmost part of the periaqueductal gray and in or outside the oculomotor nerve, suggesting axonal branching of the neurons in these effective areas. Furthermore, monosynaptic EPSPs could be induced in MR motoneurons from the reticular formation and periaqueductal gray. These results suggest that excitatory neurons projecting to MR motoneurons exist at least in these two areas.

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NEURONAL ACTIVITY IN THE LOCUS COERULEUS AND ITS ADJACENT AREAS DURING FEEDING BEHAVIOR IN THE MONKEY.
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Extracellular single neuron activity was recorded during operant feeding behavior in and near the locus coeruleus of the monkey. Neurons analysed may include those in the locus coeruleus as well as in the medial parabrachial nucleus and the lateral part of the pontine central gray. The paradigm was a high fixed ratio (FR 20) bar press task: 1) a cue light signaled a monkey to start bar presses, 2) on the 20th bar press, a cue buzzer signaled delivery of food, and 3) the monkey took and ate food. Of 21 neurons analysed, 16 (76%) increased firing rate responding to a cue light and delivery of food. Eleven cells (52%) increased firing rate after a cue buzzer. During the bar press period, 6 cells (29%) decreased, but no cells increased, firing rate. After the monkey put food into the mouth, firing rate was increased in 6 cells (29%) and decreased in 2 cells (9%). Thus, many neurons in this region responded to visual or auditory stimuli, but we could not find any cells showing firing increase during food acquisition (bar press period).

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NEURONAL RESPONSES IN AMYGDALA DURING OPERANT REWARDING STIMULI OR AVOIDANCE BEHAVIOR IN THE RAT.
MURAMOTO, K., ONO, T., SHIBATA, R., NISHINO, H. and FUKUDA, M. Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani,Toyama 930-01.

Single unit activity was recorded from the amygdala (AM) of conscious rats during a tone discrimination operant task. The rat was fixed stereotaxically, but permitted to lick a spout automatically pushed close to the mouth. In one group of rats, licking caused either a drop of juice to be released from the spout, or intracranial self-stimulation (ICSS) in the posterial lateral hypothalamus or ventral tegmental area. In another group, licking caused either ICSS or avoidance of electric footshock. Each discriminative cue tone was presented at random for 2.0 sec prior to each task. In the basolateral nuclear AM, most neurons responded to one or both rewarding stimuli, and half responded to a tone that indicated reward. Responses to both rewards were usually in the same direction, and most were excitation. In the corticomedial nucleus of the AM, although few neurons responded, excitatory or inhibitory responses to both tone and reward were observed. In the avoidance task, when the rat avoided the electric shock by licking behavior after a cue tone, neuronal responses continued from cue tone to licking behavior, but when the rat did not avoid the electric shock, no response was observed. The data suggest that AM neurons might be important in reward related learning and maintenance of avoidance behavior.

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ACTIVITY IN MONKEY LATERAL HYPOTHALAMUS (LHA) DURING OPERANT FEEDING: MODULATION BY CATECHOLAMINES AND OPIATE. OOMURA, Y., NISHINO, H., AOU, S. AND SALAMAN, C.R.P. Department of Biological Control System, National Institute for Physiological Sciences, Okazaki 444, Japan.

Correlation between single LHA neuron activity changes and bar press feeding, and modulation by electrophoretic catecholamines and an opiate were investigated. The paradigm was: i) Cue light to start bar press. ii) Bar press (fixed ratio, FR, 30). iii) Cue tone and food reward at end of bar press. iv) Taking and ingesting food. Of 171 neurons tested, 94 (55%) changed activity in one or more phases. Of these 94, 15 (16%) responded at cue light, 73 (78%) during bar press, 17 (18%) at cue tone, and 48 (51%) during reward. Bar press responses increased in trials for more palatable food, and decreased during no reward (extinction) or satiation. Dopamine (DA) excited 18%, and inhibited 26%. Phase i affected more DA sensitive than DA insensitive neurons. DA antagonist (spiperone) blocked cue light and bar press initiation responses. Noradrenaline (NA) and morphine inhibited about ½ of neurons tested. NA sensitive neurons responded more, usually phase iii, than NA insensitive ones. NA enhanced, and α-antagonist (phenoxybenzamine) reduced cue tone responses. A β-antagonist (sotalol) had no clear effect, but sometimes modulated phase ii responses. Naloxone reduced phase iv responses. Summary: Many LHA neurons responded during task; magnitude varied with motivational state. Catecholamines and opiate modulated the effects. DA is important to task initiation, NA to drive reduction (motor termination), and opiate to reward perception.

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ANGIOTENSIN II SENSITIVE NEURONS IN THE SUPRAOPTIC NUCLEUS (SON), SUBFORNICAL ORGAN (SFO) AND PREOPTIC PERIVENTRICULAR NUCLEUS (POP) IN RATS IN VITRO. OKUYA, S.#, INENAGA, K., YAMASHITA, H. and KANEKO, T.*#. Dept. of Physiol., Univ. of Occup. Environ. Health Med., Kitakyushu 807 and #Dept. of Int. Med., Yamaguchi Univ., Sch. Med., Ube 755.

Angiotensin II (AII) has several central actions which are stimulation of drinking, blood pressure increase and vasopressin release. It has been postulated that receptor sites for AII are in the SFO, anteroventral third ventricle (AV3V) and SON, etc. We investigated the AII sensitivity of neurons in the SON, SFO and POP, which is part of the AV3V, in the rat brain slice preparation by perfusing with AII (10^{-10}-10^{-6} M) containing medium. The results are summarized as follows: 1) The proportion of excited neurons by AII was significantly higher in the SFO (60 of 92) than in the SON (38 of 93) and POP (26 of 79). 2) Compared with neurons in the SON, some neurons in the SFO and POP showed clear responses at lower concentration of AII (10^{-10} M) and sharper increase in firing rate. 3) In the SON, 15 (38 %) of 40 phasic firing neurons (putative vasopressinergic neurons) and 23 (43%) of 53 non-phasic firing neurons (including putative oxytocinergic neurons) were excited by AII. These results suggest that the SFO and AV3V are very important sites for AII mediated responses and behaviors through the central nervous system and also suggest a possibility that AII stimulates both vasopressin and oxytocin release affecting directly the SON.

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DIFFERENTIAL ROUTES TAKEN BY DESCENDING PROJECTIONS OF THE LARGE AND SMALL NEURONS IN THE FEMALE RAT VENTROMEDIAL HYPOTHALAMUS.
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The spatial spread of the extracellular antidromic action potentials was measured in 36 neurons in the ventromedial hypothalamus (VMH) following electrical stimulation of the midbrain central gray in urethane-anesthetized ovariectomized female rats, which had either lateral (towards the supraoptic commissure and zona incerta) or caudal (in the periventricular system) projection of the VMH disrupted by a small knife-cut. The amplitude and other parameters of antidromic action potentials were recorded as microelectrodes travelled through the field of the activated cells. The spatial spread of the extracellular field was represented by the travelled distance of the electrode at which peak-to-peak amplitude was not less than half the maximum amplitude. Nineteen responses in the posterior-cut animals had significantly larger field spread (108 ± 44 μm) than those (n=17) in the lateral-cut animals (81 ± 34 μm) ($P<.05$). Responses with the large field spread in the posterior-cut animals were recorded predominantly from the rostral VMH.

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AN ATTEMPT TO TEST CLASSICAL CONDITIONING UNDER ANESTHESIA. SHIBUKI, K., YAGI, K. AND HAMAMURA, M. Dept. of Physiol., Jichi Med. Sch., Tochigi-ken 329-04.

Effects of conditioning (CS: photic stimuli; UCS: footshocks) on vasopressin-cell activity, hindlimb flexion and blood pressure (BP) were tested in rats anesthetized with α-chrolalose and urethane (70 and 700 mg/kg, respectively). Rats previously trained in conscious state showed a slight inhibition of supraoptic neuron discharge after CS under anesthesia but this inhibition was not differential between trained and untrained rats. Electric eye stimulation evoked hindlimb flexion both in trained and untrained rats. Effects of training under anestehsia on BP were tested also under anesthesia. A depression after CS was not potentiated or inhibited after training. Effects of a similar training conducted under continuous adrenalin infusion were not also detected. These data indicate that responses to CS under anesthesia are not markedly influenced by training. Since quantitative analyses in our previous study have demonstrated that conditioned bradycardia can be detected under anesthesia, the possibility remains that quantitatively designed experiments disclose neural mechanism of conditioning in anesthetized animals.

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PARAVENTRICULAR NEURON PROPERTIES AND HYPOTHALAMIC CONTROL OF GASTRIC ACID SECRETION. SHIRAISHI, T. Dept. of Physiology, Tokai University, School of Medicine, Isehara 259-11

It has been demonstrated that the rostral part of paraventricular neucleus (r-PVN) of the hypothalamus is a new site for modulation of central control of gastric acid secretion (GAS). Central control has been attributed to gastric related neurons in the lateral hypothalamus (LHA). This study was initiated to investigate to the character of r-PVN neurons. Electrical single stimulation of LHA evoked complex waveform potentials in 253 r-PVN units. PVN neurons that responded LHA repetitive stimulation were classified E, E-I, I and I-E types. The alteration of these PVN neuronal discharge might be modulated gastric type LHA neuron activity. Effects of electrophoretic application of norepinephrine (NE) and dopamine to PVN neurons recorded both neuronal activity and GAS were studies. Results showed that when NE applied PVN alone neuronal activity increased ($p<0.01$) and suppressed GAS, but when NE applied accompanied by tranylcypromine (TCP), its effect more enhanced than without TCP. These results suggest that NE may play as neurotransmitter or modulator for the PVN-LHA gastric related relations.

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FOOD-RELATED VISUAL RESPONSES OF MONKEY LATERAL HYPOTHALAMIC NEURONS. FUKUDA, M., ONO, T., NISHINO, H. and NISHIJO, H. Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01

Single neuron activity was recorded from the monkey lateral hypothalamic area (LHA) to investigate neuronal events correlated with food discrimination and initiation of movement to procure food during operant bar press feeding. Of 429 neurons tested, 68 (16%) responded to sight of food and/or nonfood objects. Of these, 30 (7%) responded selectively to the sight of food or non-food objects associated with a juice reward, but not to the sight of non-food or objects associated with aversive saline. The latency to food-related visual responses was 110-310 msec (mean, 191 $\pm$ 58 msec); latency of nondiscriminative visual responses was shorter (134 $\pm$ 50 msec, $n = 38$). The visual response did not depend on occurrence or arm movement of bar press behavior. Neuronal responsiveness related to discrimination decreased and eventually disappeared in extinction (9/11), reversal (5/5) or satiation (2/2). The strength of visual responses was correlated to latency of bar press initiation and speed of bar pressing. Data suggest that the LHA is deeply involved in discrimination of food or nonfood and drive to get food during operant feeding behavior, which are affected by learning and internal states such as hunger and satiety.

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VIBRISSA-DRIVEN VENTROBASAL THALAMIC NEURONS: DEPENDENCE OF RESPONSE CHARACTERISTICS ON VIBRISSA LOCATION. ITO, M. Aichi Colony for the Handicapped, Kasugai 480-03

Neurons responding to vibrissal deflection were sampled from the ventrobasal complex (VB) of the rat thalamus under urethane anesthesia. These neurons were found in the dorsal aspect of VB, occupying a region of 0.5 mm x 0.5 mm in the dorso-ventral and rostro-caudal axes respectively. For each neuron, the size of receptive field (RF) was defined by the number of whiskers that, when deflected individually, activated spike discharges, and the location of a whisker that drove the unit most strongly was determined (center of RF). Tonic/phasic nature of response was noted during deflection of the center whisker in the most preferred direction. It was found that 1) small-field (1 or 2 whiskers) neurons could be related to any one of whiskers on the contralateral snout, whereas large fields (3 whiskers or more) were confined to caudal whiskers (whiskers Nos. 1-4 of each row); 2) although tonic neurons were obtained for all contralateral whiskers, phasic responses were observed only in neurons with caudal receptive fields. Rostral whiskers may be associated with fine detection of static object.

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THE RELATIVE LATENCIES OF VB NEURON DISCHARGES SYNCHRONIZED WITH HEART BEAT AND THOSE RECEPTIVE FIELDS. AIKAWA,S., NODA,K., AKITA,H., HOTSUMI,K., OCHIAI,N., AND KOBAYASHI,S.* Dept. of Physiol., Kitasato Univ. Sch. of Hygienic Sciences, Sagamihara, Kanagawa and Section of ME, Seishin Igaku, Inst., Itabashi-ku, Tokyo*

Unitary discharges were recorded extracellulary using single or multi-microelectrode technique in the thalamic ventrobasal complex in the cat. 35 of 681 units responded to not only somatic stimuli but also heart beats (HBS) were investigated. The receptive fields of these HBS neurons were distributed on almost part of the contralateral body surface. Some HBS unitary discharges were inhibited by electric stimuli to the receptive fields on body surface. After repeated Nembutal injections (6.25 mg/kg i.v.), HBS discharges were vanished earlier than somatic responses which evoked by the electric stimuli. In some cases, the HBS unitary discharges were still remained after bilateral vagotomy at cervical level. The latencies of HBS discharges whose receptive fields were distributed on the projection area of referred pain at heart attack were significantly ($P<0.01$) shorter than those of HBS discharges whose receptive fields were on other areas.

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EXCITATORY BRAINSTEM AFFERENTS TO THE RAT THALAMIC RETICULAR NUCLEUS. KAYAMA, Y., TAKAGI, M.* and OGAWA, T., Department of Physiology, Akita University School of Medicine, Akita 010

Under urethane anesthesia thalamic reticular (TR) neurons usually fire with bursts of several spikes. Repetitive electrical stimulation of the laterodorsal tegmental nucleus (LDT), which is composed of cholinergic neurons projecting to the thalamus, stopped the burst activity and induced tonic discharges. The stopping of bursts was brought about by weak LDT stimulation which did not produce desynchronization of cortical EEG recorded simultaneously. A little stronger LDT stimulation led to EEG desynchronization with tonic discharge of TR cells. Cooling of the cortex blocked the LDT-induced tonic discharges. Acetylcholine applied iontophoretically to TR neurons also stopped the burst activity and induced tonic discharges. Considering general properties of thalamic neurons studied by Jahnssen and Llinas (1984), we interpreted these results to indicate that cholinergic stimulation depolarized TR neurons directly and partly indirectly through neocortical activation. This interpretation challenges the classical view that the ascending reticular activating system inhibits TR activity.

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KINESTHETIC NEURONS IN THE MONKEY THALAMUS.
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Responses of thalamic VPL neurons were analized in monkeys with spontaneus sustained tremor. Rhythmic burst discharges, time-locked with the contralateral tremor were found in rostral part of the VPL. Natural stimuli such as passive joint movement and compression of the corresponding muscle belly evoked the same spike responses. Electrical stimulation of the forelimb muscle produced excitation of the same VPL neurons with the latencies of 4.5-11 msec. Few of the kinesthetic neurons received convergent cutaneous input. Kinesthetic responses remained following a contra-lateral hemisection of the spinal cord at the cervical level including both the dorsal column tract and the spinocervical tract. This suggest s that the spinothalamic tract may be involved in the mediation of deep kinesthetic information. Cytometric analysis revealed that VPLo contained large darkly stained neurons (600-700um^2) distributed sparsely. The thalamic VPLo-VPLc area considered to be the relay nucleus of ascending limb mediating the tremor.

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NOCICEPTIVE NEURONS IN VENTROBASAL COMPLEX OF THALAMUS.
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Nociceptive specific (NS) and wide dynamic range (WDR) neurons were found in the shell region of the ventrobasal complex of the thalamus. Trigeminal NS and WDR neurons were located in the nucleus ventralis posteromedialis proper (VPM proper). Spinal NS and WDR neurons were encountered in the nucleus ventralis posterolateralis (VPL). Trigeminal NS and WDR neurons were antidromically excited by electrical stimulation of the trigeminal projection area in the primary somatosensory cortex. Cooling the dorsolateral surface of the medulla oblongata caudal to the obex reversibly blocked responses of trigeminal NS and WDR neurons in the VPM proper to mechanical stimulation of the peripheral receptive field. The responses were irreversibly abolished by the trigeminal tractotomy at the level of the obex. These results suggest that afferent impulses from the peripheral receptive field to trigeminal NS and WDR neurons in the VPM proper are relayed through the trigeminal subnucleus caudalis and its adjacent subnucleus reticularis dorsalis.

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THE PONTINE RETICULAR NEURON ACTIVITIES IN RELATION TO FORELIMB STEPPING OF THALAMIC CATS.
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The paralemniscal pontine reticular formation (PLRF) exerts a patterned, bilateral control of the forelimb muscles and appears to play an important role in initiating and maintaining forelimb stepping.* In this experiment, we recorded unit discharges of the PLRF neurons in 12 thalamic cats under several conditions. Thalamic cats were performed by transection at the stereotaxic A_{12} level under ether anesthesia. The PLRF neurons exhibited burst discharges when thalamic cats stepped on the moving treadmill, during stepping induced by mesencephalic locomotor region (MLR) stimulation, fictive locomotion by MLR stimulation after immobilization, and during MLR stimulation after transection at C_2 level. These results that MLR stimulation can evoke burst discharge within the brainstem which could result in an alternating limb movement via descending tract to the cervical cord.

* Shimamura, M., Kogure, I. and Fuwa, T., The role of the paralemniscal pontine reticular formation in forelimb stepping of thalamic cats. Neurosci. Res. 1 (1984) 393-410.

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INFLUENCE OF THE KINDLING EFFECT ON THE ELECTRICAL PHENOMENA (PART 2). IKEDA, H., OKAMOTO, M., NAITO, H. AND YASUHARA, M. Dept. of Physiol. Kansai Med. Univ., Osaka 570

Kindling was experimentally produced in the amygdaloid nucleus, hippocampus, septal nucleus and cerebral cortex of the rabbit and the effect on microvibration(MV) and photopalpebral reflex(PPR) evoked by flash stimulation of the palpebra, EEG, ECG and the muscular discharge evoked by stimulation of the cerebral cortex examined.
1) The EEG, MV and PPR findings indicate that kindling of the neocortex, paleocortex and archicortex all have a facilitating effect on the state of consciousness.
2) Disagreement of the MV and PPR5-8 findings suggests that the nervous tissue involved in the occurrence of these reaction may differ in part.
3) The neocortex- and paleocortex-kindling had a facilitating effect on pyramidal motor activity.
4) The changes in the ECG indicate that HPC-kindling had a facilitating effect while neocortex-kindling had an inhibitory effect on the heart rate.
5) The finding that there was not always a parallel relation between the MV and PPR results and the ECG suggests that in the process of kindling, consciousness and motor activity and cardiac function are not always interrelated.

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THE ROLE OF LIMBIC-HYPOTHALAMIC SYSTEM IN PYRUVATE METABOLIC ADAPTATION TO REPEATED HEAT EXPOSURES. HORIUCHI, C., YOSHIMATSU, K., EDASHIGE, N., OTSUKA, K., NAKAGAWA, M., TAKESHIMA, Y. and SETO, K. Dept. of Physiol., Kochi Med. Sch., Nankoku, Kochi 781-51

The effects of lesions of the limbic-hypothalamic structures on the pyruvate metabolic responses to daily repeated heat exposures in the liver of rabbits have been investigated. The experimental results were as follows: (1) The pyruvate metabolic responses to the 1st heat exposure (heat exposure on the 1st day) were altered by lesions of periventricular arcuate nucleus (ARC), ventromedial hypothalamus (VMH), stria terminalis (ST) and dorsal fornix (FX). (2) The pyruvate metabolic responses to heat exposure were completely abolished by the ten times repetition of daily heat exposures in the rabbits with the lesions of ARC, VMH and ST, as same as in each sham-operated group. (3) In rabbits with the FX lesions, the pyruvate metabolic responses to heat exposure remained even after the ten times repetition of daily heat exposures. (4) From these results, it might be suggested that the ARC, VMH, amygdala (AMYG)-ST system and dorsal hippocampus (HPC)-FX system played a certain role in the mechanisms of pyruvate metabolic responses to the 1st heat exposure, but the only HPC-FX system participated in the process of pyruvate metabolic adaptation to daily repeated heat exposures.

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MEMBRANE PROPERTIES OF AMYGDALA NEURONS AND THE EFFECT OF ESTROGEN. NABEKURA, J., MIZUNO, Y., INOUE, M. AND OOMURA, Y. Dept. Physiol., Fac. Med., Kyushu Univ. 60, Fukuoka 812

Intracellular recordings were obtained from centromedial(CM) and basolateral(BL) neurons of amygdala of rat brain slice in vitro conditions. Effect of estrogen, which modulates feeding and sexual behavior, was also examined. Under normal condition, Na spikes were observed in all of CM neurons, but both Na and Ca spikes were observed in 23% of BL neurons. Delayed return of membrane potential to the resting level, due to increase of K^+ (I_A) conductance is shown in CM and BL neurons. Afterhyperpotential (a.h.p.) with reversal potential of -93mV, disappeared on application of TEA, 4-AP(E_K=-93mV). Stria terminalis stimulation elicited EPSP-IPSP sequence in CM and BL neurons. Application of bicuculline eliminated IPSP with the reversal potential of -64mV(E_{Cl}=-64mV). External capsule stimulation elicited EPSP only in BL neurons. 17ß-estradiol hemisuccinate(17ßE_2S,10^{-8}M) showed hyperpolarizing effect with an increase of membrane conductance among 23% of female CM neurons, which persisted on application of TTX and Cd^{++}. There were no effect of 17ß-E_2S on male and female BL neurons and very slight effect on male CM neurons. With regard to the response of 17ß-E_2S on membrane potential and I-V relationships with and without 17ßE_2S, the reversal potential for 17ß-E_2S was around -90mV. This result indicates that 17ßE_2S has a direct effect with an increase in K^+ conductance to CM neuron of amygdala.

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NEURON ACTIVITY VARIATIONS IN MONKEY AMYGDALA IN POSITIVE AND NEGATIVE AFFECTIVE SITUATIONS. NISHIJO, H., ONO, T., NISHINO, H., FUKUDA, M. AND YAMATANI, K.* Dept. Physiol. and Neurosurg.*, Fac. Med., Toyama Med. and Pharmaceu. Univ., Toyama 930-01.

Neuronal activity in the monkey amygdala was analyzed during bar press operant behavior to investigate neuronal responses to various stimuli associated with reward or punishment, or neutral. Of 1651 neurons recored, 461 responded to some stimuli and were classified into six general categories: 1) 38 neurons (2.3%) indiscriminately and transiently responded to various stimuli. 2) 33 neurons (2.0%) responded primarily at the sight food and/or reward-associated objects. Of these, 28 neurons clearly responded only to familiar food, but not to reward-associated objects, and 5 neurons responded to both. 3) 22 neurons (1.3%) responded primarily at the sight of certain non-food. 4) 51 neurons (3.1%) responded only in the ingestion phase after putting food into the mouth or drinking water or juice. This response was attenuated by salted food or saline. 5) 88 neurons (5.3%) responded to various objects in proportion to their significance, whether rewarding or punishing. 6) Of 1134 neurons tested, 26 responded in the delay period of delayed pair comparison test. The results suggest that the amygdala might be important in complex information processing to recognize the significance of objects and in stimulus-affective association, and is related to maintenance of emotion or mnemonic function.

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REWARD AND AVERSION RELATED HYPOTHALAMIC NEURAL ACTIVITY OF THE RAT DURING CUE SIGNAL LEARNING. NAKAMURA, K. and ONO, T. Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01.

Single neuron activity was recorded in the lateral hypothalamus (LHA) and lateral preoptic-anterior hypothalamic area (lPOA-AHA) of the rat during discrimination learning of cue tones that predicted glucose or intracranial self-stimulation (ICSS) to the posterior LHA as rewarding stimuli, or electric shock or tail pinch as aversive stimuli. The same licking task was used to either obtain a rewarding stimulus or avoid an aversive stimulus. Of 695 neurons tested, 488 were in the LHA and 207 were in the lPOA-AHA. The responses to both glucose and ICSS rewards were in the same direction, in both areas (81%, 111/137 in LHA; 77.4%, 24/31 in lPOA-AHA). Similarly, responses to both electric shock and tail pinch were in the same direction, in both areas (96.3%, 26/27 in LHA; 95.2%, 20/21 in lPOA-AHA). Rewarding and aversive stimuli had opposite effects on the same LHA neurons (64.9%, 24/37), but had the same effects on lPOA-AHA neurons (81.8%, 27/33). Responses to cue tones were in the same direction as responses to the respective rewarding or aversive stimulation in both areas. In the lPOA-AHA, 5 (2.4%) neurons responded to one or more of the cue tones, but not to rewarding or aversive stimulation. The results suggest reward and aversion related integrative functions in the LHA, and attention or arousal functions in the lPOA-AHA.

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EMOTIONAL INVOLVEMENT OF MONKEY ORBITOFRONTAL CORTEX AND LATERAL HYPOTHALAMIC AREA IN FEEDING BEHAVIOR. YAMAMOTO,T., OOMURA,Y., NISHINO,H., NAKANO,Y. and FUJITA,I. Nat. Inst. Physiol. Sci., Okazaki 444.

To elucidate the motivational mechanism involved in the initiation of feeding behavior, single neuronal activity was recorded in the monkey orbitofrontal cortex (OBF) and lateral hypothalamic area (LHA), both of them have been reported to play an important role on control of feeding behavior, during a vocalization-initiated feeding task. The present task paradigm was as follows: the monkey (Macaca fuscata) was requested to vocalize an SE subtype of "coo" sound to turn a cue light on, which meant a "GO" sign of the task. Then the monkey pressed a bar 30 times, and food was presented as a reward after a cue tone simuli. The neuronal activity during conventional bar press feeding task signaled by a cue light on, was also studied as a control task. 24% of OBF neurons and 38% of LHA neurons showed preceding activity changes to vocalization during vocalization-initiated feeding task. Most of them also showed preceding activity changes to the bar press when the task was i nitiated by monkey's requesting bar press instead of the vocalization. These results suggest that neurons in the OBF and LHA are involved in the motivational mechanism to initiate the feeding behavior.

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VENTROMEDIAL HYPOTHALAMIC NEURON RESPONSES TO FEEDING, DRINKING AND CIRCADIAN RELATED ACTIVITY. SHIBATA, R., ONO, T., SASAKI, K., NISHINO, H. and MURAMOTO, K. Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01.

Single unit activity of the ventromedial hypothalamus (VMH) was recorded over a long term in freely moving rat using a multi-electrode consisting of 7 teflon insulated platinum-iridium wires (dia.25 μm). We investigated neuron responses related to feeding, drinking and circadian rhythm. Of 59 neurons recorded, 8 responded continuously to sequential feeding, drinking or some sensory stimuli. Of these 8 neurons, 2 were excitated during feeding, inhibited during drinking, and neuronal activity was increased by intraperitoneal injection of 1.5 M NaCl solution. Another 2 neurons were excitated during feeding, and their activity was increased by 20% glucose solution injection into a lateral ventricle. The remaining 4 neurons showed complex responses; activity varied with food content and state of hunger. Of 51 neurons which did not respond to feeding or drinking, one responded to light and dark. Activity of the other 50 depended on sleep-wakefulness, 22 increased and 25 decreased activity during sleeping and 3 did not change throughout the day. The results suggest that VMH neurons contribute to long-term processing of humoral and chemical information and are related to circadian rhythm.

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INPUTS FROM THE SUBFORNICAL ORGAN AND THE A1 NORADRENERGIC REGION TO HYPOTHALAMIC PARAVENTRICULAR NEUROHYPOPHYSEAL NEURONS. TANAKA, J., KABA, H., SAITO, H. AND SETO, K. Dept. of Physiol., Kochi Med. Sch., Nankoku, Kochi 781-51

Electrical stimulation of the subfornical organ (SFO) produced orthodromic short latency brief duration (n=6 and n=8) or long latency prolonged (n=5 and n=7) excitation, inhibition (n=11 and n=3) or antidromic response (n=3 and n=2) of the activity of rat paraventricular(PVN) units (27 phasically and 24 non-phasically firing units) that were antidromically identified by electrical stimulation of the posterior pituitary. Electrical stimulation of the A1 noradrenergic region predominantly produced excitation (n=19 and n=13) of the activity of these identified PVN units. In the units (n=19 and n=15) that responsed to stimulation of both the SFO and A1 region, neither simultaneous nor preceding stimulation of A1 region affected the short latency brief duration excitation induced by SFO stimulation. On the other hand, simultaneous stimulation of A1 region significantly enhanced the long latency prolonged excitation and inhibition induced by SFO stimulation, and abolished or attenuated the excitation induced by A1 region stimulation itself in both phasically and non-phasically firing units. These results suggest that neurohypophyseal PVN neurons receive efferent projections from both the SFO and A1 region, and that the A1region modulates the inputs from the SFO to putative vasopressin-secreting PVN neurons.

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RESPONSE OF PREOPTIC/ANTERIOR HYPOTHALAMIC NEURONS TO A1 NORADRENERGIC REGION STIMULATION, YAMASAKI, F., KABA, H., SAKAMOTO, M., SAITO, H., OTSUKA, K., AND SETO, K. Dept. of Physiol., Kochi Med. Sch., Nankoku, Kochi 781-51

Response of preoptic/anterior hypothalamic (PO/AH) neurons to electric stimulation of the A1 noradrenergic region were investigated in urethane anesthetized female rats. A1 region stimulation produced orthodromic excitation (n=37, 19.6%) or inhibition (n=39, 20.6%) of the spontaneous activity of PO/AH neurons. Latency of excitatory responses showed bimodal distribution, with one peak in the range of 0-10ms and another in the range of 40-50ms, suggesting that two types of excitatory responses are present. The excitatory responses were thus divided into two categories according to their onset latencies and were designated short-latency excitatory responses (latency<15ms) and long-latency responses (latency≧15ms). Latencies of inhibitory responses ranged from 2.5 to 90ms with a mean of 46.5ms. Both the long-latency excitatory and inhibitory responses were significantry attenuated by intravenous injection of the α-adrenoceptor antagonist, phenoxybenzamine (5mg/kg) but not by the β-adrenoceptor antagonist, propranolol (2mg/kg).

These results suggest that the long-latency excitatory and inhibitory inputs from the A1 noradrenergic region to PO/AH neurons are both mediated by α-adrenergic pathway.

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THE MODE OF PROJECTIONS OF SINGLE LOCUS COERULEUS NEURONS TO CEREBRAL CORTEX IN RATS. SAKAGUCHI, T., NAKAMURA, S. Dept. of Neurophysiol., Inst. of Higher Nervous Activity, Osaka Univ. Med. Sch., Osaka 530

Locus coeruleus (LC) is known to have widely divergent projections to cerebral cortex (CX) in rats. In the present experiments, we employed antidromic stimulation technique to further clarify the mode of projections of single LC neurons to CX. In rats anesthetized with urethane, single unit activity was recorded extracellularly from LC by means of glass micropipettes. Stimulating electrodes were implanted in ten sites of CX ipsilateral to the recording site. It was found that the extent of axon terminals of single LC neurons varied from cell to cell. The amount of the LC projections decreased from rostral to caudal in CX. LC neurons were classified into the following three groups according to their mode of entry into CX. 1) neurons entering first frontal cortex, 2) those entering first parietal and/or occipital cortex, 3) those showing simultaneous entry of 1) and 2)

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DEVELOPMENT OF RAT LOCUS COERULEUS NEURONS: SOME ELECTROPHYSIOLOGICAL AND NEUROPHARMACOLOGICAL PROPERTIES. KIMURA, F.* AND NAKAMURA, S., Dept. of Neurophysiol., Inst. of Higher Nervous Activity, Osaka Univ. Med. Sch. Osaka 530

Developmental changes of some electrophysiological and neuropharmacological characteristics of locus coeruleus (LC) neurons were investigated in rats aged 1-30 days. In animals anesthetized with urethane, single unit activity was recorded extracellularly in the LC by means of a glass micropipette. Conduction velocity of LC axons increased gradually as animals developed. The number of the spontaneously active cells increased abruptly at postnatal day (PD) 7, while the discharge rates increased gradually and the discharge pattern became tonic during development. Although α_2-adrenoceptor mediated inhibition of the spontaneous activity was already manifest at the day of birth, the inhibition after antidromic activation was not antagonized by an α_2-antagonist, piperoxane, in neonatal period but after PD 13. These results indicate that α_2-adrenoceptors are already present in the membrane of LC neurons as early as PD 1 but become functional later.

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MID-PONTINE EVOKED INHIBITORY EFFECTS UPON ALPHA-MOTONEURONS INNERVATING HINDLIMB EXTENSOR MUSCLES. TAKAKUSAKI, K., MATSUYAMA, K., SAKAMOTO, T., MORI, S. Dept. of Physiol., Asahikawa Med. Coll., Asahikawa 078-11

In acute decerebrate cats, repetitive stimulation (rectangular pulses, duration; 0.2 ms, frequency; 50 pulses/s, period; 5 to 10 s) delivered to the dorsal tegmental area of the pons in its midline suppressed extensor muscle tone of the hindlimbs. With this, membrane potentials of extensor alpha-motoneurons (MNs) hyperpolarized, induced membrane hyperpolarization outlasting the period of the stimulation. The effective pontine area corresponded to the caudal portion of the nucleus centralis superior. In response to a single stimulation to the dorsal area, IPSPs were evoked in the alpha-MNs innervating the soleus muscle. The latencies of the IPSPs were in the range between 4.5 to 6.0 ms (half time, 8 to 15 ms). A single intramuscular stimulation delivered to the tibialis anterior muscle evoked Ia-IPSPs in the soleus alpha-MNs with latencies in the range between 3.0 to 4.5 ms (half time, 2.0 to 5.0 ms). Combined subthreshold stimulation to both the dorsal area of the pons and the tibialis anterior muscle evoked a clear membrane hyperpolarization. Suprathreshold stimulations to both sites for evoking IPSPs did not elicit summation effects. All these results suggest that the inhibitory effects induced by stimulating the dorsal area of the pons are, at least in part, relayed by way of the Ia-inhibitory interneurons at the spinal cord.

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MID-PONTINE EVOKED EXCITATORY EFFECTS UPON ALPHA-MNS INNERVATING HINDLIMB EXTENSOR MUSCLES. MATSUYAMA, K., TAKAKUSAKI, K., SAKAMOTO, T., MORI, S. Dept. of Physiol., Asahikawa Med. Coll., Asahikawa 078-11

In acute decerebrate, reflex standing cats, repetitive stimulation (rectangular pulses, duration; 0.2 ms, frequency; 50 pulses/s, period; 5 to 10 s) delivered to the ventral area of the pons along the midline evoked a long-lasting increase in the level of extensor muscle tone in the hindlimbs. The location of the effective sites corresponded to the rostral portion of the nucleus raphe magnus. In this study, alpha-MNs innervating the soleus muscle were identified by stimulating their axons within the muscle, and the effects of ventral stimulation upon them were studied. With the beginning of the ventral stimulation, alpha-MNs without spontaneous discharges depolarized their membrane potentials, and when the membrane potentials exceeded critical firing levels, they started to discharge tonically. The induced stimulus effects outlasted the period of the ventral stimulation for several minutes. Alpha-MNs with spontaneous discharges increased their firing rates during and after the ventral stimulation. Intraaxonally recorded GIa muscle afferents considerably increased their firing rates during, and after termination of the ventral stimulation. All these results suggest that the long-lasting increase in the excitability of alpha-MNs is due to supraspinal and segmental excitatory inputs to them.

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VENTROLATERAL MEDULLARY NEURONS PROJECTING TO THE PREOPTIC/ANTERIOR HYPOTHALAMIC AREAS. KABA, H., SAITO, H., OTSUKA, K. and SETO, K. Dept. Physiol., Kochi Med. Sch., Nankoku, Kochi 781

Presumed A1/C1 catecholaminergic neurons were antidromically activated from both the preoptic/anterior hypothalamic areas and the medial forebrain bundle in urethane anesthetized female rats. These identified neurons were tested for their sensitivity to microiontophoretic application of noradrenaline (NA), adrenoceptor antagonists, γ-aminobutyric acid (GABA) and GABA antagonists.

NA, applied with a current of 20 nA, inhibited the spontaneous firing of all the cells tested (n = 21). The β-adrenoceptor antagonist, timolol (n = 7), applied with a current of 20 nA, abolished the inhibitory response to NA. The α-adrenoceptor antagonists, phentolamine (n = 5) and piperoxane (n = 7), on the other hand, failed to block the inhibitory effect of NA.

GABA inhibited the spontaneous firing of all the cells tested (n = 17) and the inhibition was blocked by bicuculline (n = 5).

These results suggest that presumed A1/C1 catecholaminergic neurons projecting to the preoptic/anterior hypothalamic areas possess β-adrenoceptors, rather than α-adrenoceptors, and GABA receptors to inhibit their firing in the vicinity of the cell bodies.

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CEREBELLO-CEREBRAL PROJECTION FROM THE FASTIGIAL NUCLEUS ONTO THE FRONTAL CORTEX IN THE CAT. KYUHOU, S. AND KAWAGUCHI, S. Dept. of Physiol., Inst. for Brain Res., Fac. of Med., Kyoto Univ., Kyoto

Two areas in the frontal cortex, one in the ventral bank of the cruciate sulcus, and the other in the fundus and both banks of the presylvian sulcus, were found to be responsive to stimulation of the fastigial nucleus under pentobarbital anesthesia. In both areas, the response was a surface-positive, depth-negative potential with a latency of 3.0±0.5 ms and a time-to-peak of 9.3±1.2ms. The projection to the two frontal areas from the fastigial nucleus was studied by the anterograde and retrograde HRP methods, and by recording field potentials and unit activities. When HRP was injected into either of the two areas, retrogradely labeled neurons were distributed mainly in the lateral part of the ventromedial (VM) nucleus of the thalamus. In the same area of the VM nucleus fastigiothalamic fiber terminals were densely labeled anterogradely when HRP was injected into the fastigial nucleus. In the two areas stimulation of the VM nucleus induced a response similar to that induced by fastigial stimulation. We found units in the lateral part of the VM nucleus which were activated antidromically by stimulation of the cortical areas and orthodromically by fastigial stimulation. Present findings indicate that the lateral part of the VM nucleus mediates the fastigial projection to the two areas of the frontal cortex.

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IMMUNOHISTOCHEMICAL STUDIES ON THE CEREBELLUM FROM THE NEUROPATHOLOGICAL MUTANT MICE. TAKAMIYA,Y[1]., MIKOSHIBA,K[2]., MURAKAMI,H[1]., KIMURA,H[3]., OKANO,H[2]., OTANI,M[1]., TOYA,S[1]., TSUKADA,Y[2]. Dept.Neurosurg.[1], Dept.Physiol.[2], Keio Univ. Sch. Med.Tokyo 160, Dept.Anatomy[3], Shiga Med. College, Shiga.

Recently many neuropathological mutant mice have been reported that have abnormalities in the cerebellar development. Weaver and nervous mutant mice are characterized by degeneration of granule cells and Purkinje cells,respectively, while staggerer is characterized by the absence of synaptic contact between parallel fibers and Purkinje cell dendrites. Immunohistochemical staining of the mutant cerebellum by the antibody against neurofilament protein(NF) and myelin basic protein (MBP) was performed to visualize nerve fibers or myelinated fibers. In the weaver mutant mouse,Purkinje cells were surrounded by the myelinated fibers and no parallel fibers were observed.In the staggerer, fibers were not stained clearly and degenerative figures of both myelinated and nonmyelinated fibers could be observed.In the nervous, staining patterns of nerve fibers appeared to be normal in spite of prominant loss of Purkinje cells.

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ACCESSORY-OPTIC-TRACT RESPONSE EVOKED IN THE CARP CEREBELLUM AND ITS FUNCTIONAL IMPLICATION. MORI, S., TAKABAYASHI, A., YAGI, T., TAKAGI, S. and WATANABE, S. Dept. of Aerospace Physiol., Res. Inst. Environ. Med., Nagoya Univ., Nagoya 464.

Electrical stimulation of the optic nerve in carp elicited a paired positive waves (P1, P2) on the dorsal surface of the cerebellum. The P2 wave was localized at the rostral center and inverted the polarity in the granular layer while the P1 remained almost unchanged. These responses were not much affected by removal of the optic lobe, indicating contribution of the accessory optic system which projects to the cerebellum via the nucleus of pretectal area (Vanegas and Ito, 1983). The P2 latency was short (3 msec) and its refractoriness was close to that of the response evoked by the peduncle stimulation, suggesting the intermediate neuron might be single. The dorsal light tilt reaction (DLTR) is an innate behavior of fish to tilt its back toward a light source and it is known to be controlled mainly by sensory input from the visual and vestibular systems (von Holst; 1935, 1950). The fact that the DLTR maintained without the optic lobe suggests strongly that the projection system observed may involve the function concerning DLTR.

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THE VISUALLY RESPONSIVE NEURONS IN THE FASTIGIAL NUCLEUS OF THE CAT.
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Although cerebellar vermis-fastigial nucleus(FN) system is related to control of eye movements, it remains unknown whether the FN receives input from the visual system. Using 11 cats, immobilized by gallamine, under urethane anesthesia, 111 FN neurons were recorded extracellularly that responded to electrical stimulation (bipolar:0.5 - 0.75 mA, 100 μsec) of the optic chiasm. Fifty five neurons (type-1) showed transient suppression (averaged latency(L); 10.2 msec, averaged duration(D); 34.5 msec) followed by facilitation (L=42.6 msec, D=106 msec). Thirty seven (type-2) exhibited transient burst discharge (L=13.7 msec, D=47.8 msec) and suppression (L=47.8 msec, D=141 msec). Seventeen (type-3) showed only long suppression (L=9.7 msec, D=172 msec) by the stimulation. Photic responses were also tested in some experiments, with a xenon lamp. Responses in 3 type-1 cells, 4 type-2 cells and a type-3 cells were similar to the responses to the electric stimulation. Recording sites of the cells were limited in dorsal portion of the caudal FN where the lobules VI and VII project fibers.

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INHIBITORY EFFECTS OF CEREBELLAR NODULUS(LOBULE X) STIMULATION ON SYMPATHETIC NERVE ACTIVITY AND SYSTEMIC BLOOD PRESSURE IN RABBIT
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Effects of cerebellar nodulus(lobule X) stimulation on the vermal sympathetic nerve activity and the systemic blood pressure were studied in α-chloralose-urethane(30 and 600mg/kg, respectively) anesthetized albino rabbits. The sympathtic nerve activity was depressed by stimulation of the lateral part of lobule X with a train of a few pulses. The effective sites were distributed in the contralateral dorsal part of lobule X(lateral part between 2.9 and 3.7mm from midline). The latency of the depression was about 150ms. Stimulation of these sites caused a reduction of the systemic blood pressure, but this effect required stimulation by repetitive pulses, for periods of more than 10 sec. This depressant effects on the systemic blood pressure was transient, lasting for only one or two seconds; the systemic blood pressure returned to the control level during stimulation. We reported previously that stimulation of the ipsilateral medial parts in the lobules II,III,VII and VIII caused depression of both sympathetic nerve activity and systemic blood pressure. It seems that the depressant effects caused by stimulation of lobule X on the sympathetic nerve activity was similar to, but that on the systemic blood pressure was qualitatively different from the effects by stimulation of lobules II,III,VII and VIII.

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CHANGES OF VESTIBULAR RESPONSIVENESS OF FLOCCULAR PURKINJE CELL INDUCED BY SUSTAINED OPTOKINETIC STIMULATION IN THE RABBIT.
NAGAO,S. Dept. of Physiol., Fac. of Med., Univ. of Tokyo, Bunkyo-ku, Tokyo, Japan 113

Prolonged optokinetic stimulation with sinusoidal rotation of the cylindrical drum (0.1Hz,7.5°p-p) in front of the alert pigmented rabbit induced not only adaptive changes in the optokinetic response, but also changes in vestibulo-ocular reflex. In this experiment, I recorded single unit activities of Purkinje cells and compared the amplitude of frequency modulation of simple spike discharges to sinusoidal rotation of the drum and to sinusoidal rotation of the turntable in darkness(0.1Hz,5°p-p) before and after the sustained rotation of the drum for one hour. After the end of recording, brief train pulses of negative currents were passed through the microelectrode for microstimulation. Of the Purkinje cells recorded from the area where the microstimulation induced horizontal eye movement, significant changes of modulation to drum rotation were observed in 75% cells, and significant changes of modulation to turntable rotation were observed in 57% cells. Such changes were never observed in the Purkinje cells sampled where the microstimulation failed to induce any horizontal eye movement.

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EYE MOVEMENTS EVOKED BY CEREBELLAR MICROSTIMULATION IN THE CAT.

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Eye movements evoked by microstimulation of the vermis were investigated in the encephale isolé cat. We also studied the effects of stimulation of the vermis on discharge of prepositus hypoglossi neurons(PHN) encoding eye position. Repetitive stimulation(50-500Hz) of the vermis evoked saccadic eye movements that brought the eye to the same final goal irrespective of the initial positions. Saccadic latencies varied from 40 to 60ms, the threshold stimulus current being 20-30μA. The goal of the eye movements changed eccentrically with increasing stimulus current and pulse frequency. The direction of the goal varied with electrode locations from straight up in the midline of lobe V, through horizontal(ipsilateral) on lobe VI, to down on the midline of lobe VII. Single shock stimuli in lobe VI consistently inhibited the spontaneous discharge of the contralateral PHN. The discharge rate of these neurons was highly correlated($r=0.92$) with eye position along the ipsilateral horizontal direction. Low intensity repetitive stimulation below threshold current for the eye movements in lobe VI also significantly decreased the discharge rate of the contralateral PHN. On the other hand the ipsilateral PHN were activated by single shock stimuli in lobe VI.

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Tactile neurons with receptive field in the oral cavity, obtained at the frontal operculum of the cerebral cortex of macaque monkeys. Shin'ichi Ito and Hisashi Ogawa. Dept. of Physiol., Kumamoto Univ. Med. Sch., Kumamoto 860.

Using 11 macaque monkeys with an Evarts-type well installed on the head beforehand, we recorded single or multiple unit activities from the frontal operculum of the cerebral cortex, and examined the response properties to tactile, gustatory stimulation of the oral cavity and to visual stimulation. Monkeys were lightly anesthetized and immobilized. All the pressure points and incision sites were locally anesthetized. Tactile neurons were somatotopically arranged on the exposed surface of the frontal operculum (EFO); the teeth were represented rostro-dorsally, the tongue rostro-ventrally, and the palate caudally. Most rostrally were situated visually-activated neurons. On the other hand, tactile and gustatory neurons were obtained in and around the transition zone between the buried part of the frontal operculum and the insula. Tactile neurons could be classified into several groups according to the receptive field (RF), but the same group of neurons were found as clusters at two or three regions. Gustatory neurons were mainly found at the rostral part of the transition zone. Whether they were at the EFO or the transition zone, most of tactile neurons had ipsilateral or bilateral RFs, except lip neurons on the EFO. Lip neurons, mostly with contralateral RFs could hardly be located on a particular region in either EFO or the transition zone.

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INPUT-OUTPUT RELATIONSHIP BETWEEN THE FACE AREAS OF THE SECOND (SII) AND FIFTH (SV) SOMATIC SENSORY CORTICES IN THE CAT. MORI, A., MATSUURA, N., HANAJIMA, H. AND SUMINO, R. Dept. of Physiol., Sch. of Dent., Nihon Univ., Tokyo 101.

We examined the physiological properties of the neurons of the face regions in the surrounding areas of the second (SII) and the fifth somatic sensory cortices. Under ketamine anesthesia a chamber was installed over the skull surrounding the SI, SII, SIII and SV. A single microelectrode was inserted into the SI, SII, SIII and SV. A tungsten in glass microelectrode was used for intracortical microstimulation (ICMS), and also for recording unitary spikes from the cortex.

ICMS of the face region in the medial bank of the ASSS produced eye blinking on the contralateral side of the face with threshold currents as low as 2uA. In addition, ICMS of the face region in the medial bank of the ASSS produced movement of the whiskers on the contralateral side of the face with threshold currents as low as 8uA. However, ICMS of the first somatic sensory area (SI) did not show any movement (30uA or less). On the other hand, ICMS of the SII produced eye blinking on the contralateral side of the face with threshold currents as low as 2uA. Neurons in the effective area received afferent input from receptors in the skin structures in the region of the face which was related to that movement.

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CORTICAL FIELD POTENTIALS ON AUDIO-INITIATED HAND MOVEMENT IN THE MONKEY. GEMBA, H. AND SASAKI, K. Inst. Brain Res., Fac. Med., Kyoto Univ., Kyoto 606

Monkeys were trained to respond to auditory stimulus by lifting a lever (audio-initiated hand movement), and field potentials were led from various cortical areas with electrodes implanted on the surface and in 2.5-3.0 mm depth of respective areas. In association with the movement, characteristic potentials were recorded respectively in primary auditory, auditory association, prefrontal, premotor, motor and somatosensory cortices. Initial surface-positive (s-P), depth-negative (d-N) potentials appeared in sensory and association cortices as short as 15-20 ms after the onset of auditory stimulus (ca 500 or 10 ms duration), and they were often followed by s-N, d-P potentials. By cerebellar hemispherectomy ipsilateral to the moving hand, s-N, d-P potentials in the forelimb motor cortex contralateral to the hand were eliminated and reaction times prolonged. The same monkeys were also trained to perform the visuo-initiated movement, and results were compared with each other. Primary sensory and sensory association areas activated on respective movements were certainly different, and the prefrontal association cortex appeared to participate less predominantly in the audio- than visuo-initiated movement. Reaction times were generally longer and more variable on the audio- than visuo-initiated movement. Nevertheless the cerebello-thalamo-motor cortex projection was found commonly recruited prior to both movements.

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ELECTROPHYSIOLOGICAL EVIDENCE FOR FASTIGIAL PROJECTIONS TO THE CAT INSULAR CORTEX.
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Electrical stimulation of the cerebellar fastigial nucleus (FN) produced surface-negative field potentials (latency: 5-6 ms) bilaterally in the cerebral cortical regions around the anterior ectosylvian sulcus (AES) in the cat anesthetized with pentobarbital. Laminar field potential analysis revealed that the active synaptic site for the FN-evoked potentials is located within the cortical layer I of the ventral bank of the AES and the adjacent anterior sylvian gyrus, i.e., the granular insular cortex. Moreover, stimulation of the thalamic ventromedial nucleus (VM) also produced surface-negative field potentials in the insular cortex (latency: 3 ms). Laminar field potential analysis revealed that the VM-evoked potentials showed the same depth profile as the FN-evoked potentials. These results indicate that the VM mediates the FN projections onto layer I of the insular cortex. On the other hand, stimulation of the thalamic suprageniculate nucleus (SG) produced surface-positive field potentials in the insular cortex, and the depth profile of them was quite different from that of the FN- or VM-evoked potentials. It was suggested that the SG-evoked potentials originate from excitatory thalamocortical inputs to deeper layers, probably layers III and IV of the insular cortex. Intracellular recording from neurons in the insular cortex showed some input convergence between the FN/VM and the SG at a single neuronal level.

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RESPONSE PROPERTIES AND MORPHOLOGICAL FEATURES OF PYRAMIDAL NEURONS IN THE CAT PARIETAL CORTEX.
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Neurons in the cat parietal cortex(areas 5 & 7) were examined with intracellular recording and intracellular HRP staining under Nembutal anesthesia to clarify their response properties and morphological features. Responses were studied by stimulation of the cerebellar nuclei(CN), the VA-VL and LP nuclei of the thalamus and the pontine nuclei(PN). In this study, morphologically identified pyramidal neurons from layers II to VI were mainly analyzed. For neurons in layer V, those responded antidromically to PN stimulation were included. All neurons except for layer II were evoked EPSPs by both CN/VA-VL and LP nuclear stimulation. The mean latencies of CN- and VA-VL-EPSPs were not different in neurons from layers II to V;i.e., 4.7-5.4ms for CN- and about 2.8 ms for VA-VL-EPSPs respectively. Those for layer VI neurons were 6.1 and 4.7ms respectively and were slightly longer than those in layers II to V. The mean latencies of LP-EPSPs were 3.2ms in layer III, 3.8ms in layer V and 2.7ms in layer VI.

Morphological features of pyramidal neurons in layers II to VI in the parietal cortex were almost the same as those in the motor cortex. In layer V, two types of pyramidal neurons were found;i.e., one has spiny apical dendrites and the other has aspinous ones. This may indicate that these two types of layer V pyramidal neurons are analogous to slow and fast pyramidal tract neurons in the sensorimotor cortex.

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GABAergic inhibition in the control of voluntary movement in the primate motor cortex.
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GABAergic inhibition of neuronal activities in the primate motor cortex was studied while monkeys were performing a visual reaction time task. GABA and/or its antagonist Bicuculline were applied to neurons of which activities were related to lever press and/or lever release. Seventy-nine movement-related neurons, including 12 PTNs, were sampled from the hand area of the contralateral motor cortex in three macaque monkeys. These were related uni- or bi-directionally to the hand movement. In more than 90% of 45 neurons GABA showed an activity decrease (current 30nA), and in more than 95% of 73 neurons Bicuculline showed an activity increase at lever release or lever press phases. In six neurons, Bicuculline induced uni-directional discharge patterns into bi-directional ones. These results suggest that GABAergic inhibitory neurons play an role in spatially and temporally organized reciprocal discharge patterns in the motor cortex.

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DUAL MODE OF PROJECTIONS FROM THE PARIETAL TO THE MOTOR CORTEX IN THE CAT. KANG, Y., ENDO, K., ARAKI, T. and *MITANI, A. Depts Physiol. and *Anat., Kyoto Univ. Fac. Med., Kyoto 606

The cortico-cortical projection from area 5 to area 4γ was studied in anesthetized cats. EPSPs produced in a total of 54 fast PT cells following intracortical microstimulation of area 5 were analysed. The rising phase of these EPSPs was often composed of fast and slow components. Fast-rising EPSPs (fast component) were produced predominantly by stimulation within layer III of area 5 while slow rising EPSPs (slow component) were evoked predominantly by stimulation within layer V of area 5. The amplitudes of the fast and slow components of EPSPs produced during repetitive stimulation within layers III and V of area 5 decreased and increased, respectively, with an increase in the stimulus frequency without any appreciable changes in their latency and time-to-peak. The slow component was much less influenced by membrane hyperpolarization than the fast component. Retrogradely labeled neurons were found not only in layer III but also in layer V of area 5 following HRP injection centered on superficial layers (I-III) of area 4γ. It is concluded that there are two groups of cortico-cortical neurons in layers III and V of area 5, which may make monosynaptic contact with the proximal and distal portions of fast PT cells in area 4γ, respectively.

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THE ORGANIZATION OF MONKEY FRONTAL EYE FIELD REGARDING ELECTRICALLY EVOKED SACCADIC EYE MOVEMENTS. AZUMA, M., NAKAYAMA, H. and SUZUKI, H., Dept. of Pysiol., Hirosaki Univ. Sch. Med., Hirosaki 036

In the frontal eye field (FEF) of the alert monkey, intracortical microstimulation to various depths parallel to the radial fibers elicited the saccadic eye movement (SEM) of constant amplitude and direction. By contrast, the SEM vector was successively changed on the stimulation of various depths oblique to the radial fibers, indicating that the vector depended on the stimulated site in the FEF. We then examined the relation between the SEM vector and the cortical site. Small-amplitude SEMs were evoked by the stimulation of the lateral part in the FEF. SEM amplitude became larger on the shift of the stimulated site toward the medial direction along the arcuate sulcus. We further found that the generated SEMs brought the visual line onto the part of the environmental space occupied by the receptive field of the visual neurons located at the stimulated site. This may indicate that the FEF organization regarding the SEM is suitable for the generation of the visually guided saccades.

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MONOCULAR MOVEMENT EVOKED BY INTRACORTICAL MICROSTIMULATION OF THE BURIED CORTEX IN THE CORONAL SULCUS OF THE CAT. NAKAI, M., TAMAI, Y. and TSUJIMOTO, T. Dept. of Physiology, Wakayama Medical College, Wakayama 640, Japan

We have reported that the contralateral monocular movements were elicited by stimulation of the buried cortex in the coronal sulcus of the cat and that the direct corticofugal pathway to the ipsilateral pontine reticular formation was suggested by the electrophysiological method and the HRP tracing method. In the present study, we applied an intracortical microstimulation to the cortex where the monocular movement was induced (CIMM) and examined the details of the monocular movements and the characteristics of the CIMM. The contralateral eye moved rapidly and overall adducted with converging on the horizontal plane from the different original eye positions. The threshold was 8 to 40 μA and the latency was 18 to 22 msec. The CIMM was adjacent to or overlapped by the cortices where facial muscular movements, especially contralateral blink, were evoked. No apparent topographical relations were, however, suggested between the directions of the monocular movement and the sites of the cortex stimulated.

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PROJECTION PATTERN OF NEURONS IN THE CORTICAL MASTICATORY AREA AND ITS VICINITY FIRING IN CORRELATION WITH MASTICATORY MOVEMENTS IN THE CHRONIC CAT. TAIRA, M., KATAYAMA, T. AND NAKAMURA, Y. Dept. Physiol., Fac. Dent., Tokyo Med. Dent. Univ., Tokyo 113

During ingestion of food in 10 unanesthetized cats, single unit activity was recorded from 23 neurons in the cortical masticatory area (CMA), where repetitive microstimulation (RMS) (40 uA) induced rhythmical masticatory jaw movements (RMJM), and from 38 neurons in the adjacent sensorimotor area (non-CMA), where RMS induced a tonic jaw-opening. Activities of 13 CMA and 26 non-CMA neurons showed no correlation with RMJM (unrelated neuron), while the remaining 10 CMA and 12 non-CMA neurons showed actvities related to RMJM: 8 CMA neurons and 6 non-CMA neurons tonically increased the firing rate during RMJM (non-phasic group). No CMA neurons responded with antidromic spikes to stimulation (100 uA) of any of the cerebral peduncle (CP), the lateral amygdaloid nucleus and the red nucleus (RN) on the ipsilateral side, and the bulbar reticular formation and the spinal trigeminal nucleus (subnucleus interpolaris) on the contralateral side, except for 1 unrelated neuron antidromically responding to the RN stimulation. In contrast, 3 phasic group, 12 non-phasic group and 16 unrelated neurons in the non-CMA antidromically responded to the CP stimulation, and 1 non-phasic group and 2 unrelated neurons in the non-CMA did so to the RN stimulation. We concluded that neither phasic group nor non-phasic group neurons in the CMA are pyramidal tract neurons.

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RESPONSE PROPERTY AND DISTRIBUTION OF NEURONS RECEIVED CONTRA-,IPSI-,AND BILATERAL MASSETERIC MUSCLE INPUTS IN SI OF CAT. IWATA, K.,IKUKAWA, A.,ITOGA,H.,KAMOGAWA,H.,and SUMINO,R.Dept.of Physiol.,Sch.of Dent.,Nihon Univ.,1-8-13 Kandasurugadai,Chiyoda-ku,Tokyo 101, Japan.

Single neuron activities driven by contra-, ipsi-, and bilateral masseteric nerve stimulation (MDN) were recorded in SI of cat lightly anesthetized with ketamine. MDNs received inputs from the contralateral masseteric muscle(CONTRA-MDN) were distributed three separate cortical areas (the posterior, the middle, and the anterior projection areas), while those from ipsilateral one(IPSI-MDN) were in the most ventral part of the anterior projection area. MDNs from bilateral masseteric inputs were located in between the contrala- and the ipsilateral projection areas. More than half (60 %) of IPSI-MDNs were fired at intensity of less than 0.1 x stimulus threshold for jaw opening reflex (T). On the other hand, only small number of CONTRA-MDNs (12%) did at 0.1 x T. The peak latencies of spike potentials of IPSI-MDNs (mean;5.4 msec) were two times shorter than those of contralateral ones (mean;11.6 msec). Twenty of sixty three (32%) IPSI-MDNs received exclusively from a muscular input, while almost all CONTRA-MDNs received inputs from the orofacial skin,the periodontal membrane,and TMJ as well as a muscular input.

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LOCATION OF SITES IN CORTICAL AREA 20 WHICH INHIBIT THE PUPILLARY CONSTRICTION EVOKED FROM PMLS AREA IN CATS. BANDO,T. Dept. Physiol., Yamanashi Med. Sch., Tamaho, Nakakoma, Yamanashi 409-38, Japan.

By preceding stimulation of cortical area 20 of cats, pupillary constriction responses evoked by PMLS-stimulation were reduced in amplitudes, up to 30% of the control responses. This inhibitory interaction was seen when the area 20-stimulation preceded PMLS-stimulation by less than 100 msec. In this study, the zone was delineated in area 20 from which inhibition on PMLS-evoked pupillary responses was produced.

Cats were anesthetized by α-chloralose and urethane. Pupillary responses were monitored by using an infrared pupillometer, calibrated by an infrared video system. A tungsten microelectrode was used for stimulation of area 20 and PMLS.

Sites from which inhibitory interaction was produced were identified in histologically reconstructed electrode tracks. Inhibitory zone was found in the postero-lateral parts of area 20, corresponding to the stereotaxic coordinates P5-P7, and lay just caudal to the pupillo-constrictor zone in area 20, which occupied the rostro-dorsal parts of area 20.

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SEXUAL DIMORPHISM IN VOCAL CONTROL AREAS OF THE BRAIN OF THE ORANGE WEAVER. TANIGUCHI, I., ARAI, O., and SAITO, N. Med. Res. Inst., Tokyo Med. & Dent. Univ., Tokyo 113, and Dept. of Physiol., Dokkyo Univ. Med. Sch., Tochigi 321-02.

The male of the orange weaver (Pyromelana franciscana) has beautiful nuptial plumage in breeding season. The male, when out of color, resembles the female. We studied the correlation between the eclipse of nuptial plumage and the volume change of vocal control areas in orange weavers. Four vocal control areas (HVc, RA, MAN and X) are well developed in males but not recognizable in females. A fourth, area X of males of this species is remarkably large in comparison with male canaries and zebra finches. However, the volume of the vocal control areas in males decreases with the eclipse. This suggests that the volume of vocal control areas is influenced with the seasonal change of hormonal secretion.

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SET-DEPENDENT ACTIVITY OF THE PUTAMEN NEURONS DURING CONDITIONED MOVEMENT IN THE MONKEY (Macaca fuscata). KIMURA, M.
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In order to assess the role of the putamen in voluntary movements, neuronal activity was recorded in the putamen during visually triggered repetitive extension and flexion of the right arm and acoustically triggered orofacial movement of a monkey. Somatotopic organization in the putamen was identified by microstimulation (300 Hz, 10 pulses, <200 uA) which evoked diserete movement of body musculatures. Cells with tonic spontaneous activity (3-7 Hz, Type I, n=77) showed either excitation followed by inhibition or inhibition to the click (latency 70 msec) which accompanied delivery of juice reward and licking movements of monkey and to the visual cue (90 msec) triggering extension and flexion of arm. These responses to the sensory cue appeared only when monkey initiated conditioned movements by the cue. Cells with low spontaneous discharges (< 2 Hz, Type II, n=67) showed phasic discharges locked to the body movement depending on the somatotopic location of the cell. Most type II cells, however, exhibited phasic discharges at only initial period of repetitive arm or orofacial movements triggered by sensory cue and were almost silent at unconditioned movement. These set-dependent nature of putamen cell activities suggested a role of the putamen in linking sensory stimuli to behavioral responses.

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VISUAL RESPONSES OF MONKEY CAUDATE NEURONS. HIKOSAKA, O. and SAKAMOTO, M. Dept. Physiol., Toho Univ. Sch. Med., Ota-ku, Tokyo 143

Recent studies (*) have suggested the role of the basal ganglia in the voluntary initiation of saccadic eye movements. Cells in the monkey substantia nigra pars reticulata discharge rapidly thereby tonically inhibiting saccade-related superior colliculus cells, and in response to visual or mnemonic signals cease their discharges thereby removing the inhibition and allow a saccade to occur. The present study suggests that the origin of the visual signals is the caudate nucleus.

While the monkey was fixating a small central spot of light on the screen, another spot of light was presented to investigate visual responses of caudate cells recorded. Visual cells were clustered in the caudate nucleus forming an antero-posteriorly elongated column. Their receptive fields were usually large but centered in the contralateral visual field. Cells recorded along a given electrode penetration tended to have similar receptive fields. Latencies of the visual responses ranged mostly between 90 and 190 msec. The visual responsiveness of caudate cells strongly depended on the behavioral significance of the stimulus. For example, a group of cells responded to a visual stimulus only when the monkey was required to remember its position in order to make a saccade to the stimulus after a several second delay.

(*) Hikosaka and Wurtz, J. Neurophysiol. 49: 1230, 1983.

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VISUAL DISCRIMINATION-RELATED RESPONSES OF THE MONKEY ORBITOFRONTAL CORTEX. KOGA, T., ISHIKAWA, T., MIYAZAWA, T., *AOU, S., *OOMURA, Y., *NISHINO, H. Dept. Physiol., Nihon Univ. Sch. Med., Itabashi, Tokyo 173, *Dept. Humoral Control, Nat. Inst. Physiol. Sci., Okazaki 444

The neuronal activity of the monkey orbitofrontal cortex (OBF) in visual discrimination tasks were studied. A monkey was trained to press a bar repeatedly for food (FR-30) on a red cue light and press a bar continuously to avoid a shock on a green cue. Of 94 units in the left OBF, 58% showed changes in activity, and of them, 16% showed differences in activity. The discharge rate in bar pressing phase increased tonically in the feeding task and phasically in the avoidance task. In discrimination phase, it increased after cue followed by decline at the first bar pressing in the feeding task, while it increased moderately like a plateau followed by increase at the 1st bar pressing in the avoidance task. Though when the green and red light was applied simultaneously to the left and right eyes respectively the same changes as seen in the avoidance task were seen, when they were applied reversely the same changes as seen in feeding and avoidance tasks were seen. By statistic methods, it was revealed that the peak after cue and the following decrease may be concerned to input process of the red light and initiation of the feeding task. The plateau and the following peak may reflect input sequence of the green cue and initiation of the avoidance task.

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REWARD-RELATED NEURONAL ACTIVITY OF THE MONKEY AMYGDALA AND THE EFFECT OF ELECTROPHORETICALLY APPLIED ACETYLCHOLINE, MORPHINE AND GLUCOSE. NAKANO, Y., OOMURA, Y., LENARD, L., NISHINO, H. YAMAMOTO, T. and AOYAGI, K., Dept. of Biological Control, Nat. Inst. Physiol. Sci., Okazaki 444.

Extracellular single neuron activity was recorded during operant feeding behavior, and the effect of acetylcholine (ACh), morphine (Mo) and glucose (Gluc) was studied. The behavioral paradigm was a high fixed ratio (FR20) bar press task signaled by a cue light and rewarded by a cue tone. Of 292 cells tested, 198 (68%) changed in activity during the task. The cells showed changes in activity not only to the external cue stimuli (25% of the responsive cells) but also during bar press (43%) and reward period (24%). The predominant effect of ACh was an increase (32%) in firing while Mo (31%) and Gluc (12%) was a decrease. Gluc-sensitive cells were located in and around the central nucleus. Mo- and Gluc- sensitive cells in the centromedial part of the AMG showed decrease in activity during bar press period more frequently than insensitive cells. ACh sensitivity did not correlate any firing pattern during the task, but continuous application of ACh enhanced the increasing response during the task. These results suggest that Mo- and Gluc-sensitive cells are possibly involved in the neuronal processing during food aquisition (bar press period) and cholinergic inputs may affect the neuronal excitability during the performance of the feeding task.

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THE DENDRITIC SPIKE IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONS.
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The laminar profile of extracellular field potentials evoked by the alveus or the Shaffer collaterals stimulation were recorded at the CA1 region of guinea-pig hippocampal slices. In order to interpret the result, the current source density analysis method was applied.

When the alveus was stimulated, the location of inward membrane current (sink) moved from the cell body layer to the dendritic region for at least 200 μm at the velocity of 0.13 ± 0.02 m/sec. This sink movement represents the propagation of action potential along the dendrite. It was not blocked in low Ca (0.2mM) medium. But it was blocked by tetrodotoxin applied locally to the dendritic region by a newly developed method.

Though not clearly, the sink movement from the dendritic region to the cell body layer also appeared when the Schaffer collaterals were stimulated. The sink movement from the cell body layer to the dendritic region was not seen after the cell body spike. These results support the notion that when activated synaptically, an action potential propagates from the dendrite to the cell body.

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POSITIVE SHARP WAVE BURST AND UNIT ACTIVITY IN CAT HIPPOCAMPUS DURING SLOW WAVE SLEEP. EGUCHI, K. & SATOH, T. Dept. Physiol., Aichi-Gakuin Univ. Dental Sch., Nagoya 464

Two or three glass micropipettes simultaneously inserted into the cat hippocampus picked up sporadic bursts of 100 Hz positive sharp waves (PSWs) which occurred almost synchronously at different hippocampal sites during slow wave sleep. In the CA 1, the amplitude of the PSW was highest in the stratum radiatum, and the waves showed no phase reversal when the electrodes were advanced ventrally to the dentate gyrus. Twenty-two units were isolated in the regions exhibiting PSW bursts. Nine units tended to discharge selectively at the junction between two successive PSWs. Four units showed a tonic increase in discharge probability during the entire course of a PSW burst. One unit was inhibited during PSW bursts. In 8 units their discharge was apparently not correlated with the occurrence of PSW bursts. Cross-correlation analysis of discharge timing was performed in 9 unit pairs. Synchronous or time-locked discharges were observed in 5 pairs, but these correlations did not seem to be particularly related with the presence of PSWs in the background.

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INHIBITORY EEG SPIKES PRODUCED BY ELECTRICAL STIMULATION OF THE RAPHE NUCLEI. MINAMI, S., FUJITA, Y. Department of Physiology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113, Japan.

EEG spikes, whose intracellular correlates are hyperpolarizations of pyramidal cells (referred to as inhibitory EEG spikes), are observed not only in the normal hippocampus at a lower activity level, but also in the kindled hippocampus (interictal spikes) (Fujita et al., Jpn. J. Physiol., 31 (1981) 879-889, ibid., 33 (1983) 227-238, Brain Res., 278 (1983) 313-317). At a lower activity level of the brain low frequency electrical stimulation (2, 5, 50/s) of the brain stem produced inhibitory EEG spikes in both hippocampi of the normal rabbit with suppression of theta waves. Intracellular counterparts of these EEG spikes were Cl-non-dependent hyperpolarizations. The most effective sites of stimulation were examined from a rostral portion at the level of nucleus of the occulomotor nerve to a caudal portion at the level of nucleus of the facial nerve, and proved to be nucleus raphe centralis superior and nucleus raphe magnus. Outside the raphe nuclei, there existed the sites where the stimulation could produce no such effects or enhancement of theta waves. It is therefore concluded that nuclei raphe centralis superior and magnus are the specific sites in the brain stem which supress the hippocampal theta rhythm and produce the inhibitory EEG spikes.

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EFFECTS OF ANTICONVULSANTS ON DRUG-INDUCED EPILEPTOGENIC POTENTIALS IN THE HIPPOCAMPAL SLICE. CHUJO, T., Dept. of Neurol., Kanazawa Med. Univ., Uchinada, Ishikawa 920-02

Synaptic potentials in CA1 neurons of the hippocampal slices were evoked by stimulation in CA3 neurons. The epileptogenic drugs (PCN: penicillin 3000Unit/ml, PTZ:pentylenetetrazol 5mM, ACh 10mM, 4-AP: 4-aminopyridine 5mM) produced epileptiform burstgeneration in hippocampal neurons. Phenytoin (DPH 80uM) suppressed both synchronized field potentials and drug-induced epileptiform bursts. But DPH did not completly depress 4-AP-induced burstgeneration. These results suggested that DPH migh stabilize inhibitory interneurons or decrease hyperexcitability of hippocampal neurons.

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MORPHOLOGICAL STUDY OF THE COBALT-INDUCED EPILEPTOGENIC FOCAL NEURONS WITH INTRACELLULAR HRP INJECTION. *KOYAMA, I., **NISHIOKA, S. AND *SHIRAFUJI, Y. *Dept. of Physiol., Tokyo Women's Medical College., Shinjuku-ku, Tokyo. **Institute of Toxicological and Environmental Science, Midori-ku, Yokohama.

Epileptogenic foci were produced by the topical application of metallic cobalt-powder to the sigmoid gyrus of cat. During the convulsion period the pyramidal neurons in the focus with Golgi staining appeared abnormal; such as the nodal formation with attenuated shaft of the apical dendrite, as well as the depletion of the dendritic spine. The present sutdy is meant to know whether or not this abnormal neuron can become hyperexcitable. For this purpose HRP is injected electrophoretically into the focal neurons appeared in high frequency discharges. From our limited number of identified neurons penetrated the average value of resting potential was -35.3 mv (-10 to -52 mv). Such detected HRP stained cells were Betz cell, medium sized pyramidal neurons, stellate cell and spindle cells in the III through VI layers of the neocortex. The abnormality which appeared in Golgi staining, of dendrites was also detected but with lesser extent.

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INDUCTION OF A DOMINANT FOCUS BY ONE TRIAL OF ANODAL POLARIZATION IN THE MOTOR CORTEX OF CHRONIC RABBIT. HAYASHI, Y., HORI, Y., HATTORI, Y., MORIWAKI, A. AND SUNAMI, T. Dept. of Physiol., Okayama Univ. Med. Sch., Okayama 700

R. A. Pavlygina personally communicated that applying 30 μA of anodal direct current to surface of the motor representation of the forelimb (anodal polarization) for more than 60 min caused augmentation of microvibration in the contralateral forelimb in response to extraneous stimuli in high percentage of experimental rabbit after only one trial. The latter was regarded as to indicate appearance of dominant focus at polarized area. We examined Pavlygina's and other methods of polarization not on microvibration but on flexion response of the forelimb in chronic rabbits. By anodal polarization with 30 μA, we obtained statistically significant change in the pattern of forelimb flexion response in 5 out of 13 rabbits. Anodal polarizations with 3 μA and 0.7 μA to the premotor area induced statistically significant change on forelimb flexion in one trial in 1/5 and 1/6, respectively, of animals. There was statistically no significant difference among these 3 animal groups.

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ALTERATIONS OF NOREPINEPHRINE-SENSITIVE CYCLIC AMP SYSTEM BY INDUCTION OF AN EPILEPTIC FOCUS IN RAT CEREBRAL CORTEX. HATTORI, Y., MORIWAKI, A., YASUHARA, H., NISHIDA, N. AND HORI, Y. Dept. of Physiol., Okayama Univ. Med. Sch., Okayama 700

Electrocorticographic spike activity was produced by unilateral injection of $FeCl_2$ solution into the sensorimotor cortex of the rat. In slices of anterior cortical areas of rats showing dominant spike activity on either side of the cortex 8-10 days after the injection (early stage), accumulation of cyclic AMP elicited by norepinephrine was greater on the side of dominant spike activity than on the other. The relationship was reversed 30-60 days after the injection (late stage). The results of adding α- or β-adrenergic antagonist to cortical slices indicated that the regional differences at the early and late stages were due primarily to β- and α-adrenergic mechanisms, respectively. In slices of anterior cortical areas of rats showing nearly equal spike activity on two sides of the cortex at the late stage, norepinephrine-elicited accumulation of cyclic AMP was greater on the side ipsilateral to the injection site than on the other. In these rats, the regional difference was due primarily to β-adrenergic mechanism.

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TOPOGRAPHY OF FAST ACTIVITIES INDUCED BY THIOPENTAL AND DIAZEPAM.

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Dept. Anesthesia, Tokyo Women Medical College, Shinjiku, Tokyo.

Topographies of fast activities in human EEG induced by thiopental, diazepam and simultaneous administration of the two were studied making two-dimensional maps by unbiased polynomial interpolation for the purpose to find the relation between EEG changes by the two drugs and GABA-Benzodiazepine-Barbiturate receptor. Subjects of this study were six patients of abdominal diseases. The drugs were administered in two ways: (A) thiopental 25mg/30sec was i.v. injected (total 100 mg) and after 5 min diazepam 15 mg was i.v. injected. (B) diazepam 15mg/30sec was i.v. injected and after 5 minutes thiopental 25mg/30sec was i.v. injected. EEGs were recorded before and during the administration from 12 locations and A/D conversion was made with 4 msec sampling rate. EEG records containing artifacts were discarded. Area of large amplitudes increased by diazepam in the order of frontal > central > occipital for 17 -19 Hz activities, and added thiopental increased the area further but the order was unchanged. Constructed thiopental map (averaged both drug map - averaged diazepam map) showed approximate similarity to thiopental difference map. Future analysis into various topographical features is necessary to elucidate the above-mentioned relation.

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Some characteristics of responses of EEG theta component to light stimuli.

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Theta and alpha components of EEG are very important for studying maturational or developmental processes of brain. We can assume that the origin of theta component will be different from the alpha one. The present study intended to report on characteristics of responses of theta component to continuous light stimuli. The subjects were mentally retarded adults without any pathological findings and any paroxysmal abnormal waves. We considered them as stopping or retarding on the way of their maturation of EEG. For example, we had the cases who indicated the theta component only or both the theta and alpha ones. To these cases, we gave a light stimuli for 15 seconds several times and obtained auto-power spectra by the minicomputer(PDP 11/34). The frequency resolution was 1/3 Hz and analyzed time was 3 seconds. The " blocking " effects and the habituation to the light stimuli appeared in the theta frequency band. But the appearance of "blocking " effects and habituations depended on the frequency of theta components. The theta components seem to be divided into two kinds : one is a component like alpha which responses to visual stimuli and the other is an ordinary theta component. The latter might be different from the origin of the former and alpha component.

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RESPONSIVENESS OF MONKEY PREFRONTAL CORTEX NEURONS TO COMPLEX LEARNED BEHAVIOR.

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Our previous study (Brain Res, 311, 323-331, 1984) indicated that integration occurs in the prefrontal cortex (PFC) to induce the complex behavior needed for feeding. The present study was focused on elucidating higher order integration for decision making based on learning and memory. Single neuron activity was recorded from the PFC of monkey during TV-controlled and bimanual tasks including: 1) delayed matching-to-sample (DMS); 2) spatial and color discrimination with left/right and go/no-go responses (Go/No-Go); and 3) bar-press feeding dependent on discrimination of food or non-food. Responses of the same neuron in all of these tasks were compared. Half of the responding DMS neurons that were ascertained to be affected by neither the character of the figures to be matched nor the direction of response (which required the use of the corresponding hand) responded exclusively or oppositely to each pair of Go and No-Go cues and/or discrimination of food and non-food. Most were located in the principal sulcus and the area surrounding it, where most DMS delay responsive neurons are centered. Results suggest that the principal and periprincipal sulcal area might be the highest integration site in the brain for decision making related to complex learned behavior.

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S-ADENOSYL-L-METHIONINE (SAMe) TREATMENT IMPROVES DELAYED RESPONSE DISTURBANCE AFTER PREFRONTAL CORTEX LESIONS IN MONKEYS. TAKAHASHI, J., NISHINO, H., ONO, T. AND FUKUDA, M. Dept. of Physiol., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01

It is said that SAMe is important as the main methyl donor in biological transmethylating reactions and has a variety of physiological effects. In the present study, we evaluated the effects of SAMe on the course of recovery from delayed response disturbance after bilateral dorsolateral prefrontal cortex lesions (1st stage, including a part of principal sulcus; 2nd stage, including whole principal sulcus) in four monkeys. Monkeys treated with SAMe (30 mg/kg/day, i.m.) had lighter impairment and faster recovery than controls. Even in the chronic state (76-150 days postoperatively), SAMe facilitated recovery from delayed response disturbance. SAMe enhanced recovery from delayed response disturbance in reserpinized monkeys with prefrontal lesions. Histological findings in rat experiments showed that SAMe (50 mg/kg/day, s.c.) facilitated the infiltration of activated phagocytic macrophages in lesioned areas. From the results, we suggest that SAMe is beneficial to recovery from behavioral and histological disturbances due to brain damage.

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COMPARISON OF SPREAD OF NEURONAL ACTIVITIES WITHIN CAT MOTOR CORTEX INDUCED AFTER THALAMIC AND EPICORTICAL STIMULATION.
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The encéphale isolé cat preparation was atraumatically maintained in a semi-chronic stereotaxic apparatus. Primary responses of excitatory (EPSPs) and inhibitory postsynaptic potentials (IPSPs) to single shock stimulation of the ventrolateral nucleus of the thalamus (VL) were recorded from precruciate motor cortical neurones. The laminar distribution of these neurones with different response latencies were viewd as a spatiotemporal pattern of intracortical spread of excitation and inhibition, being compared with that induced after epicortical stimulation (EPICS) applied near the recording site. In contrast with activities by EPICS spreading from superficial to deep cortical layers, activities by VL afferents were initiated by monosynaptic EPSPs and disynaptic IPSPs in lamina V and the lower half of lamina III, and spread therefrom bidirectionally to both superficial and deep layers with more synaptic delay. Two intracortical routes carrying activities by VL stimulation and EPICS shared a large population of cell assemblies that received convergent EPSPs and/or IPSPs. Abundant supply of convergence may indicate a good deal of potentiality of the cortical network in producing different functional patterns.

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ALTERATIONS OF THE PERFORANT PATH-DENTATE SYNAPTIC TRANSMISSION AND DENTATE GRANULE CELL EXCITABILITY FOLLOWING PERFORANT PATH KINDLING. MARU, E., TATSUNO, J. AND GODDARD*, G.V. Dept. Physiol., National Defense Medical College, Tokorozawa, Saitama 359 and *Dept. Psychol., Univ. Otago, 56 Dunedin, New Zealand.

The purpose of the present experiment was to examine the changes in (i) transmission efficacy at the perforant path-dentate granule cell (PP-DG) synapses (ii) granule cell excitability and (iii) hippocampal commissural inhibition of the granule cells during and after perforant path kindling, with use of field potential recording in freely moving rats. Wistar rats were kindled by brief electrical stimulation of 100 Hz through an electrode implanted in the perforant path once each day for 3 weeks. The dentate field potentials evoked by single pulse to the perforant path were recorded throughout kindling and 1 month after kindling. The population EPSP evoked at PP-DG synapses was strikingly potentiated by the first few kindling stimulations and then gradually increased with successive kindling trials. The population EPSP remained elevated at least for 1 month after the cessation of kindling treatment. In contrast to the long-lasting excitatory synaptic potentiation, the excitability of the dentate granule cells gradually decreased as kindling progressed, and recovered 1 month after kindling. The commissural inhibition of granule cell firing was strengthened by kindling stimulations and declined slowly over the course of 1 month after kindling to almost control level.

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PLASTIC CHANGES BY ELECTRICAL STIMULATION IN HIPPOCAMPAL NEURONS OF GUINEA PIG.
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Plastic changes of hippocampal pyramidal neurons caused by paired electrical stimuli were studied by recording population spikes of the CA1 region in slice preparation. Paired stimuli were consisted of orthodromic stimulation placed on the st. radiatum and antidromic stimulation placed on the st. alveus. Paired stimuli were applied 100 times with 5 sec interval. The results were as follows.

(1) Paired stimuli with 20 msec interstimulus interval (ISI) caused increase in the population spikes evoked by the st. radiatum stimulation.
(2) This increase was apparent during paired stimuli and reached to 123 % of control level after 10 min paired stimuli. Then, these were maintained for more than 30 min.
(3) Paired stimuli with 500 msec ISI caused little change.

These observations indicate that the ISI of paired stimuli is important for the facilitation of population spikes evoked by the stimulation of the st. radiatum.

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THE FIRING PATTERN OF THE PUTATIVE HIPPOCAMPAL INTERNEURONS DURING PENICILLIN-INDUCED SEIZURE.
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Single unit activities were studied in the hippocampal CA1 area of lightly anesthetized and immoblized rabbits. Fifty-eight units, which responded with repetitive firing to the supramaximal contralateral hippocampal stimulation, were recorded as the putative interneurons. Twelve of those were examined during the penicillin-induced seizure (PS) (240 mM; direct application) as well as the electrically induced seizure (ES). They were classified into two types. TYPE I units (N=6) which were located in or near the pyramidal cell layer, showed a prolonged firing (about 150 msec duration) corresponding to the penicillin spikes. When PS was induced, they were gradually depressed and ceased to fire during the early part of PS, subsequently followed by re-firing during the late part. TYPE II units (N=6) which were located in the stratum oriens, also exhibited a more prolonged firing (about 500 msec) corresponding to the penicillin spikes. When PS was induced, they showed a sustained firing without any depression. These behaviors of two types of the putative interneurons during PS were well consistent with those during ES reported previously.

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DEFICITS IN THE FACIAL MOVEMENT INDUCED BY MUSIMOL INJECTION IN THE FACIAL REGION OF THE CONSCIOUS MONKEY SOMATOSENSORY CORTEX(SI).
HIRABA,H., TANAKA,M. AND IWAMURA,Y. Dept. Physiol. Toho Univ. Sch. Med., Ota-ku, Tokyo 143

A monkey was trained to protrude his tongue toward a perioral point touched with a stick and a small piece of food. Single neuronal activities were recorded in the facial region of SI using a glass-coated platinum-iridium microelectrode and their receptive field charactristics were determined. A small amount of musimol(GABA agonist) was injected into the same site through the same hole on the dura as that of the just preceding recording electrode penetration. After musimol injection, the protrusion of tongue following the perioral tactile stimuli to the receptive field of neurons just recorded, was not observed if the monkey's eyes were obstructed. Similarly, eye blinking or ear retraction, normally evoked by tactile stimulation of the eyelid or peribuccal skin respectively, was not observed after musimol injection into loci where neuronal receptive fields were on either one of these facial areas. These observations indicate that the sensory information is organized in each locus of the facial region of SI to subserve selective motor acts of the face.

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SEPTO-HIPPOCAMPAL CONNECTIONS TO FIELD CA1 OF RATS EXAMINED BY LAMINAR FIELD POTENTIAL ANALYSIS AND RETROGRADE LABELING WITH HRP. OKA, H. and YOSHIDA, K., Dept. of Physiol., Fukui Med. Sch., Fukui 910-11

Electrical stimulation of the medial septal nucleus produced field potentials in the hippocampal CA1 region in the rat. In the laminar field potential analysis, the negative responses to the medial septal stimulation were found to be localized in the alveus and the stratum oriens (the territory of basal dendrites of hippocampal pyramidal cells). The positive responses recorded as in a mirror image to the negative ones were distributed widely in the stratum radiatum and the stratum lacunosum-moleculare (the territory of apical dendrites of pyramidal cells), the neighboring fascia dentata and the underlying thalamic region. Reverse in the polarity from negative to positive occurred always around the stratum pyramidale. These findings suggested that the electromotive force of the septal-induced responses might be attributable mainly to EPSP currents generated in the stratum oriens. Following iontophoretic injection of HRP into the stratum oriens, retrogradely labeled cells were confirmed in the medial septal-diagonal band complex. However, after injections of HRP into other CA1 strata, labeled cells were scarcely found there. Thus, it was indicated that the septo-hippocampal projection sends excitatory inputs to the basal dendrites, but not to the apical dendrites of CA1 pyramidal cells.

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POSTNATAL DEVELOPMENT OF CORTICAL PROJECTION FROM THE PULVINAR IN THE CAT. KATO, N. and KAWAGUCHI, S. Inst. for Brain Res., Fac. of Medicine, Kyoto Univ. Kyoto 606

We examined postnatal development of cortical projection from the pulvinar (Pul) in the cat using the orthograde and retrograde HRP methods, and the field potential method. After HRP injection into Pul of kittens at any age and an adult cat, labeled terminals were observed most densely in the middle suprasylvian gyrus and, to a lesser extent, in the lateral wall of the lateral sulcus, area 19 and the splenial visual area, indicating that topographical arrangement of the projection from Pul is established before birth. Cortical laminar distribution of the terminals, by contrast, changes with age. In kittens of less than 2 weeks old (N=13), the terminals were distributed mostly in layer I, whereas they were distributed largely in layers III and IV in kittens of more than 1 month old (N=8) and an adult cat. Correspondingly, after HRP injection into layer I of MSSG, distribution of neurons retrogradely labeled in the thalamus was different between younger and older kittens. The neurons were located largely in Pul (32.1%) and VA-VL (35.7%) in younger kittens, while they were located largely in VA-VL (87.1%) and only sparsely in Pul (4.9%) in older kittens. Stimulation of Pul elicited in MSSG two different types of responses according to animals' age. The response was surface-negative, depth-positive in younger kittens, whereas it was surface-positive, depth-negative in older kittens. These electrophysiological changes correspond well with the anatomical findings.

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STABILITY OF THE M-AND H-RESPONSES DERIVED FROM DORSO-VENTRAL PART OF THE LEG IN MAN.

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The aim of this study was to record M-and H-responses derived from a homonymous muscle without interference of evoked EMG from other muscles in lower extremity.

The M-and H-responses were used to be led bipolarly from two electrodes, placed 3 cm apart on the skin over the soleus muscle and the gastrocnemius muscle, where the amplitude of the reflex was measured on largest deflection. The responses in this electrode position, however, were labile in amplitude and its polarity because of a mixture of potentials derived from both of the soleus and the gastrocnemius. Therefore, we proposed a suitable recording site between the skin over the tibia and the skin over the soleus, about 2 cm below the point where the gastrocnemius with two heads joints the Achilles tendon. Both of the M-and H-responses were almost derived from the soleus, showed the same biphasic pattern in wave and kept it on increasing in electrical stimulation required for the recruitment curve for the M-and H-responses. In the data collected from 5 persons, the amplitude of the H-response fell in the range of 20-24 mV in maximum, while M-response in the range of 30-34 mV. And when the M-and H-responses were recorded at every 5 seconds for 2 hours, the fluctuations of amplitudes of both responses ranged from 0 to 12 mV and their average values were 4-6 mV.

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MOTOR UNIT PROPERTIES OF MEDIAL GASTROCNEMIUS MUSCLE IN THE AGED RATS.

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Isometric contractile properties of motor units were measured in medial gastrocnemius (MG) muscle of the aged rats (Fischer, male, 26- to 30-month-old) anesthetized with urethane and chloralose. Blood pressure and end-tidal CO_2 level were monitored throughout the experiment. The MG muscle was carefully dissected so that good blood circulation to the muscle was maintained, and was covered with warmed mineral oil. The ventral root was dissected until it was ensured that it contained only one motor fibers to MG muscle. Motor units were classified by using the same criteria as those previously employed for cat hindlimb muscles.

When compared with a motor unit sample of the younger animals (11- to 14-month-old), there was a large increase in percentage and in mean tetanic tension for type S motor units in the aged animals, whereas a slight decrease for type F motor units. There was no alteration of twitch contraction times in any unit type. Post-tetanic potentiation of type F units significantly declined in the aged animals. A considerable decrease in the average conduction velocity of motor axons was observed in aged animals. The more marked decrease was found among the type F units.

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BEHAVIOR, POSTURE AND PERIPHERAL NERVOUS ACTIVITY OF NIEMANN-PICK MOUSE.

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The Niemann-Pick mouse is a mutant of C57BL/KsJ mouse. We studied behavior and posture of this mouse, and measured the conduction velocity and threshold of the sciatic nerve. Moreover the spontanious discharges from motor units were recorded from the sural muscle. Behavioral and postural changes were divided into two steps in the cource of this disease. First change appeard in about 7 weeks. We could observe bradykinesia, asynergia, intension tremor and dysmetria in this age. These symptoms were observed more frequently in the lower extremities than in the upper, and progressed as this mouse grew older. Second change appeared in about 9 weeks. This mouse showed abnormal locomotion, in which hip, knee and foot joints took in flexural positions. This posture was due to the decrease of tonus in the sural muscle, while this mouse preserved postural reflexes in this age. In electrophysiological studies, we obtained two results.
I) There were no statistical difference in the conduction velocity and threshold of sciatic nerve between the Niemann-Pick and the normal mouse. II) In the Niemann-Pick mouse, the phasic activity of the sural muscle was preserved, but we observed the decrease of the tonic activity of this muscle in EMG.

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CHANGES OF MUSCLE FATIGUE AND EVOKED ACTION POTENTIAL IN CALF MUSCLE DURING TETANIC CONTRACTIONS. MURO, M., NAGATA, A. AND YONA, M. Bio-dynamics Lab. Tokyo College of Pharmacy, Hachioji 192-03.

The experiments were designed to study the myo-physiological effect on the blood flow in the contracted muscle resulting from tetanic stimulation(f=20Hz & 80Hz,d=0.3ms), tibial nerve, in triceps surae muscle in human. The tetanic force and evoked potential of gastrocnemius(MG) recording were made at the three times under each circulatory conditions that during tetanic stimulation was performed with a free circulation and with continuing ischaemia for 60s and release of circulation at after 30s. When the circulation was relesed from occlusive ischaemia on thigh after 30s at 20Hz stimulation, the constant force was evidently decreased to blood flow immediately. However, the blood fluid entering the muscle of tetanic contraction, tetanic force was decreased synchronous about 10% the amount of the initial level of force. On the other hand, the tetanic force, being in occlusive ischaemic state, was not approximately decreased to initial level. The force of tetanic contraction is graded according to both the force generated in each muscle fiber and the number of fibers recruited. These results suggest that the decreased force are attributed to change in conduction mechanism of neuro-muscular junction as the muscle temperature increased of the released blood flow.

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THE EFFECT OF CONTENT OF ATP WITHOUT CIRCULATION DURING TETANIC CONTRACTION INVESTIGATED BY ^{31}PNMR. SAKUMA,T., MURO,M., YONA,M., NAGATA,A. Analytical Science Center,Tokyo college of Pharmacy,Hachioji 192-03

The influence of muscle contraction,induced by electrical atimulation,on the contents of high energy phosphates and amount of blood flow have been investigated in plantar flexion muscles in rats. The depression of ATP in rat muscle,gastrocnemius(fast muscle) and soleus(slow muscle),after tetanic contraction(20Hz stimulation)for one minute with and without circulation was measured.

The content of ATP in gastrocnemius and soleus muscle was not decreased after tetanic contraction throughout each conditions.On the contrary,the content of CrP after stimulation was decreased in gastrocnemius than in soleus but inorganic phosphate(Pi) resulted in three-to seven-fold higher release after tetanic contraction.The increase of Pi contents is of importance for increasing the glycogenolytic rate as flood circulation was increased,it follow that concentration of ATP in contractile muscles was maintained approximetly content of control level.

Therefore,depression of ATP in contractile muscle was indepent on blood circulation that during tetanic contraction blood flow was limited as complete occlution and incomplete circulation.However,slow muscle(soleus) was slightly increased than in fast muscle (gastrocnemius).

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FROG MUSCLE SPINDLES INNERVATED BY FUSIMOTOR (γ) NERVE FIBER. ITO, F. Dept. Physiol., Nagoya Univ. Sch. Med., Nagoya 466

It has been known that the mammalian muscle spindles are innervated by γ and β (skeleto-fusi) motoneurones whilst the ampibian spindles are done only by β motor system without γ system. However, some muscle spindles in semitendinosus muscles of the frog (Rana catesbeiana) are innervated by a γ motor nerve fiber. A single or repetitive stimulation of a γ fiber in ventral root resulted in an increase in the spontaneous activity of the muscle spindle without detectable contractions of the extrafusal muscle fibers. When the muscle spindle was stretched during the repetitive stimulation, the static response in the rate of afferent discharges was increased.

The γ innervation was certified by light and electron microscopic observations of a glycogen depletion method. Those muscle spindles were found to be located superficially in the vicinity of the entrance of nerve trunk and to be innervated by a thin nerve branch in which a sensory axon and a γ axon without branch were involved.

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RELATION BETWEEN FORCE SPEED AND DISCHARGE RATE OF SURFACE EMG DURING VOLUNTARY ISOMETRIC CONTRACTION.
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The relation between force speed and discharge rate of integrated surface e.m.g. was studied when force speed matching tasks were performed with thumb(adductor pollicis) flexion and foot(tibialis anterior) dorsiflexion in man. Subjects were instructed to exert forces with various amplitudes matching at some target force speeds. Surface e.m.g., integrated e.m.g., force curve and force velocity were recorded simultaneously.

The amount of discharges increased with increment of exerted force. Furthermore, the discharge rate increased with increment of force speed gradually. However, characteristic high discharge rates were found when voluntary force exertions were performed within very short time to peak force, although force amplitude and force speed were low. It is suggested that the shortness of the time to exert force, besides the force speed, might cause the specific change for the modes of muscle discharge.

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CHARACTERISTIC OF DISCHARGE FREQUENCY IN HUMAN MOTOR UNITS DURING BALLISTIC CONTRACTION.
KIMURA,M.,YONEDA,T.& ISHIDA,A. Dept.of Physiol., Sch.of Physical Edu. Juntendo Univ. Narashino, Chiba.

The motor unit frequency responses of foot muscle(Tibialis anterior) during voluntary isometric contraction at various speeds and force amplitudes were investigated. Plastic embedded fine bipolar electrodes which were made from copper wires were inserted into the muscle for recording of motor unit discharges. Twelve motor units from 5 adult men during force exertion under 25% of MVC were studied. Each motor units revealed almost same low firing rate(less than 20 Hz) when force exerted over 150 msec time to peak force, although the force amplitudes and force speeds increased. However, when forces exerted within 150 msec time to peak force, firing rate increased extremely(50-85 Hz) even if amplitude and speed of force were high or low. It is suggested that the recruitment might play main role in the control of voluntary contraction in tibialis anterior, furthermore, the shorteness of the time to peak force might cause the motoneurones to modulate the specific frequency response.

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THE RELATION BETWEEN THE DISCHARGE PATTERNS AND THE CONTRACTILE PROPERTIES OF HUMAN MOTOR UNITS IN VOLUNTARY ISOMETRIC CONTRACTION.
OGAWA,Y., KURATA,H. AND YONEMOTO,K. Lab. Sports Med., Jikei Univ. Sch. Med., Chofu, Tokyo 182

To examine the contractile properties and the modes of discharge of single human motor units(MUs), action potentials of MUs of anterior tibial muscle were recorded during voluntary isometric contraction using coil-shaped inserted electrodes. The contraction time(C.T.), the peak tension(P.T.) and the recruitment threshold(Fth) of MUs were determined similar to previous report. To determine the discharge modes, tension of dorsiflexion of ankle was held constant at various levels. The mean discharge intervals($\bar{\tau}$) and the standard diviations(S.D.) were calculated. S.D./$\bar{\tau}$ were plotted against the $\bar{\tau}$, and were analyzed the relation between S.D./$\bar{\tau}$ and $\bar{\tau}$ by quadratic regression analysis. The top of y coordinates means the degree of regularity in discharge intervals and the top of x coordinates means the discharge intervals at the minimum y values. The negative correlation between x values at minimum y values and y values were observed among each MU. x values of MUs with higher Fth was shorter than that of lower Fth. The correlation between x values and C.T. was no significant. y values were greater in the MUs with higher Fth and shorter C.T. than that of MUs with lower Fth and longer C.T.. It is suggested that the regularity of discharge intervals affects the speed of twitches of motor units.

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VARIATIONS IN THE RECRUITMENT FORCE THRESHOLD OF SINGLE HUMAN MOTOR UNITS WITH KNEE AND HIP JOINT ANGLES.

KURATA, H., OGAWA, Y. and YONEMOTO, K.
Lab. Sports Med., Jikei Univ. Sch. Med., Chofu, Tokyo 182

To examine the property of motor unit activities at different knee (45°-135°) and hip (0°-90°) joint angles, action potentials of single motor units (MUs) from medial vastus, lateral vastus and rectus femoris muscles were recorded. The values of torque output during an isometric contraction in various knee and hip angles were recorded by force-displacement transducer which secured to the lower leg. The force threshold (FT) in each MU was measured. Ratio of the FT in %MVC relative to maximal FT increased from 10% to 60% in the medial vastus muscle, increased from 15-25% to 55% in the lateral vastus muscle, and decreased from 100% to 30-40% in the rectus femoris muscle when the knee angle changed from 135° to 45° at 90° of hip joint angle. The ratio at 45° of knee angle increased in the medial and lateral vastus muscles and the ratio at 135° and 120° decreased in the rectus femoris muscle when the hip angle changed from 90° to 0°. These data support that the inhibition to α motoneurons of medial and lateral vastus muscles at the extended position of the knee is powerful at the extended position of the hip, inversely, the inhibition at the flexed position of the knee is weakend in the rectus femoris muscle at the extended position of the hip.

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MUSCLE FIBERS LENGTH AND CONDUCTION VELOCITY IN HUMAN SINGLE MOTOR UNITS.

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The length of muscle fibers is dependent on the joint angle. Haines (1934, 1935) reported that the length of the vastus intermedius muscle in the human body changed from 80mm to 185mm when the knee joint moved from the fully extended to flexed position. However, there is no method to measure muscle length from the surface of the living human body. Therefore, first of all, the author attempted to measure the muscle length in the living human body by analysing the shape of an action potential of a single motor nuit.

The effect of the change in length on the conduction velocity has been investigated. In nerve truncks of the slugs, the stretching has no significant effect on the conduction velocity of the action potential (Jenkins and Carlson 1904, Bullock et al. 1950). With respect to muscle, Martin(1954) reported that the conduction velocity was unaltered by the application of the stretch, while many investigators found an increase in the conduction velocity during stretching the muscle (Wilska and Varjoranta 1940, Hakansson 1957). Therefore, the author investigated the relation between the conduction velocity and the length of muscle fibers.

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THE EFFECT OF EMG-REACTION TIME OF CENTRAL-PERIPHERAL NEUROMUSCULAR SYSTEM ON IPSILATERAL- AND CONTRATERAL-RESPONSES IN THE HUMAN UPERARM.

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Our purpose was to test the hypothesis that simple EMG-reaction time (EMG-RT) of healthy adult men is correlated with respondency of ipsilateral and contrateral, or with the difference of ablearm and unablearm for subjects. The visual stimulation is from righ eye, left eye, and both eyes simultaneity. The reaction arm is right and left arm, respectibly. EMG activity is recorded with paris of closely spaced silver disc surface electrodes applied over thebiceps brachii muscle. EMG-reaction time measurements are made from stimulation signal to EMG onset . EMG-RT was determined the different experiment conditions. The results indicate that the stimulation condition of three types was no significant in EMG-RT or premotor time because this results are attributed to athleted subject at every day. On the other hand, the EMG-RT was longer in the sedentary subjects and then EMG-RT of contrateral-response was evidentry decreased to compare ipsilateral and both eyes response.

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REFLEX RESPONSES OF LEG EXTENSOR AND FLEXOR MUSCLES TO CUTANEOUS ELECTRICAL STIMULATION IN MAN. OKANO, K., TANJI, J. and KATO, M. Dept. Physiol. Sch. Med. Hokkaido Univ., Sapporo, 060

Reflex responses of leg extensor (triceps surae) and flexor (tibialis anterior) muscles to cutaneous electrical stimulation were studied in man with special attention to motor units (MUs) with different recruitment thresholds. In both of the muscles, responses falling into two categories were obtained, one with short (about 50 ms) and the other with long (70-80 ms) latencies. The short latency reflex effects in low threshold MUs were predominantly excitatory in the flexor and inhibitory in extensor muscles. Since the number of high threshold MUs successfully recorded in isolation long enough for quantitative study was rather small, the effects on them were estimated mainly on the basis of surface recording EMG at forceful contractions. In most cases their short latency effects were different from those of low threshold MUs; inhibitory in the flexor and excitatory in the extensors. The long latency responses were largely inhibitory in both low and high threshold MUs recorded from flexor as well as extensor muscles. Thus the reflex patterns in human leg muscles were basically in accord with those reported previously in cats. Some additional analyses were made on reflex effects obtained with different stimulus intensities and stimulus sites.

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RECIPROCAL Ia INHIBITION UPON INITIATION OF VOLUNTARY MOVEMENT IN MAN. KAGAMIHARA,Y. and TANAKA,R., Department of Neurobiology, Tokyo Metropolitan Institute for Neurosciences, 2-6 Musashidai, FUCHU, Tokyo 183.

Reciprocal inhibition of the soleus motoneurons upon initiation of voluntary ankle dorsiflexion appears almost simultaneously with the onset of the agonist (m.tibialis anterior) emg (Neurosci. Lett., in press). For revealing its underlying mechanism, activity of the reciprocal Ia inhibitory pathway was investigated in seven normal subjects. Reaction time method was used for controlling the movement initiation and Lloyd's method with H-reflex for testing excitability of the soleus and pretibial motoneurons, and of the Ia inhibitory interneurons which are activated by the G-Ia afferents from the ankle flexors. Facilitation of Ia interneurons started several tens msec prior to the onset of the agonist emg, in parallel with the facilitation of the agonist motoneurons. Amount of the Ia inhibition increased as the voluntary effort was strengthened. Around 70-100msec after the emg onset when excitability of the antagonist motoneurons briefly recovered due to a phasic stretch of the ankle extensor muscles by dorsiflexion movement, depression of the Ia inhibitory pathway was also observed. We conclude that, upon movement initiation, the Ia inhibitory pathway is activated in parallel with the agonist motoneurons by supraspinal motor centers and contributes to the reciprocal inhibition of antagonist motoneurons.

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NEUROANATOMICAL AND NEUROPHYSIOLOGICAL RESEARCH ABOUT THE KEIKETSU. HATAMOTO, H. Dept. of Physiol., Fac. of Med., Kyoto Univ., Kyoto 606

It is said that there are 14 channels and 354 KEIKETSUS in the human body wall. By dissecting 91 cadavers, it was found that KEIKETSUS were located in the epithelial, connective, muscle and, especially, nervous tissues. For example, SANRI is located at the deep peroneal nerve, YORYOSEN at the superficial peroneal nerve, GOKOKU at the radical nerve, JIRYO at the dorsal ramus of S_2, etc. When the nerve density in a square of 1 cm between the head of fibula and the tuberosity of tibia was measured, it was high in SANRI and YORYOSEN. SANRI is a point where the deep peroneal nerve penetrates the connective tissue between the ext. digitorum longus and tibialis anterior muscles. Some fibers of the deep peroneal nerve climbs up and arrives at the deep fascia under the skin. YORYOSEN is a point where the superficial peroneal nerve enters in the peroneus longus muscle. EMG was recorded with a surface electrode at SANRI and YORYOSEN on voluntary movement. Its amplitude was large at SANRI on foot dorsiflexion and inversion, but on foot plantar flexion and eversion EMG did not appear at SANRI. This pattern was opposite when recorded at YORYOSEN. Immediately after sticking an acupuncture needle into SANRI and YORYOSEN, EMG was observed without voluntary movement. By single electric stimulation of SANRI, the blood flow of the foot was remarkably reduced, but it was scarcely changed by stimulation of other areas near SANRI.

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MUSCULAR DYSTROPHIC MICE AND DWARF ONES: A COMPARISON BETWEEN THE MATURATIONAL DEFECTS OF THEIR MUSCLES.

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For the pathogenesis of murine muscular dystrophy, we have proposed a bone-muscle imbalance hypothesis consisting of two steps, the primary stage (a maturational defect of muscles) and the secondary one caused by the almost normal growth of bones. This hypothesis was in part evidenced by the fact that muscular dystrophy was almost latent in genotypically dystrophic-dwarf mice. Similar facts were reported in man and animals. In the present work, NADH-dehydrogenase (NADH-DH) activities in hindleg muscles of dystrophic (C57BL/6J dy/dy), dwarf (DW/J dw/dw), and their respective normal control mice were compared in view of maturation. While NADH-DH activies decreased with age in dwarf muscles, of which growth had been arrested, similarly as in the normal controls, they changed little with age in dystrophic muscles.

The present results provide further evidence that dystrophic muscles fail to mature and that, paradoxically, dwarf muscles of which growth is arrested mature almost normally, in support of our previous findings.

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PERIOD OF THE CIRCADIAN RHYTHM CHANGED AFTER TRANSPLANTATION OF SUPRACHIASMATIC NUCLEI IN RATS. SAITO, Y.*, NIHONMATSU, I.* and KAWAMURA, H., Lab. Physiol. Psychol., Mitsubishi-Kasei Inst. Life Sci., Machida-shi, Tokyo 194.

After transplantation of neonatal suprachiasmatic nuclei (SCN) into the 3rd ventricle of SCN lesioned rats, we have observed reappearance of circadian rhythmicity in wheel running activity as reported previously (Sawaki, Y. & Kawamura, H., J. Physiol. Soc. Japan, 45: 462, 1983). Up to now, we have at least 4 successful cases which showed totally different circadian periods before and after transplantation. In all cases, the period of circadian rhythmicity originally longer than 24th changed to the period shorter than 24th after transplantation and *vice versa*, showing the activity of a totally new circadian rhythm oscillator contained in the transplanted tissue. Immunohistochemical study verified that these grafts contained both vasopressin-like and vasoactive intestinal polypeptide-like immunoreactive areas, which were found in intact SCN. Histological examination of all 12 grafts, which enabled to reproduce the circadian rhythm, revealed that in all but one case, grafts were located caudal to the SCN. Also, the ependymal layer did not invade between the graft and the host hypothalamus. These findings suggest that the location of our transplantation is a suitable site for SCN grafts in terms of axonal ingrowth and reinnervation to receptor sites near the wall of the 3rd ventricle after dennervation by SCN lesions.

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ROLE OF RETINAL PHOTORECEPTORS IN GENERATION OF OFF-RESPONSE IN THE LOCOMOTOR BEHAVIOR OF THE GIANT LAND SNAIL, ACHATINA FULICA. OGASAWARA, T. AND TASAKI, K. Dept. of Physiol., Tohoku Univ. Sch. of Med., Sendai 980

In order to elucidate the role of retinal photoreceptors in generation of off-response (transient light-off suppression of locomotor activity), the behavioral response was examined on intact, one-eye removed and eyeless snails, presenting light pulses in the dark periods of LD 12:12 cycles. Threshold intensities of blue and red lights which could elicit off-response were about 10^{-8} and 10^{-4} $\mu W/cm^2$ respectively in intact snails and abuot 3×10^{-7} and 3×10^{-3} $\mu W/cm^2$ respectively in one-eye removed ones. Habituation phenomena could be observed when 5 minutes duration of light pulses were presented every 10 minutes, and off-response was recovered with a longer duration (10 minutes) of pulse given. However, eyeless snails did not show off-response and responded to light pulses (10^{-2} $\mu W/cm^2$) with light-on suppression of locomotor activity.

These results suggest that off-response of Achatina might be mediated by sustained type retinal photoreceptors which have high photosensitivity (Tasaki and Suzuki, 1979).

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THE UNITARY ACITIVITY IN THE VENTRAL TEGMENTAL AREA DURING COPULATORY BEHAVIOR IN THE MALE RAT. HORIO,T.,SHIMURA,T. AND SHIMOKOCHI,M. Dept. of Behav.Physiol., Fac. of Human Sci., Osaka Univ., Suita, Osaka 565.

The ventral tegmental area (VTA) has been indicated to modulate the male copulatory behavior of the rat by the previous studies using lesion technique as well as electrical stimulation technique. In this study, we recorded the unitary activity of the VTA neurons during free copulatory behavior of the rat. Five diffenrent patterns were found in the activities, compared with the baseline activity prior to the introduction of a female rat. 1.A transient increase during pursuit-mounting behavior. 2.A transient decrease during pursuit-mounting behavior. 3.A transient decrease after mount. 4.A sustained increase after the introduction of the female until the ejaculation. 5.A sustained decrease during refractory period. These results suggest that VTA might play a regulatory role in execution of male copulatory behavior.

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EFFECTS OF KAINIC ACID ON LEARNING BEHAVIOR IN MICE (II). MURAKAMI, T.H. Dept. of Biol., Kagawa Med. Sch., Kagawa, 761-07

Mice of ddN-strain, weighing between 25~30 grams, were used in this experiment. Kainic acid (0.2 μl of saline containing 0.075 μg) was slowly injected bilaterally into several places of the brain at different depths. Two weeks after administration each mouse was tested in the locomotor activity and the avoidance training. Upon completion of the avoidance training, all the brains were examined for morphological alterations, and compared with training scores and histological results. Morphological examination of coronal sections with light microscopy revealed a close association with the rate of learning deficits and with the degree of the pyramidal cell damage to the homotopic CA3, and ipsilateral CA3 and CA1 regions of the hippocampus.

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BEHAVIORAL CHANGES OF RAT WHICH DEVELOPED NEURONAL DISTURBANCE BY ADMINISTRATION OF CLIOQUINOL AT ITS NEONATAL PERIOD. HORI, S., OHTANI, S. and SUGIURA, H. Dept. Neurochem., Tokyo Metropolitan Institute for Neurosciences, Fuchu-city, Tokyo.

Clioquinol (5-chloro-7-iodo-8-hydroxyquinoline) develops neuronal disturbance on animals such as human, rabbit, monkey and rat. However, toxity of clioquinol is extremely variable in each individual of animal. Then, to study biochemically the process of neural disturbance induced by neurotoxicity of clioquinol, selection of animal group with less variation in neural disturbance is essentially necessary. In this paper, we tried to estimate the neural disturbance of rat by observation of behavior, sensitivity of the skin or muscular strength. Some rats which were subcutaneously injected clioquinol (100-200 mg/kg.body weight) once a day during 20days after birth and were breeded for several weeks after the final shot of clioquinol showed significantly high activity in daily spontaneous movement, although no change on the circadian rhythms of spontaneous movement. Sensory disturbance was observed on the test of heat sensitivity, that is, threshold to heat stimulation was shifted to high temperature. The endurance of walking behavior, which was tested by the rotor road, was no difference between control rats and rats suffered ataxia by clioquinol. In the process to induce the neural disturbance, rats could be hereditarily separated into three groups such as highly sensitive, intermediately sensitive and resistant to toxicity of clioquinol.

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METHAMPHETHAMINE EFFECTS ON CIRCADIAN LOCOMOTOR RHYTHM OF RATS. HONMA. S., HONMA. K. and HIROSHIGE, T. Dept. Physiol. Hokkaido Univ. Sch. Med. Sapporo, 060

Effects of chronic administration of low doses of methamphetamine (Met-Amp) were studied on the circadian locomotor rhythm of rats.
Materials and Methods : Met-Amp dissolved in drinking water (0.0025-0.01%) was administered to rats continuously. Met-Amp effects were examined in the light-dark cycle (LD) and in the constant darkness (DD). Effects of Met-Amp withdrawal was also observed.
Results : Under the LD cycle, the phase-angle difference between the onset of locmotor activity and the time of lights-off increased after the Met-Amp treatment. In an advanced stage, signs of relative coordination, such as an unstable phase relation to the LD cycle and periodical phase-jumps appeared. In some rats, splitting of locomotor rhythm into two activity components was observed, one component entraining to the LD cycle while the other free-running. Met-Amp withdrawal resulted in an reentrainment of the circadian rhythm with a transient period of several days. Elongation of the free-running period beyond the limit of entrainment, and a change in the sensitivity to light were excluded as the main reason, because the changes in the circadian rhythm continued in DD. A change in the coupling between two activity components which comprise the locomotor rhythm, or the interaction between the circadian rhythm and other oscillation induced by Met-Amp was more probable explanation for these changes.

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ENTRAINMENT OF HUMAN CIRCADIAN RHYTHMS TO AN ARTIFICIAL LIGHT CYCLE IN A TEMPORAL ISOLATION UNIT. HONMA, K., HONMA, S., *WADA, T., AND HIROSHIGE, T. Dept. Physiol., Sch. Med., *Coll. Med. Technol., Hokkaido Univ., Sapporo, 060

Entrainability of an artificial light cycle to human circadian rhythms was examined in a temporal isolation unit, using a bright light of 5,000 lx during the light period. Materials and Methods : Two healthy male students (23 and 24 years old) lived alone in an isolation unit for 2 weeks without knowing the time of day. Contact with the subject was carried out by letter. Meals were supplied to the subject. Rectal temperature was measured continuously by a thermister, which was transmitted to a recorder outside the unit. Sleep time was recorded by inputing the signals of sleep-onset and waking-time by the subject himself. In the unit, the subjects were allowed to turn-on and -off the lights of low intensity (about 500 lx) at any time. An artificial light cycle was set by illuminating the unit with a 5,000 lx bright light for 8 hours every day (LD 8:16), which was imposed on the subject during the second week in the unit. Results :The circadian rhythms free-ran with a period longer than 24 h during the first week in the unit. The rhythms entrained to the artificial light cycle in the second week. The phase angle difference between the sleep-wakefulness cycle and the temperature rhythm was essentially the same during the periods of free-running and entrainment. The results indicate that a bright light is albe to entain human circadian rhythms.

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Phase shift and phase response curve of pineal-dependent locomotor activity rhythm. SAMEJIMA, M., UCHIDA, K. and MORITA, Y. 1. Dept. Physiol., Hamamatsu Univ. Sch. Med., Hamamatsu 431-31

River lamprey, Lampetra japonica, which has a directly photosensitive pineal organ, is nocturnal. The effect of light on its locomotor activity was investigated. Lampreys were separately hold in plastic cylinders in aquariums. The movement of the cylinder was picked up by a strain gauge. Six hours phase delay or advance of light-dark 12:12 cycle produced re-entrainment with one or two delaying or advancing transient days. That of 12 hr produced re-entrainment without clear intervening transient. Bilateral enucleation had no effect on re-entrainment. This indicates the pineal organ may be a photoreceptor for entraining light. To obtain a phase response curve, two hours light pulses (250 lx inside the cylinder) were applied at every seven days to lampreys which showed free-running under the continuous darkness. The average free-running period was 21.1±0.7 hr. The light pulse given at 9 o'clock in circadian time (CT) resulted in 8 hr advance of the free-running and that at 4.5 o'clock resulted in 9 hr delay. The phase shift almost linearly related to the CT in the range from 9 to 4.5 o'clock.

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CIRCADIAN RHYTHM OF UTERINE CONTRACTIONS IN RABBITS. FUJITANI,Y., HOSOGAI,M., OIKAWA,T. Dept. of Physiol., Tottori Univ. Sch. Med., Yonago 683

Spontaneous contractions and electrical activities of the uterus were continuously recorded during several days up to 20 days in the longest under unanesthetized and unrestrained state of 4 non-pregnant rabbits. The records of uterine contractions were obtained using a water-proof staingauge sewed onto the uterine wall. Usually, a light (L) and dark (D) cycle of each 12 hrs was used as room illumination. Time series of large contraction waves with multipeaks and 10 mins' or longer intervals gave simpler findings, though single waves with variable heights were brought about in the order of min. Analyses on the average intervals of large contraction waves revealed that they were significantly lengthened in the D period compared to those in the L period. The differences of average intervals in large contraction waves between D and L periods, however, became to be insignificant in natural illuminating circumstances. The trends in the interval series of large contraction waves were ascertained also by the methods of autocorrelation function and power spectrum.

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SEASONAL CHANGES OF CIRCADIAN RECTAL TEMPERATURE RHYTHMS IN SUBJECTS SPENDING A NORMAL REGULAR LIFE UNDER NATURAL CONDITIONS. TAMURA, N. AND TOKURA, H. Lab. of Physiol., Dept. of Clothing Sci., and Div. of Human Life and Environ. Sci., Graduate Div. of Human Culture, Nara Women's Univ. Nara 630

We studied effects of season on circadian rectal temperature rhythm in subjects spending a normal regular life under natural conditions. Two males (45 yrs, 38 yrs) and a female (22 yrs) served as subjects. Rectal temperature was continuously recorded by means of "Environmental Body Temperature Recorder (YM-1)" for 7 to 10 days every 5 min during spring, summer, autumn and winter, respectively. The morning rise of rectal temperature rhythm in a male began at 0:00 a.m. in spring, 6:00 a.m. in summer, 4:00 a.m. in autumn and 2:00 a.m. in winter. Thus, significant delay of morning rise time occurred in summer in all 3 subjects. These seasonal changes might be ascribed to interaction of endogenous and exogenous components such as circannual changes of freerunning τ in the rhythm and seasonal changes of photoperiod length and ambient temperature.

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THE DIFFERENCE OF CIRCADIAN OSCILLATION SYSTEM BETWEEN SLOW WAVE SLEEP(SWS) AND PARADOXICAL SLEEP(PS) IN THA RAT. YAMAOKA, S. Dept. of Physiol. Saitama Medical School, Moroyama-cho, Iruma-gun, Saitama 350-04

In earlier reports, we suggested that the circadian oscillation system of SWS was different from that of PS in the study of hypothalamic lesion, phamacological manipulation and aging process. Namely, 1),the circadian phase of SWS and PS dissociated during returning from three months exposure of constant illumination. 2),the SCN lesion disrupted the circadian SWS rhythm, but not the PS rhythm. 3),the intra-SCN injection of PCPA disrupted the circadian PS rhythm, but not the SWS rhythm. 4),the changing pattern of SWS in aging process differed from that of PS. 5),however, the intraventricular transplantation of newborn SCN restored the circadian cyclicity of the rhythm disrupted aged rat. From these results, it is speculated that both circadian oscillators exist in SCN and have the functional differentiation. On the other hand, it is known that the VIP neuron and the vasopressin neuron are the efferent from SCN. In the preliminary experiment, the Brattleboro rats(about one year old) did not show the circadian cyclicity in both SWS and PS. The intraventricular injection of dPTyr(Me)AVP (the antagonist of vasopressin) supressed the circadian amplitude of SWS and enhanced the circadian cicliicity of PS, but the anti-VIP(made by Dr. Arita) did not affect the sleep rhythm. But it needs further experimental studies to clarify this functional difference.

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MECHANISMS CONTROLLING THE PARADOXICAL SLEEP RHYTHM. HANADA, Y. and KAWAMURA, H., Lab. of Physiol. Psychol., Mitsubishi-Kasei Inst. Life Sciences, Machida-shi, Tokyo 194.

To study the locus of a pacemaker of paradoxical sleep rhythm, polygraphic records of fast wave (FW) ECoG, hippocampal theta wave, EOG and EMG atonia of neck muscles taken for 6 hours a day from 0 p.m to 6 p.m were analyzed in 5 chronic cerveau isolé (C.I.) male rats. The total time and averaged duration of FW ECoG and EMG atonia in C.I. rats were similar to those in 4 control intact rats. The FW ECoG representing forebrain activity were totaly independent from neck muscle EMG representing hindbrain activity in C.I. rats. Total time, averaged duration and averaged interval of the neck muscle atonia, were similar to paradoxical sleep in intact rats. Recording for 24 hours a day indicated that the circadian rhythm in the forebrain ECoG remained unchanged, whereas the rhythm in the hindbrain was abolished in C.I. rats. The similarity of the total time of FW ECoG both in intact rats and in C.I. rats suggests that the FW in C.I. rats contains not only wakefulness but also paradoxical sleep component. In the previous study, we found that the multiple unit activity recorded from the posterior hypothalamus increased earlier than the ECoG at the onset of paradoxical sleep. Such changes were also found in C.I. rats. Functional relationship of pacemaker mechanisms between the forebrain and hindbrain for induction of paradoxical sleep in intact rats awaits further study.

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AROUSAL FROM SLEEP IN CATS (II) : A BRAIN ELECTROSTIMULATION STUDY. MATSUMOTO, H., MORITA, Y., SENO, H., *MATSUMOTO, J. AND **SATO, K. Dept. Physiol., Univ. Tokushima, Sch. Med., Tokushima 770, *Dept. Human Culture, Naruto Univ. Teachers Education, Naruto 772 and **Res. Foundation on Traffic Med., Tokyo 170

We aimed at presenting further basic data on the relation between arousal mechanism and sympathetic activity with brain electrostimulation in chronic cats. For the brain electrostimulation, we used low voltage rectangular pulses (0.5-5 V, 0.2 msec) of gradual increase frequencies varied from 14 Hz to 80 Hz, in order to examine the arousal threshold from a deep sleep state under polygraphic recordings. The autocorrelogram, autoregressive power spectra of EEG and of its component waves were also computed with an autoanalysing system. Through examination of brain electrostimulations, we found that both EEG activation and behavioral arousal were elicited with the lowest current intensity of 0.05-0.15 mA in the stimulation of the ventromedial nucleus of the hypothalamus (VMH), a main sympathetic center. Furthermore, the current intensity of stimulation which triggers the initiation from EEG activation to behavioral arousal was lower in VMH than in the anterior ventral nucleus of the thalamus, in the mesencephalic reticular formation and in the cerebral cortex, respectively. From these facts, it is suggested that the excitation of the sympathetic activity plays an important role in the mechanism of the initiation from sleep to arousal.

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SLEEP IN AGED RATS. SANO, A. AND MORITA, Y. Dept. Physiol., Sch. Med., Univ. Tokushima, Tokushima 770

We have studied age-related sleep changes in rats. Five young adult (2-4 mounths) and five aged (20-30 mounths) Wistar male rats with chronically implanted electrodes for polygraphic recordings were subjected to a 6 a.m.- 6 p.m. light, 6 p.m.- 6 a.m. dark schedule for two weeks before a 24 h recording session. Percentages of time spent in awake (A), slow wave sleep (SS) and paradoxical sleep (PS) were computed and compared in the above two groups.

Aged rats spent significantly ($p<0.05$) more time in A, but significantly ($p<0.05$) less time in SS and PS. PS in aged rats was frequently interrupted by episodic A or SS. The number of PS episodes increased and its duration was shortened in aged group. Also, the examination of the time spent in various PS episodes (<1, 1-2, 2-3, >3 min) was carried out. The number of PS of a shorter duration than one min was significantly increased and the number of PS of a longer duration than 3 min significantly decreased in aged rats.

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A THEORY ON THE EVOLUTION OF SLEEP AND SLEEP SUBSTANCES. INOUÉ, S., HONDA, K., OKANO, Y. and KOMODA, Y. Inst. for Med. Dent. Engineering, Tokyo Med. Dent. Univ., Chiyoda-ku, Tokyo 101

Based on the experiments in fish and insects, a theory is proposed on the evolutionary aspects of sleep regulation. A 24-h rest deprivation caused a marked reduction in swimming activity in 2 species of cyprinids, the crucian carp and the topmouth gudgeon. This may be comparable to the change occurring after sleep deprivation in mammals. Fish were exposed for 24 h to DSIP, DSIP-P or uridine. All substances were effective either lowering or in activating swimming activity. The activity reducing action was comparable to the quiescence induced by the rest deprivation. Giant Japanese rhinoceros beetles were intraabdominally injected with uridine, which caused either hyper- or hypoactivity in nocturnal locomotion according to the dosage. The experimental data suggests that the rest-activity regulating mechanism in lower animals may be similar to the mammalian sleep-waking mechanism. It may be postulated that sleep-specific messengers are endogenously produced and distributed to the entire central nervous system via the cerebrospinal fluid. Such a neurohumoral regulatory system of sleep may have evolved from the basic circadian rest-activity mechanism in parallel with the development of sleep, which represents a recently acquired function in higher animals.

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SLEEP-PROMOTING EFFECTS OF URIDINE AND SPS-B IN RATS. HONDA, K., KOMODA, Y., OKANO, Y. and INOUÉ, S. Inst. for Med. Dent. Engineering, Tokyo Med. Dent. Univ., Chiyoda-ku, Tokyo 101

Sleep-promoting substance (SPS) was extracted from the brainstems of 24-h sleep-deprived rats. Two effective fractions of SPS, uridine which had been designated as SPS-A-1 and SPS-B which was partially purified but unidentified, were infused into the third ventricle of freely moving saline-infused male rats for a 10-h nocturnal period. Ten-pmol uridine administration resulted in a significant increase in slow wave sleep (SWS) and paradoxical sleep (PS) during the dark period; the increment of SWS and PS was 21.0% and 68.1%, respectively, as compared to the previous baseline night. This was due to an increase in frequencies of both SWS and PS episodes but not to their durations. Brain temperature was not affected by the uridine administration. A lower dose of uridine (1 pmol/10 h) exerted no effect, while higher doses (100 and 1,000 pmol/10 h) exhibited slight but insignifcant increases in SWS and PS. Two-brainstem equivalents of SPS-B exerted a dramatic sleep-modulating effect. The increment of SWS and PS was 38.6% and 49.9%, respectively, as compared to the previous baseline night. Thus, the nocturnal administration of SPS induced excess sleep, although the neurohumoral mechanism associated with the sleep-promoting effect of each sleep substance remains largely unknown.

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CHANGES IN SCHEDULE CONTROLLED OPERANT-TYPE LEARNING BEHAVIOR OF SPONTANEOUSLY HYPERTENSIVE STROKE PRONE RATS (SHRSP). NOMURA,M., and OKAMURA,K. Dept.of Physiol., Fujita-Gakuen Health Univ., Sch. of Med., Toyoake, Aichi 470-11

Humans with high blood pressure in some severe cases show considerable memory retardation. The SHRSP were very useful models for the study of the controlling mechanisms of the blood pressure and cerebral angiovascular disorders in humans. Behavioral changes in the SHRSP were examined by using the operant-type brightness discrimination learning schedule of Multiple VI 15 sec. extinction (Mult. VI 15 ext.) and a fixed ratio 50 (FR-50) schedule, and results were compared with that in normotensive controls of the Wistar Kyoto strain (WKY). The SHRSP showed less ability than the WKY in performing the Mult. VI 15 ext. learning test. Whereas the WKY showed an advancement of the responses in the FR-50 schedule test : from 20 to 40 responses per min after 30 sessions, the SHRSP achieved only 10 to 20. Therefore, SHRSP are considered to be retarded in performing learning tasks, probably due to severe hypertension.

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IS THE VENTROMEDIAL NUCLEUS OF HYPOTHALAMUS (VMH) INVOLVED IN THE PREFEEDING CORTICOSTERONE PEAK IN RATS SUBJECTED TO RESTRICTED FEEDING SCHEDULE? *NAGASAKA, T., HONMA, K., HONMA, S., KATSUNO, Y., HIROSHIGE, T., AND *OIKAWA, K. Dept. Physiol., Sch. Med., *Dept. of Pediat. Dent., Sch. Dent., Hokkaido Univ., Sapporo, 060

Previously, Krieger,D.T. demonstrated that the prefeeding corticosterone peak induced by restricted daily feeding (RF) did not appear in VMH lesioned rats, and suggested an involvement of VMH in the hormone peak. We reexamined the effect of VMH lesion on the formation of prefeeding hormone peak in RF.

Materials and Methods : Adult male rats were lesioned electrically in VMH bilaterally. The amount of food intake and increment of the body wight were measured. Food intake exceeding the mean of the control rats by 2 SD was defined as a sign of hyperphagia. A 24 h profile of plasma corticosterone level in individual rats was determined by serial blood sampling at 2 hours intervals. RF schedules of free-access to meal for 4 hours (4h-RF) and for 1 hour (1h-RF) were imposed on VMH lesioned rats.

Results : The prefeeding corticosterone peak did not appear in VMH lesioned rats when 4h-RF was imposed during the dynamic phase, whereas the hormone peak was clear when the same RF schedule was performed during the static phase. In contrast, 1h-RF developed the prefeeding peak even in the dynamic phase. The results indicate that VMH is not necessary for the development of prefeeding corticosterone peak in rats.

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PHYSIOLOGICAL ROLES OF THE PREFEEDING CORTICOSTERONE PEAK INDUCED BY RESTRICTED DAILY FEEDING. *NOE, Y., HONMA, K., HONMA, S., KATSUNO, Y., HIROSHIGE, T., AND *OIKAWA, K.
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An anticipatory corticosterone peak is known to develop before meal when restricted daily feeding (RF) is imposed on rats. Roles of this prefeeding hormone peak were examined in feeding behavior and insulin response to meal.
Materials and Methods : Male rats were adrenalectomized bilaterally 2-3 weeks before RF experiments. RF (meal; 10:00-12:00 h) was imposed on the adrex rats for 2 weeks, and plasma corticosterone, insulin, and glucose levels were determined before and after meal. Then, the effect of corticosterone replacement (2.5 mg/kgb.w./day) was examined. The replacement was performed daily for 10 days at two different times ;08:00 or 16:00 h. Feeding behavior (approach to food cup) and food intake were measured as well.
Results : One third of the adrex rats died during RF. The plasma glucose level before meal and insulin response to meal intake decreased in the survived adrex rats. Food intake and feeding behavior were suppressed in these rats. The corticosterone replacement just prior to meal (8:00 h) restored both the glucose level and the insulin response to the control level. These results indicate that the prefeeding corticosterone peak auguments the insulin response to meal probably by activating feeding behavior which may result in an incsease of food intake.

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SUPPRESSIVE EFFECT OF ENCAPSULATED 2-DEOXYTETRONIC ACID ON FEEDING IN RATS. TERADA, K., SAKATA, T., ARASE, K., FUJIMOTO, K.†, OOMURA, Y.†* OSANAI, T.X AND NAGAI, K.***X 1st Dept. of Int. Med.* and Dept. of Physiol.†* Faculty of Med., Kyushu Univ., Fukuoka 812, and Dept. of Biochem., Faculty of Med., Tokyo Univ., Tokyo 113***

2-Deoxytetronic acid (2-DTA) has been found to be an endogenous satiety substance. Intraperitoneal injection of 2-DTA, however, showed no suppressive effect on feeding. We attempted the liposome-mediated 2-DTA delivery to the brain. The rats used were housed in a room illuminated daily during 0800-2000 hr and infused with test solutions at 1940 hr to 1950 hr. While intraperitoneal injection of 2500 μmol 2-DTA showed no effect on feeding. At 3 doses of 2500,1250 and 625 μmol, encapsulated 2-DTA showed dose-related suppressive effect on feeding. Feeding suppression was also induced by intra-carotid arterial injection of 2500 μmol 2-DTA. These data indicate that feeding center in the hypothalamus must be a major site for 2-DTA and that exogenous 2-DTA might not reach the brain sufficiently enough due to its trapping or degradation in some organ tissues.

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PRINCIPAL COMPONENT ANALYSIS OF THE IMAGES DURING THINKING PROCESS.

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Images used during thinking process were described by 72 testee on enquêt reports on 39 problems. Problems of thinking process were of four types; logical, creative, episodic and sensitive. Images were classified into verbal and non-verbal: and into three types; visual, auditory and of other senses. The principal component analysis of the images revealed four components; the first component named " visual factor," the second "sensitive thought," the third "remembering," the fourth "thought". The people with high score of the first principal component were pure visual type. The nature of the second component are different between Japanese and Thai testee.

The visual images were used most frequently by 67% testee, however the visual images in total images were 34.5%. The visual and verbal images were used on logical problems relative frequently 50.7% and 24.9% respectively. The visual and auditory coexistent type image and non-verbal were used on sensitive problems relative frequently 71.9% and 42.4% respectively.

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MORPHOLOGICAL AND FUNCTIONAL PROPERTIES OF THE DUM NEURONS IN THE MESOTHORACIC GANGLION OF THE COCKROACH. TANAKA, Y. and WASHIO, H., Lab. of Neurophysiol., Mitsubishi-Kasei Inst. Life Sci., Machida 194.

Dorsal unpaired mediun (DUM) neurons of Periplaneta americana have cell bodies on the dorsal surface of the ganglia. They have bifurcating axons which project symmetrically onto left and right peripheral nerve roots of the ganglia. Intracellular stimulation of the DUM neurons caused either significant decrease in the amplitude of the tetanic tension of the coxal depressor muscle or increase in the amplitude of the basal tension of this muscle. It has been known that some DUM neurons are octopaminergic. Application of 10^{-9} -10^{-4} M octopamine reduced the tetanic tension of the muscle. The α-adrenergic antagonist, phentolamine blocked the decrease in the amplitude of the tetanic tension but was without effect on the increase of the basal tension. Therefore, it appears that some DUM neurons are not octopaminergic. The effect of various pharmacological agents on the muscle contraction have been examined. Synephrine, adrenaline, noradrenaline, histamine and GABA reduced the amplitude of the tetanic tension, but 5-HT, dopamine and acetylcholine had no effect on the contraction. The neuropeptide proctolin produced the increase in the basal tension. Therefore, it has been suggested that proctolin could be the transmitter of some DUM neurons of the cockroach thoracic ganglion.

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POSTURAL CONTROL OF CARPS IN ROTATING WATER TANK。 TAKAGI,S。, WATANABE, S。 MORI, S., TAKABAYASHI, A。 SATAKE,H*, UEKI,S*, ITO,K*, YAMAZAKI,T*, SEKIMOTO,T*, AND MITARAI,G** Dept。 of Aerospace Phyiol。, Res。 Inst. Environ。 Med。, Nagoya Univ., Nagoya 464。, Inst。 Equilibrium Res.、, Gifu Univ* and Dept. of Physical Ed。, Chukyo Univ**

By rotationg a water tank contained a unrestrained carp, the postural control of carp was investigated. The water tank illuminated constantly from a light source laid on one face (a ceiling in normal position) was revolved with 0。3 or 0.6 rpm around the long axis of fish (head to tail axis)。 Carps were divided into 4 groups : an intact group of 10 carps, a bilateral labyrinthectomized group of 11 carps, a bilateral eye enucleated group of 3 carps, and a bilateral labyrinthectomized and enucleated group of 2 carps. When rotating the water tank, the intact carp initially tilted with the rotating light, until maximal tilt angle of 34.3±11。8° and holded a little while, then righted vertically after exceeding about 90° - 100° of the light angle. This result shows three different states; firstly the initial phase of the dorsal light response (DLR),secondly the visuo-vestibular interaction phase of DLR, and thirdly labyrinthine righting reflex phase. The labyrinthectomized group revealed mostly the visual dominant DLR. Blinded two groups maintained the righting posture toward gravitational force all the time。 In comparing with results of the four groups, it was discussed the dominancy between vision and vestibular sensation by controlling the posture.

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DEVELOPMENTAL CHANGES IN MORPHOLOGY OF THE MEDIAL GIANT FIBER OF THE CRAYFISH. CHIBA, A., YAGINUMA, S. and CHICHIBU, S. Dept. Physiol., Kinki Univ. Sch. Med., Sayama, Osaka 589

As the crayfish (Procambarus clarkii) grows, the dominant response patt;rn to noxious stimuli changes from escape to defense. We examined the possible neural correlates of this behavioral change. Morphology of medial giant fiber (MG), which is critically involved in the escape response, were studied by injection of a fluorescent dye, Lucifer Yellow CH, and by electrical recording techniques on the mature and immature crayfishes. Small collaterals extended from the MG toward the contralateral circumesophageal connective were found only in the immature crayfish (body length, 3-6 cm). By contraries, small branches originated nearby the MG soma and projected to the optic nerves bilaterally were found only in the mature crayfish with the body length of more than 6 cm. In addition, dye-couplings between the bilateral MGs and between the MG and the medial hemi-giant axon (C-99) were more frequently observed in the mature crayfish than in the immature one. The differences in the MG morphology in different maturation stages may mediate the change of behavioral pattern with growth.

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EFFECTS OF α-FLUOROMETHYLHISTIDINE, A SPECIFIC HISTAMINE SYNTHESIS INHIBITOR, UPON THE COPULATORY BEHAVIOR OF RATS. SHIMURA, T., HORIO, T., AND SHIMOKOCHI, M. Dept. of Behav. Physiol., Fac. of Human Sciences, Osaka Univ., Suita 565

Effects of α-fluoromethylhistidine (α-FMH), an irreversible inhibitor of histidine decarboxilase, upon masculine and feminine copulatory behavior of rats were examined. In male rats, α-FMH (50mg/kg i.p.) significantly decreased mount latency and intromission latency. On the other hand, post-ejaculatory mount latency and post-ejaculatory intromission latency were significantly increased by α-FMH. Other copulatory behavioral measure such as ejaculation latency, mount frequency, intromission frequency and interintromission interval did not change significantly. In ovariectomized estrogen plus progesterone-primed female rats, α-FMH (50mg/kg i.p.) did not modify lordosis quotient and soliciting behavior such as darting and hopping. These results suggest that histamine may have a minor facilitatory effect upon the recovery from sexual refractoriness of male rats.

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SLEEP-PROMOTING EFFECTS OF INTRAPERIOTONEALLY ADMINISTERED URIDINE IN RATS. OKANO, Y., HONDA, K., KOMODA, Y. and INOUÉ, S. Inst. for Med. Dent. Engineering, Tokyo Med. Dent. Univ., Chiyoda-ku, Tokyo 101

Uridine, an active component of sleep-promoting substance (SPS), induces excess sleep if its optimal dose (10 pmol) is steadily infused into the brain via an intracerebroventricular route. This fact leads to a question whether an intraperitoneal (i.p.) injection of uridine also causes excess sleep, since it is known that some of systemically administered substances may be prohibited by the blood-brain barrier from entering into and acting on the central nervous system. Hence saline solutions of 5 different doses (0.01-100 nmol) of uridine were i.p. injected 70-75 min before onset of the dark period in each group of 8-9 young male rats. Hourly summated records of slow wave sleep (SWS) and paradoxical sleep (PS) were compared to those of the previous day, on which saline only was similarly injected. A single injection of 0.1 nmol uridine resulted in a significant increase by 62.9% in SWS within 1 h after the administration. PS increased by 50.0% but the difference was not significant. Sleep latency was significantly shortened by 9.8 min. Changes in numbers and durations of SWS and PS episodes were statistically not significant. A single injection of smaller and larger doses exerted no remarkable effect. It is concluded that an i.p. injection of uridine did acutely cause excess sleep.

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EFFECTS OF ACTINOMYCIN S_2 ON SLEEP PATTERNS OF MICE. TSUBOSHITA, T.* AND UEZU, E. Dept. of Physiol. Sch. of Med. Univ. of the Ryukyus, Okinawa 903-01.

Eight C57BL/6J and three BALB/c strain mice were used. Under Nembtal anesthesia the electrodes for doing polygraphic recording were implanted. At the same time a cannula for administrating Actinomycin S_2 (an inhibitor of RNA synthesis, AcS_2) was also implanted into right lateral ventricle. The animals were housed under the conditions of a 12-hour light-dark cycle (light, 8:00-20:00). They were subjected to the polygraphic recordings ten days after the surgery. AcS_2 (1 mg/kg) in physiological saline was infused through the cannula during the 8:00 to 10:00 period. Changes in sleep and wake states were compared with control data recorded before the treatment. The C57BL strain showed clear diurnal variation in sleep: the amounts of slow wave sleep (SS) and paradoxical sleep (PS) were greater in the light period than in the dark period before AcS_2 treatment, and the amounts of both phases significantly increased during the dark period after the treatment. On the other hand, the same light-dark SS and PS rhythms were not observed in the BALB strain, and the amounts of SS and PS were not affected by AcS_2. These results suggest that the circadian rhythms of SS and PS are influenced by AcS_2 in the C57BL strain mice.

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NEUROTRANSMITTER RELEASE IN THE RAT STRIATUM DURING CONVULSION INDUCED BY PENTYLENETETRAZOLE (PTZ) USING IN VIVO DIFFERENTIAL PULSE VOLTAMMETRY. FUJIKAWA, N.*, YAMAMOTO, M.*, SHIRASU, A.*, YOKOI, I., Dept. of Neurochem., Inst. for Neurobiol., Okayama Univ. Med. Sch., Okayama 700.

Differential pulse voltammetry was used together with treated carbon fiber electrodes to study the in vivo dopamine and serotonine release in the rat striatum during convulsion induced by PTZ (60 mg/kg, i.p.). The working electrode was implanted into the striatum according to the following coordinate : anterior 7.8 mm, lateral 3mm, ventral 2.5 mm.

In the chronic experiment, an in vivo oxidation current of DOPAC that peaks at 130 mV (peak 2) significantly decreased during tonic convulsion and the minimum lasted for about 30 min. A trace of the oxidation current of 5-HIAA that peakes at 300 mV (peak 3) was detected, and this peak 3 markedly increased during tonic convulsion. In the acute experiment, rats were immobilized by succinyl choline, measurements were made under EEG recordings from epidural electrodes. The peak 2 increased after paroxysmal discharges on EEG and lasted for 3 to 6 min. A trace of the peak 3 was detected, and it markedly increased during paroxysmal discharges on EEG.

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF INTEGRAL GLYCOPROTEINS OF PERIPHERAL NERVE MYELIN. WANG,Y., SAKAMOTO,Y., KITAMURA,K., AND UYEMURA,K. Dept. of Physiol.,Saitama Medical School, Saitama 350-04

Peripheral nerve myelin contains integral glycoproteins, P0 and PASII, and basic proteins, P1 and P2. These glycoproteins are not amenable to isolation procedures, because of their insolubility. Previously we developed a purification method of these glycoproteins by SDS gel filtration chromatography. We attempted to isolate them more effectively by HPLC with SDS. The glycoprotein fraction of bovine peripheral nerve myelin was applied on a TSK 3000SW column and eluted with 0.1M phosphate buffer containing various concentration of SDS. Two glycoproteins, P0 and PASII, were separated adequately in 2% SDS, while it was inadequate with lower concentration of SDS. The high concentration of SDS presumably diminished strong hydrophobic interaction between these glycoproteins. The SDS-Tris, LDS-phosphate and SDS-lithium citrate were also examined for the elution buffers. The tryptic peptides of the P0 protein were separated by HPLC using a macroreticular resin. The amino-terminal peptide,the carbohydrate containing peptide and the fatty acid binding peptide were isolated.

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PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES TO 2',3'-CYCLIC NUCLEOTIDE 3'-PHOSPHODIESTERASE (CNPase). FUJISHIRO, M., KOHSAKA, S., NAGAIKE, K., MIKOSHIBA, K. and TSUKADA, Y. Dept. of Physiol., Keio Univ. Sch. Med., Tokyo 160

CNPase is considered to be a maker enzyme of CNS myelin and the activity is correlated well with the level of myelination. Here, we report the production and characterization of monoclonal antibodies to CNPase, and immunohistochemical staining of the brain of dysmyelinating mutant mouse (shiverer) is also presented. As an antigen, CNPase was partially purified from bovine white matter by modified method of Suda and Tsukada (1980). Lewis rats were immunized by intraperitoneal injection of partially purified CNPase with complete Freund's adjuvant and the spleen cells were fused with mouse myeloma cells (SP2) using polyethlene glycol. The cultured supernatants were screened for antibody production to CNPase by ELISA(anti rat IgG), immunohistochemical staining, immunoblotting and by binding assay(CNPase activity). Two monoclonal antibodies were obtained which recognize CNPase molecule of human, rat, mouse and bovine. In adult shiverer mouse, certain cells in the white matter, possibly oligodendrocytes, could be visualized by immunohistochemical staining.

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ACTIN FILAMENTS PLAY A SUBSTANTIAL ROLE IN AXOPLASMIC TRNASPORT.

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We permeabilized single fin nerves of the squid with 0.01 % Saponin for 2 minutes, and succeeded in reactivation of particle transport by ATP. It is now possible to conduct intra-axonal pharmacological experiments using such a perforated model axon. The composition of the artificial intracellular fluid was 300 mM K-Aspartate, 250 mM Glycine, 20 mM Na-Aspartate, 10 mM $MgCl_2$, 20 mM HEPES (pH 7.2). A dark field microscope was used for the observation of the particle movement. Microfilament depolymerizer, 88K protein/actin complex which was isolated from porcine brain (J. Biochem., 94,1671,1983) blocked the particle movement at the concentration of 5 μ M. Cofilin whcih inhibits the interaction between actin and myosin (Biochemistry,23,5307,1984) also showed an inhibitory effect on the particle movement at the concnetration of 60 μM. These results suggest that actin filaments do not play only a role of a structural support, but also a role of a transport machinery.

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TRANSPLANTATION OF SCIATIC NERVE INTO THE BRAIN OF DYSMYELINATING MUTANT MOUSE (SHIVERER).

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Shiverer mutant mouse has a severe disturbance in myelination in the CNS which lacks myelin basic protein (MBP) genetically. Pieces of sciatic nerve from a normal 10-day-old mouse were transplanted into the corpus callosum of a 4-week-old shiverer mouse, and the myelination by the Schwann cells in the host brain was analysed immunohistochemically and electron microscopically at 5 weeks after the transplantation. It was shown that the corpus callosum was stained with anti-MBP serum. Electron microscopic examinations revealed that most of the fibers in the region were surrounded by Schwann-like cells and myelin-like structures could also be detected. In conclusion, Schwann cells are able to survive in the host brain, have a capacity to migrate out of the graft, express MBP and possibly synthesize myelin along the CNS axons.

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IMMUNOHISTOCHEMICAL STUDIES ON THE NEURAL GRAFTS BETWEEN DIFFERENT SPECIES. (THE EFFECT OF CYCLOSPORIN-A)

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Newborn mouse cerebral cortex was transplanted into the third ventricle of adult rat brain. After the operation, the immunosuppressant cyclosporin A (Cy-A) was administered daily for 2 weeks, and the grafted tissue was examined immunohistochemichally. Hematoxylin staining revealed that the transplanted mouse brain tissue was rejected by 4 weeks after the transplantation in the control group. While, in the Cy-A-treated group, a large mass of grafted tissue was clearly detected in the third ventricle even at 4 weeks after the transplantation, and the tissue was heavily stained with Thy-1,2 antibody. Moreover the neural elements, neuron, astroglia and oligodendroglia, were positively stained by antibodies against neuro filament, GFAP and CNPase, respectively. In addition, it was suggested by the CNPase staining that myelination had occurred in the grafted tissue. These results revealed that the neural elements can survive and mature in the third ventricle of the different species upon administration of Cy-A.

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CELLULAR DISTRIBUTION OF GANGLIOSIDES IN CULTURED NEURONS AND GLIAL CELLS.

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The distribution of gangliosides was studied in different type's cell cultures, where the majority of astroglial cells are present after plating from 21-day-old cerebral hemispheres and the majority of neuronal cells are present before completing glial cells migration in cultures.

It is concluded that ganglioside GM1 is absent in astroglial cells. The presence of small amounts of di-sialoganglioside(GD3,GD1a) in the "pure" astrocytes. In addition, we inferred that ganglioside GM3 and GM2 are absent in neuron-riched cultures,which means that before completing glial cell proliferation. But GM3 was enriched in astrocytes. Onthe other hand, GT1a and GD1a were enriched in neuronal-cell cultures.

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ONTOGENY OF VIP, SOMATOSTATIN AND SUBSTANCE P IN JAPANESE MONKEY (MACACA FUSCATA FUSCATA) CEREBRAL CORTICAL SUBDIVISIONS. HAYASHI, M. and OSHIMA, K., Dept. of Physiol., Primate Research Institute, Kyoto Univ. Inuyama, Aichi 484

Concentrations of vasoactive intestinal polypeptide (VIP), somatostatin (SRIF) and substance P (SP) were determined in various cortical subdivisions of Japanese monkey at three different ages (embryonic 4 months, embryonic 5 1/2 months and adult). The three neuropeptides were detectable in the cerebral cortex at embryonic 4 months. It is of interest that SP concentration was particularly high in somatosensory cortex and visual cortex at this time. Between 4 months and 5 1/2 months, the three peptides increased dramatically: VIP (5- to 22-fold), SRIF (7- to 21-fold) and SP (4- to 23-fold). In adult, peptide levels decreased. The ratios between 5 1/2 months embryo and adult were ebout 1/2-1/8 for VIP, 1/3-1/6 for SRIF and 1/4-1/14 for SP, respectively. In contrast, choline acetyltransferase activity significantly increased from embryonic 5 1/2 months to adult in the various cortical subdivisions. This may be due to cell death or elimination of synapses which contain neuropeptides in monkey cerebral cortex during ontogeny.

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REM SLEEP AND ORGANIC BROMINE SUBSTANCE IN HUMAN PLASMA. OKUDAIRA, N., YAMANE, M.*, YANAGISAWA, I* AND TORII, S. Dept. of Physiology, Toho University, School of Medicine and *Dept. of Biochemistry, Tokyo Medical College, Ohta-ku, Tokyo 143.

1-methyl heptyl-γ-bromoacetoacetate (MHBAA), existed endogenously in human cerebrospinal fluid (CSF) (Yanagisawa & Yoshikawa, 1973), was shown to induce rapid eye movement (REM) sleep in cat (Torii et al., 1973) and a competitive inhibitor of the acetylcholinesterases (Yamane & Yanagisawa, 1984). Endogenous MHBAA increased in cat's CSF and blood after the 72 hours REM sleep deprivation (RSD) (Okudaira et al., 1984). In the present study, 7 healthy male volunteers, ranging in age 20 to 28 years, were recruited for the RSD and total sleep deprivation (TSD) studies. Venous samples were drawn at 10:00, 16:00, 22:00 and 4:00 in TSD study, and before and after nocturnal sleep, i.e. at 22:00 and 7:00, in RSD study. The amount of MHBAA in plasma was measured by a radiodilution method combined with fluorometry (Yamane and Yanagisawa, 1982). MHBAA level in human plasma, 0.02 - 1.23 n mol/ml, showed a diurnal fluctuation, highest at 10:00 and lowest at 22:00. The MHBAA level tended to decrease during TSD and to increase after RSD. MHBAA correlated negatively to the amount of REM sleep in their nocturnal sleep. These results suggested that MHBAA, being increased after sleep onset, is expended during REM sleep and waking.

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BIOCHEMICAL CHANGES IN THE BRAIN AND LEARNING ABILITY OF THE RATS TREATED PRENATALLY WITH METHYLAZOXYMETHANOL (MAM). NAGAYOSHI, M., TAMARU, M., SUGIMOTO, S.*, HIRATA, Y. and MATSUTANI, T. Dept. of Develop. Physiol., Inst. for Comprehensive Med. Sci., Fujita-Gakuen Health Univ. Sch. of Med., Toyoake, Aichi 470-11, *Dept. Behavioral Sci., Fac. of Integrated Arts and Sci., Hiroshima Univ., Hiroshima 730.

Brain weight and DNA content per brain in MAM-treated group (MAM-G) to which MAM was administered on the 11th to 13th days of gestation was about 75% in cerebral hemisphere and about 80% in brain stem of the control. In the brain stem the contents per brain of Norepinephrine and Dopamine showed a trend of reduction compared with the control and a reduction to about 70% of the control with 5-hydroxytryptamine (5-HT) ($P < 0.001$). The learning test was started from the age of 80 days, MAM-G showed a remarkable depression in reactivity especially on FR 30 schedule under the condition of light on and also spontaneous locomotion activity under the light condition (from 6:00 a.m. to 6:00 p.m.) in a day measured with Automex apparatus reduced to about 65% of that in the control. It was suggested that in MAM-G production of monoaminergic cells in brain stem was disturbed administration of MAM, which induced a reduction of 5-HT content per brain giving certain effect on the learning behavior.

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CHEMICAL DETERMINATION OF CARBOXYL-TERMINAL AMIDATED NEUROPEPTIDES TAKANO,H.,NISHIZIMA,T., and UYEMURA,K. Dept. of Physiol.,Saitama Medical School,Saitama 350-04

Recent studies revealed that chemical methods were as useful for detecting bioactive neuropeptides as bioassay. We examined a sensitive procedure for chemical determination of neuropeptides containing an amidated carboxyl-terminal,because carboxyl-terminal amino acids of many neuropeptides are known to be blocked. The peptide sample was digested with proteases (subtilisin and/or thermolysin), dansylated, extracted with ethyl acetate under alkaline conditions, and separated by a reverse phase high performance liquid chromatography (HPLC: Develosil C8-5 column) using a linear gradient of methanol in Tris-HCl buffer. This HPLC system was sensitive enough to detect up to pmol level of dansylated amino acid amides with a Shimadzu spectrofluorophotometer. The acid hydrolysate of the extract was also analysed for dansylated amino acids by the HPLC system described by Kaneda. Phe-amide and Met-amide were demonstrated in the degradation products of CCK-8 and Substance P, respectively. We found Phe-amide,Met-amide, and Ile-amide in peptide fractions,which were obtained from bovine spinal cords.

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MITOTIC CELLS IN DISSOCIATED CELLS CULTURES FROM EMBRYONIC RAT CEREBRAL HEMISPHERES EXPRESS NEUROFILAMENT PROTEIN(NF), MYELIN BASIC PROTEIN(MBP), AND GLIAL FIBRILLARY ACIDIC PROTEIN(GFAP). MUTOU, N., ASOU, H., HIRANO, S. 2nd Dept. of physiology, Toho Univ. Sch. of Med., Ohmori nishi, Tokyo 143

The purpose of this study is to demonstrate that neuronal or glial precursor cells have a ability to proliferate by mitosis in dissociated cell cultures from embryonic rat cerebral hemispheres. In conclusion, the results indicated that neuronal precursor cells have a mitotic ability with different stages of mitosis at 15-day-old primary cultures, and also observe the survival of long-term period(for 53 days) in these cultures. On the other hand, non-neuronal cells which are GFAP-positive astroglial cells and MBP-positive oligodendrocytes were being generated throughout at least the first 21 days or 53 days respectively, in these dissociated cells cultures. These findings suggested that some neurons and glial cells have a mitotic potential and differentiation in the cell cultures after plating.

These cultures may also provide a convenient model system for studies of the role of cell-cell interaction involved in myelination.

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CLASSIFICATION OF DIRECTIONALLY SELECTIVE CELLS INTO FIGURE, FIELD AND NON-SELECTIVE TYPES IN THE MT-RECIPIENT ZONE OF THE MACAQUE STS CORTEX. HIKOSAKA,K.,YUKIE,M.,IWAI,E., TANAKA,K.*,SAITO,H.*,FUKADA,Y.* Dept. of Behav. Physiol. Tokyo Metropolitan Inst. for Neurosci., Fuchu, Tokyo 183, * NHK Sci. and Tech. Res. Labs., Setagaya, Tokyo 157

We have shown that three classes of directionally selective cells cluster in a part of the MT recipient zone (area MST). D-cells responded to frontparallel straight movements of patterns, whereas S- and R-cells responded only to size change and rotation, respectively. In the present study, we examined their responses to two kinds of visual stimuli; a movement of a single slit and that of a wide-field dot pattern. D- and S-cells were divided into three types: Figure type cell were activated by a slit movement, but suppressed by the wide-field movement. By contrast, Field type cells were activated only by the wide-field movement. Non-selective type cells showed comparable responses to both stimuli. The distributions of Figure, Field and Non-selective types for D- and S-cells were 2:2:3. All R-cells, except one (Non-selective type), were classified into Field type. Thus, in this area both object and field motion can be analyzed in parallel; object motion by Figure type cells, and field motion by Field type cells.

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INTEGRATION OF VISUAL MOVEMENT SIGNALS IN THE SUPERIOR TEMPORAL SULCUS OF THE MACAQUE MONKEY. FUKADA,Y., SAITO,H., TANAKA,K., YUKIE,M.*, HIKOSAKA,K*, IWAI,E*, NHK Sci. and Tech. Res. Labs., Setagaya, Tokyo 157, *Dept. of Behav. Physiol., Tokyo Metropolitan Inst. for Neurosci., Fuchu, Tokyo 183

Visual movement signals are retinotopically analyzed into small components of linearly directed motion in the macaque MT area. Cells in the area anteriorly adjacent to the two-thirds of MT (DSR area) have large receptive field. They reassemble the fragments of the motion signal which have been sent from MT area and tell how and in what direction the visual world moves: D-cells respond to a specifically directed straight movement of a visual object and/or a flow of the wide field throughout their receptive field; S-cells, to an expansion or a contraction; Rf-cells, to a clockwise or counter-clockwise circular movement in the frontoparallel plane. Further, rotation of patterns (textured flat board) in depth in a certain direction is extracted by Rd-cells. Since Rd-cells responded strongly to monocular stimulation, the binocular disparity is not necessary to activate them.

In the area which adjoins laterally to the DSR area there cluster cells which particularly sensitive to the movement of the real object such as face, or hand. This suggests that the integration of movement and shape signals takes place in this area.

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RESPONSES OF THE ROTATION-SENSITIVE NEURONS OF THE MONKEY PARIETAL CORTEX TO THE ROTATION IN DEPTH. Sakata, H., SHibutani, H., Ito, Y. and Tsurugai, K. Dept. of Neurophysiol., Tokyo Metropolitan Inst. for Neurosci.

The majority of the rotation sensitive neurons (19/28) found in the posterior part of area 7a of alert monkeys responded maximally to the rotation in depth rather than in the frontoparallel plane. The optimal plane of rotation was either horizontal (6/19), saggital (6/19) or diagonal (7/19). They responded to the rotation of a pair or a single spot as well as to a solid bar, but responded poorly if any to the shearing movement (a pair of linear movements in opposing directions) of a pair of spots. Therefore we concluded that the changing direction of the movement of each point of the stimulus is the most important factor to discriminate rotation from the linear movement. Their responses to the depth rotation decreased in monocular vision compaired to those in binocular vision. However the residual responses in monocular vision indicated that some monocular cues of rotary movement were also effective to activate these neurons. When we rotated a trapezoid window-shaped plate (Ames' window) in depth in monocular vision, the preferred direction of rotation of one of these neurons reversed when the longer edge moved behind the center of rotation, just as we perceived the reversal of rotation.

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THE EFFECT OF ATTENTION AND STIMULUS-STIMULUS INTERACTION APPEARED IN THE ACTIVITY OF INFERO-TEMPORAL CORTICAL NEURONS IN MACAQUE MONKEYS.
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Two monkeys were trained to perform eye-fixation and pattern dicrimination tasks. In the pattern discrimination task, the animals must respond to one of the patterns quickly by lever-releasing, and to the other, wait for the dimming of the pattern. The light stimulus preceded the pattern stimuli in one trial, and in other trial, it was presented while the animal was watching and waiting for the dimming of the pattern. The position of pattern stimuli was fixed at one of three positions (center, contralateral 5 or ipsilateral 5 degrees in the visual field) in one task session. The response of neurons to the light stimulus decreased when it was presented while the animal watching the pattern stimulus. This effect appeared in more than half infero-temporal neurons tested when the pattern stimuli presented at the center or at the contralateral position in the visual field. However, when the patterns were presented at the ipsilateral field, this effect disappeared. This results suggest that the effect of this type of selective attention may base on the neural circuit constructed indpendently in each cerebral hemisphere.

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A GABA ANTAGONIST UNCOVERS VISUAL RESPONSES OF NEURONS IN THE VISUAL CORTEX OF DARK-REARED KITTENS.
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In dark-reared kittens the majority of neurons in the visual cortex are unresponsive or only weakly responsive to visual stimulation. This might be due to loss of afferent connections or to dysfunction of afferent inputs in the dark-reared cortex. To test these possibilities we studied effects of ionophoretic application of bicuculline methiodide (BIC), a γ-aminobutyric acid (GABA) antagonist, on visual responsiveness of neurons in 4 dark-reared kittens at 14-19 weeks of age under N_2O anesthesia. Fifty-nine of the 110 cells recorded were unresponsive or very weakly responsive to visual stimuli. Most of the others had visual responses unselective to stimulus orientation. About 80% of the unresponsive cells become visually responsive during BIC application. The originally responsive cells also enhanced their visual responses during BIC application. Considered together with the fact that the stimulus selectivity depends on the intracortical GABAergic inhibition in the normal cortex, the present results suggest that in the dark-reared cortex, inhibitory synapses responsible for the selectivity may be immature or retarded but those for the tonic inhibition may so well be developed as to mask visual responses.

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PROPERTIES OF REINNERVATED GENICULATE RELAY CELLS IN NEONATALLY ONE-EYE-REMOVED CATS.
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When one eye was removed shortly after birth the remaining optic nerves show translaminar growth and reinnervate relay cells in denervated laminae. To know how such ectopic retinogeniculate synapses function we studied response properties of reinnervated relay cells of the denervated lamina (lamina A1) in six one-eyed adult cats. Relay cells were identified by orthodromic response to single optic chiasm shocks and antidromic response to visual cortical shocks. Based on receptive field properties reinnervated relay cells were identified as either Y or X type. For both types response latencies to orthodromic and antidromic stimuli were in normal range. However, receptive field center diameter of each cell type was twice as large as that of the corresponding type recorded in the intact lamina (lamina A). In vertical penetrations with semimicroelectrodes responsive fields did not shift much when the tip entered into lamina A1 from lamina A. From these observations we concluded that both Y and X types of retinal axons make translaminar growth and their terminals make functionally active but somewhat broader synaptic connections with target relay cells in denervated lamina.

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CHROMATIC AND SPATIAL PROPERTIES OF THE MEDULLA NEURONS IN THE SWALLOW-TAIL BUTTERFLY LARVA. ICHIKAWA, TOSHIO Dept. of Biol., Fac. of Sci., Kyushu University, Fukuoka 812

Chromatic and spatial properties of the neurons in the 2nd optic neuropile (medulla) were intracellularly examined by stimulating six stemmata individually by monochromatic flashes. About 50 neurons were identified by the profiles of chromatic inputs from the individual stemmata. They were divided into two major groups: monostemma dominant neurons and multistemmata dominant neurons. 33 neurons belonging to the former group were examined in detail in the present study. The monostemma dominant neurons receive strong inputs from one stemma and weak inputs from other stemmata; the weak inputs are usually antagonistic to the strong inputs when tested by white light stimuli. They were subdivided into 18 types depending on the spectral properties of their strong inputs. 8 types of them are color-opponent neurons which show notable color antagonisms in the dark. Other seven are narrow-band neurons which do not show notable color antagonism in the dark, but show it under certain light-adapted conditions. Remaining three are broad-band neurons which show similar excitatory or inhibitory responses to 330-600 nm.

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CEREBRAL GLUCOSE METABOLISM IN SENSORY DEPRIVED MAN AS MEASURED WITH POSITRIN EMISSION TOMOGRAPHY. [1]TASAKI, K., [2]UEMURA, K., [2]KANNO, I., [3]TSUKAHARA, Y., [4]FUKADA, Y. and [5]TAMAI, M. [1]Dept. of Physiol., Tohoku Univ. Sch. of Med., Sendai, [2]Akita Res. Inst. of Brain and Blood Vessels, Akita, [4]N H K Broadcast. Sci. Res. Labs., Kinuta, Tokyo and [5]Dept. of Ophthalmol., Tohoku Univ. Sch. of Med., Sendai.

Regional cerebral metabolic rate for glucose (rCMRgl) was measured using ^{18}F-fluoro-deoxyglucose and a positron emission tomograph of high quantitation and resolution (HEADTOME III) under the strictly controlled state of sensory deprivation in 10 normal and one visually handicapped subject. At least 30 min before injection and 50 min after injection of the radiotracer the subject lay supine on a bed in a sound-proof darkened room. During this period the subject's eyes were covered with an individually made black mask and each external auditory meatus was plugged with a steel ball which was further covered with a large mass of rapid-cure silicon rubber (Otoplastic, Dreve). EEGs were monitored throughout. The mean rCMRgl of the whole brain of 5 normal subjects is 7.89 mg/100g/min. Although the frontal cortex shows the well-known high rate of glucose metabolism (hyperfrontality), the most unexpected finding is that the highest value was found at the cingulate gyrus and the corpus striatum. Regions which show low metabolism are the temporal and occipital lobes, thalamus and cerebellum.

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FINE DISTRIBUTION OF GABA IN VISUAL CORTEX, DORSAL LATERAL GENICULATE NUCLEUS AND SUPERIOR COLLICULUS IN CAT. KANNO S., OKADA, Y., Dept. of Physiology, Sch. Medicine, Kobe University, Chuo-ku, Kobe, 650

Distribution of GABA(γ-amino butyric acid), an inhibitory neurotransmitter was studied in the visual system in adult cat. Microassay method to measure GABA (10^{-12} mol) was established using enzymatic cycling of NADPH-NADP. Small area (300ng) was dissected from the freezed-dried tissue section from the visual cortex, dorsal lateral geniculate nucleus (d-LGN), superior colliculus (SC), and the GABA distribution was mapped after measuring the GABA content in each sample. In the d-LGN, GABA concentration was the highest in the lamina A (23-27mmol/kg dry) and the lowest in the lamina C. In the visual cortex (Area 17, Area 18), the concentration of GABA was 10-11mmol/kg dry in the layers I-IV, and 5-8mmol/kg dry in layers V and VI. Not so much difference was found in the GABA distribution in Area 17 and Area 18. In the SC, the highest amount of GABA was found in superficial grey layer (30-40mmol/kg dry), whereas other deep layers contained GABA at the concentration of 23-28mmol/kg dry. GABA in the lamina A in d-LGN and the superficial grey layer in SC may play an important role in controling the function of visual system.

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HPLC ANALYSIS OF THE CHROMOPHORE OF THE VISUAL PIGMENT FROM THE FRUITFLY DROSOPHILA MELANOGASTER. ISONO, K., TANIMURA, T.*, TSUKAHARA,T. Res. Ctr. Appl. Inf. Sci., Tohoku Univ., Sendai 980,*Inst. Basic Biol., Okazaki 444

Chromophore of the visual pigment of the fruitfly Drosophila melanogaster was studied by high performance liquid chromatography (HPLC). Oximes were successfully analysed with a more polar solvent than what has been used for retinaloximes. Preceeding irradiation resulted in a reversible interchanges in the amount of the oximes that reflected the photointerconversion of rhodopsin and metarhodopsin of the peripheral photoreceptors (Rl-R6). Synthetic all-trans, 3-hydroxyretinal was used to identify the chromophore from metarhodopsin. Results further supported the idea that the chromophore of the visual pigment of Rl-R6 is 3-hydroxyretinal as has been proposed in larger flies.

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RHODOPSIN METABOLISM DURING THE DAILY CYCLE OF MEMBRANE TURNOVER IN LIGIA EXOTICA PHOTORECEPTOR. HARIYAMA,T.,ISONO,K.,HARADA,A.*,TSUKAHARA,Y. Research Center for Applied Information Sciences, Tohoku Univ.,Sendai980

Diurnal changes in the total amount of retinal isomers and in the relative amount of each isomer were studied by high performance liquid chromatography (HPLC) in the eye of Ligia exotica. The relative amount of 11-cis form of retinal oxime to total amount of 11-cis and all-trans forms of retinal oximes increased with time after midday and decreased after midnight. The smallest vaule was 0.6 and the largest value was 0.9. This changes seem to result from mainly by the change in the amount of 11-cis form of retinal oxime. An endogeneous rhythm in the changes of the amount of 11-cis oxime was also observed when the animals were kept in continuous darkness. These changes seem to occur preceeding the morphological changes. The average sensitivity of single cells defined by the normalized V-logI curve obtained by a small spot on the visual axis of each penetrated cell did not show much difference throughout the day suggesting that the rhodopsin concentration in the photoreceptor membrane does not change much in spite of the drastic morphological change in the rhabdomere.

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ISOELECTRIC POINTS OF VISUAL PIGMENT. KOYAMA, K.[1], HIRAKI, K.[2], FUJISHITA, S.[1] AND KITO, Y.[3] [1] Dept. of Health Science, Osaka Kyoiku Univ., Osaka 547, [2] Dept. of Biophysical Engineering, Faculty of Engineering Science, Osaka Univ. Osaka 560, and [3] Dept. of Biology, Faculty of Science, Osaka Univ. Osaka 560

Isoelectric points of squid rhodopsin, Watasenia scintillans, and its photoproduct, metarhodopsin, were estimated by means of chromatofocusing. The purified specimens solubilized in 1 % solution of lauryl ester of sucrose (C_{12} ester) were chromatofocused. They were separated in two peaks at pH 5.04 for rhodopsin and at pH 4.75 for metarhodopsin on the elution profile, respectively. This result suggests that 3 to 5 carboxyl groups are dissociated through the conversion from rhodopsin to metarhodopsin. The rhodopsin was chromatofocused at 15°C using palmityl (C_{16}) and oleyl ($C_{18:1}$) monoester of sucrose, in order to estimate the effect of the hydrocarbon chain on the pI value. The pI value in C_{16}, $C_{18:1}$ is almost the same as that in C_{12} ester at 15°C. The proteolysed rhodopsin which lost about 10,000 dalton C-terminus peptide showed the broad peak of more acidic region on the chromatofocusing.

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IDENTIFICATION OF CHROMOPHORES IN RETINAL AND PINEAL PHOTORECEPTORS
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1. Dept. Physiol., Hamamatsu Univ. Sch. Med., Hamamatsu 431-31

The electrophysiological examination suggested the pigment conversion in the pineal photoreceptors of the river lampreys, Lampetra japonica between larvae and adults. 11-cis-Retinal (11_1) and 11-cis-3-dehydroretinal (11_2), chromophores of vitamin A_1-based pigments (ex. rhodopsin) and vitamin A_2-based pigments (ex. porphyropsin), respectively were analyzed in the retina and in the pineal organ with high-pressure liquid chromatography (HPLC). The amount of 11_2 was almost the same as that of 11_1 in the retina of upstream adults in May and less than 11_1 in the young adult, immediately after metamorphosis in October. 11_1 was main component in the adult in October. This implies that the visual pigment seasonally changes in the retina of the adult lamprey. Both chromophores were also confirmed in the pineal photoreceptor, and 11_2 exceeded 11_1 in December, whereas the retina had more 11_1 than 11_2 in the same month. This result shows that the component of the visual pigments in the pineal was different from that in the retina even in the same season.

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CALCULATION OF THE CONTENT OF FLAVINS IN RETINA AND CHOROID OF A FROG BY MEANS OF HEPATIC FAD, SERUM GLUCOSE AND SERUM FATTY ACID. FUJISHITA, S. Dept. of Health Science, Osaka Kyoiku Univ. Hirano-ku Osaka 547

In the present study, the influence of FAD contained in a frog liver to that of flavins in the choroid and retina is discussed. When the activity of the liver of a frog is well, the quantity of the total riboflavin in the choloid of a frog (CHR) may be expressed as follows, CHR = 0.425 (FAD) + 4.4 x 10^{-5} mol. On the contrary, when the activity of the liver of a frog is not so well, CHR may be expressed as follows, CHR = 0.29 (FAD) + 0.4 x 10^{-5} mol where (FAD) is the total FAD in a liver. So, the value of (FAD) may be determined by means of liver activity and the total content of FAD in the liver.

The content of FMN per g of retina of a frog (R) may be expressed as follows, R = 0.194 (chr) $^{1/3}$ x (G + FFA) $^{1/2}$ - 0.837 x 10^{-5} mol, where (chr) is the riboflavin content per g of choloid. And G and FFA are the concentration of glucose and fatty acid in the serum of the frog respectively.

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GLIAL TOXICITY OF α-AMINOADIPIC ACID IN THE CARP RETINA. KATO, S., MATSUKAWA, T., SUGAWARA, K. AND NEGISHI, K. Dept. Neurophysiol., Neuroinformation Res. Inst. (NIRI), kanazawa Univ. Sch. Med., Kanazawa 920

To estimate the gliotoxicity of α-aminoadipic acid (α-AAA), we investigated a biochemical (glutamine synthetase; GS) and an electrophysiological (b-wave of ERG) glial marker after intravitreal injection of α-AAA. At first we prepared an antiserum against the GS purified from sheep brain and immunohistochemically ascertained the GS was exclusively localized in glial Müller cells. The GS activity was inhibited to 30% of control at 1-2 days after L-AAA injection. The minimum effective dose of L-AAA was about 0.5 μmoles. The gliotoxic effect of L-AAA was not permanent, but transient. The GS activity was recovered from the L-AAA treatment within a few week. D-AAA was less potent in about one order than L-AAA. Electrophysiologically, L-AAA produced a complete loss of b-wave, while S-potential recorded from horizontal cells remained intact to light stimuli. Some of neuroexcitants, L-glutamate, L-aspartate and kainic acid were no effect on the GS activity, although the b-wave and S-potential were completely blocked by them. These data strongly suggest that L-AAA can be used as a specific glial toxin and as a useful tool to study a neuron-glial interaction in the carp retina.

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DOSE-DEPENDENT AUGMENTING AND BLOCKING EFFECTS OF OUABAIN ON THE ERG C-WAVE. KIM, H., SATO, T.*, YONEYAMA, T., MATSUMOTO, N.* AND SUZUKI, T.A.* Dept. of Ophthal., Sch. Med., *Dept. of Oral Physiol., Sch. Dent., Iwate Med. Univ., Morioka, Iwate 020.

For clarifying the effect of ouabain on the ERG c-wave, this experiment was performed using the isolated chick retina. Although the c-wave was abolished by higher dose of ouabain (10^{-4} M), it increased in amplitude in the presence of ouabain at low concentration of 10^{-7} M, which was within the range of clinical use for cardiac glycoside.

On the other hand, the standing potential of the retina did not change appreciably until 10^{-6} M order and then decreased gradually at higher concentrations. In the presence of 10^{-4} M ouabain, the level having a complete blocking effect of Na-K-ATPase, both the c-wave and the standing potential were almost abolished. These phenomena were more conspicuous when ouabain was applied to the vitreous side than in the choroidal side.

Noradrenaline, a stimulant of the adenylate cyclase, augmented the c-wave as much as that of ouabain but this effect was largest at 10^{-9} M. After augmentation of the c-wave by 10^{-9} M noradrenaline, there was no additional augmenting effect of 10^{-7} M ouabain. This fact suggests that the ouabain-induced augmentation of the c-wave may be due to the stimulation of adenylate cyclase. In the presence of 10^{-7} M ouabain, light sensitivity of the retina elevated to 0.5 log unit and the maximum response increased about 30%. This may be one of the sign for visual complications of ouabain, such as chromatopsia.

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INJECTION OF CYCLIC GMP-DEPENDENT PROTEIN KINASE INTO ROD OUTER SEGMENTS (ROS) CAUSES DEPOLARIZATION THAT IS INHIBITED BY ILLUMINATION. SHIMODA, Y., Dept. Physiol. Kyorin Univ., Sch. Med., Mitaka 181, MILLER, W.H., Dept. Ophthal. and Visual Sci., Yale Univ. Sch. Med. New Haven, CT 06511.

Previous biochemical and physiological studies have suggested that cyclic GMP depolarizes ROS and that cyclic GMP hydrolysis, regulated by a light-dependent phosophodiesterase, cuases hyperpolarization. We have investigated the role of cyclic GMP-dependent protein kinase(GK) purified from bovine lung was injected from glass microelectrode into ROS of isolated Bufo marinus retina. Injection of the protein kinase cuases (1) a depolarization and (2) a reduced amplitude of the photic response. Both the depolarization and the reduced amplitude of the light response were found to recover on a time scale of minutes. Application of a light flash during the depolarization caused by injection of GK immediately prevented further depolarization.

The above data indicate that the activity of the injected kinase depends on the high endogenous ROS level of cyclic GMP, which is significantly reduced by activation of the light-dependent phosphodiesterase. The data are consistent with the interpretation that endogenous ROS cyclic GMP normally depolarizes the ROS through the activation of endogeous cyclic GMP-dependent protent kinase.

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CORRELATION BETWEEN IN SITU PHOSPHODIESTERASE ACTIVITY AND THE PHOTORECEPTOR POTENTIAL IN GECKO RETINA. KAWAMURA, S. AND MURAKAMI, M., Dept. Physiol., Keio Univ. Sch. Med., Tokyo 160

Intracellular injection of cGMP in the dark depolarizes gecko photoreceptor and increases membrane conductance to sodium. When cGMP was injected immediately after a light flash, the duration of cGMP-induced depolarization was short, indicating that cGMP phosphodiesterase (PDE) remained active and rapidly hydrolyzed the injected cGMP. When the injections were made at various time intervals after the flash, the duration became longer as the interval was increased, showing that the PDE activity gradually decayed to the dark level. Quantitative estimation of the PDE activity was made by measuring the time required for the complete repolarization from the cGMP-induced depolarization, since it is the time necessary for the PDE to completely hydrolyze the injected cGMP and is inversely proportional to the PDE activity. The results showed that the PDE activity recovered following first order reaction and that its time course after a light flash paralleled with the recovery of the photoreceptor sensitivity defined as the return of the response amplitude. Theoretical analysis indicated that this parallelism can be explained by assuming cGMP to be the transduction messenger in the gecko photoreceptor.

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SYNAPTIC INPUTS TO FROG ON-OFF GANGLION CELLS. NAKA, K.-I., NIBB, Okazaki 444 and SAKURANAGA, M., Physiol., Nippon Med. Sch., Tokyo 113

White-noise analysis was used to identify the linear and nonlinear components underlying the generation of ganglion-cell responses from extracellularly recorded spike discharges. We found that the responses of on-off ganglion cells from the frog are made up of a nonlinear depolarizing component and a linear hyperpolarizing component. Since transient amacrine cells respond nonlinearly to white-noise modulated stimuli, we suggest that these cells are the primary source of excitation to the on-off ganglion cells in frog. The linear inhibitory component could result from bipolar or sustained amacrine cell input to the ganglion cells, since the responses of both of these neurons to white-noise stimuli contain mainly linear components.

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STUDIES OF RETINAL NEURONS USING VOLTAGE CLAMP BY LIGHT. YASUI, S. and YAMADA, M. Dept. of Biological Regulation, National Inst. of Basic Biology, Okazaki, Aichi, 444 and Applied Optics Sect., Electrotechnical Lab., Tsukuba Science City, Ibaraki, 305.

To study nonspiking retinal neurons, a voltage clamp system has been developed which utilized the natural stimulus of light. The error signal which represents the difference between the command and measured membrane votages drives a PLZT electronic shutter that can modulate rapidly the retinal irradiance. A sign-inverting manual switch is provided to handle both hyperpolarizing (e.g., vertebrate photoreceptors and L-type horizontal cells) and depolarizing (e.g., vertebrate C-type horizontal cell and bipolar cells, and invertebrate photoreceptors). Using this system, dc and ac spectral sensitivities were measured of different horizontal cell types, by reading off directly from a single uninterrupted raw record in contrast to the time-consuming conventional procedure. In L-type horizontal cells, the dc and ac results were quite similar, which suggests a shift of the V-log I curve along the log I axis with stimulus wavelengths. The present system was suitably combined with the bridge-balance technique to measure the membrane resistance under voltage clamp by light. This application revealed a gradual resistance increase after a light-induced voltage step in the crayfish photoreceptor. This result suggests the existence of a voltage-activated and time-dependent membrane conductance which has a positive reversal potential.

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ANOMALOUS RECTIFIER CHANNELS IN ISOLATED HORIZONTAL CELLS OF GOLDFISH RETINA. SHINGAI, R., Pharmacology, Sch. of Med., Yamaguchi Univ., Ube 755

The ion channels resposible for inward rectification in horizontal cells were studied using the patch clamp technique applied to isolated cells from goldfish retina. A high external K^+ solution was used in order to facilitate the opening of channels. In addition, a blocking cation was introduced to provide for frequent open-close current transitions. Most experiments employed the inside-out patch clamp. The temperature range for the experiments was 9 to 14°C. The single channel conductance was 20 pS in 125 mM external K^+. The null current potential shifted with changes in the K^+ concentration was expected for a channel permeable to K^+. Reductions in internal Na^+ did not shift the reversal potential as expected for a channel permeable to Na^+. As the membrane was hyperpolarized from the K equilibrium potential, the channel was more likely in the open state.

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SYNAPTIC TRANSMISSION FROM HORIZONTAL-CELL AXON TERMINALS TO THE AMACRINE CELLS. SAKAI, H., NAKA, K-I National Institite for Basic Biology, Okazaki Aichi 444

In teleost retinas, the axon terminals of the horizontal cells lie along the border of the inner nuclear layer and inner plexiform layers. Although the axon terminals are structurally prominent, their function is not known. We report morphological and functional evidence that signals from catfish horizontal-cell axon terminals can be transmitted directly to amacrine cells.

Electron microscopic observations showed that typical chemical synapses were made by the horizontal-cell axon terminals onto the postsynaptic elements in the inner nuclear layer and along the border of the inner plexiform layer. Two thirds of the postsynaptic elements were identified as amacrine cell processes or perikarya.

Current injected into horizontal cell axon terminals produced responses from both transient and sustained amacrine cells. These current elicited responses were similar to those elicited by light stimuli.These data suggests that amacrine cells in the catfish retina recieve two inputs, one from bipolars and the other from horizontal-cell axons.

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ELECTRICAL PROPERTIES OF GAP-JUNCTIONAL MEMBRANE OF HORIZONTAL CELL IN THE CARP RETINA. TAKABAYASHI, A., WATANABE, S., & MITARAI, G.* Dept. Aerospace Physiol., Inst. Environ. Med., Nagoya Univ. and *Dept. Physical Ed., Chukyo Univ.

The large distant potential spread through the horizontal cell (HC) layer has been attributed to the presence of gap-junctional contacts between cells. To elucidate its functional significance, the voltage dependent properties of junctional membrane between external HCs were studied in the isolated carp retina. Current pulses were injected into one HC (C_1) and the induced potentials were recorded from the neighbouring HC (C_2). Hyperpolarizing voltages yield larger coupling ratios than depolarizing voltages, showing rectification of the lateral spread. When the membrane potential of C_1 was clamped at the dark resting level, the coupling was facilitated with background light. These results suggest that the conductance of junctional membrane is voltage dependent. To clarify these properties, pairs of C_1 and C_2 were voltage clamped, and junctional current was measured during transjunctional voltage steps. The steady-state junctional conductance slightly decreases as a function of depolarizing transjunctional voltages, and increases as a function of hyperpolarizing transjunctional voltage. From these results, we propose that the connection between HCs is voltage-sensitive.

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IONIC MECHANISMS OF GABA-INDUCED CURRENTS IN SOLITARY TURTLE CONES. KANEKO, A. and TACHIBANA, M. National Institute for Physiological Sciences, Okazaki 444

It is suggested that γ-aminobutyric acid (GABA) is the neurotransmitter of the feedback synapse from monophasic horizontal cells to red-sensitive and green-sensitive cones. Ionophoresis of GABA induced current responses in these types of solitary cones voltage-clamped using "patch" pipettes (pipette solution; 138 mM K^+, 120 mM Cl^-). The polarity of the GABA-induced current was inward at negative membrane potentials, and reversed at 6 ± 4 mV (mean ± SD, n = 19) in the control medium ($[Cl^-]_o$, 120 mM). The reversal potential followed very closely to the equilibrium potential of Cl^-, when either $[Cl^-]_i$ or $[Cl^-]_o$ was changed. Changes in either $[Na^+]_o$ or $[K^+]_o$ affected neither the reversal potential nor the amplitudes of GABA-induced responses. Immediately after the rupture of the patch membrane, when the diffusion of Cl^- from the recording pipette remained negligible, GABA induced membrane hyperpolarization.

Cones had the type-A GABA receptors: muscimol was as effective as GABA (threshold dose, 100 nM), but baclofen (>0.1 mM) was ineffective; GABA was antagonized by bicuculine (<10 μM) competitively, and by picrotoxin (<10 μM) non-competitively. It is concluded that GABA induces a hyperpolarization in in situ cones by combining with the type-A receptors.

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IONIC MECHANISMS OF ELECTRICALLY EVOKED DEPOLARIZATION IN FROG RODS. TAKAHASHI, K.-I. AND MURAKAMI, M., Dept. Physiol., Keio Univ. Sch. Med., Tokyo 160.

In a frog rod, we have found that extracellularly applied transretinal current pulse, flowing from the receptor side to the vitreous side, evoked a transient depolarization. We tentatively concluded that the depolarization was mainly due to a Ca current activated by a depolarizing current at the rod terminal. In order to reveal in detail ion channels activated by the stimulus current pulse, intracellular current injections were carried out. Transient depolarizations were observed not only at the onset of the depolarizing current (DE-ON response) but also at the termination of the hyperpolarizing current (HYPER-OFF response). Experiments in perfusates containing ion channel blockers, Co, TEA and Cs, showed that the depolarizations evoked in situ contained voltage-dependent Ca current, voltage-activated and Ca-activated K currents and possibly h current, all of which have been established in dissociated rod preparation. Furthermore, the depolarization evoked by the transretinal current pulse is mainly attributed to HYPER-OFF response rather than DE-ON response. This suggests that the site of Ca current generation is not restricted to the rod terminal considering the direction of the stimulus current flowing through the rod.

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SITE OF ORIGIN OF RETINAL SPREADING DEPRESSION. FUJIMOTO, M. and TOMITA, T. Dept. of Physiol., St. Marianna Univ. Sch. of Med., Kawasaki 213

The field potential associated with retinal spreading depression (SDP) has been interpreted in terms of extracellular K^+ increase in the proximal retina (one-origin hypothesis, Mori et al., Jpn. J. Physiol., 26, 219, 1976; Tomita, An. Acad. Brasil. Ciênc., 56, 505, 1984). Do Carmo and Martins-Ferreira (An. Acad. Brasil. Ciênc., 56, 401, 1984), on the other hand, persist in their two-origin hypothesis, based on their recent observation in the chick retina that the maximum of the SDP which normally is in the proximal retina shifts to the distal retina in a reversible way, when lowering the temperature of the preparation. We repeated the experiment of temperature change using both the frog and chick retinas, and were unable to reproduce their observation. The SDP maximum was always in the proximal retina, irrespective of temperatures. As a control experiment, we injected K^+ in the chick proximal retina and analyzed the K^+-induced field potential. Results from all the above experiments showed that the SDP could be fully explained by the one-origin hypothesis.

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MORPHOLOGICAL STUDY ON THE ISOLATED RETINAL CELLS. UEKI, K. Dept. of Physiol. Tokyo Women's Medical College, Shinjuku-ku, Tokyo 162

Morphological studies on the isolated cones of the turtle (Geoclemys reevesii) and horizontal cells of the gold fish (Carassius auratus) have been attempted by using an improved convenient method for observations of the fine structure of the isolated retinal cells. All conventional procedures were carried out leaving the pieces of glass with isolated cells intact. After polymerization of resin, the glass was detatched from the resin block. This method enables us to identify the isolated cells clearly as a whole mount with microscope and to trim the target cell for serial sections.

Freshly isolated cells fixed within a few hours after isolation showed almost similar pictures to those of non-isolated retinal cells. Most of six different cones had lost the outer segments but some pedicles remained. The inner segment contained its normal complement of cytoplasmic organelles and inclusions: oil droplet, the ellipsoid packed accumulation of mitochondria and paraboloid contained abundunt glycogen particles. Synaptic vesicles and synaptic ribbons of pedicles gave normal morphology. The processes of the horizontal cells fixed after one day culture changed into slightly rounded form and their fine structure did not significantly differ from that of the non-isolated cells. Some processes included vacuoles and stacking membranous strata.

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PHOTORESPONSE AND MORPHOLOGY OF AMACRINE CELLS OF CARP RETINA. TERANISHI, T., NEGISHI, K. and KATO, S. Dept. Neurophysiol., Neuroinformation Res. Inst., Sch. Med. Univ. Kanazawa, Kanazawa 920

By means of intracellular marking technique with Lucifer yellow (LY) for isolated carp retinas, relationships between photoresponse and morphology of amacrine cells were investigated in wholemounts and cryosections. The photoresponses were classified into 2 main types (transient and sustained), and subclassified into 5 types (fast ON-OFF, 'slow' ON-OFF, center-ON, center-OFF and sustained 'ON'). In their spectral responses, a maximum amplitude was found at 621 nm for all types except for the ON type, which exhibited it at 517 nm. Each response type was attempted to correspond to a particular cell type. Cells marked with LY were tentatively grouped according to somatic shape, dendritic field and dendritic stratification in the IPL. In the present study, the results were agreed with those previously reported, while some new findings emerged: (i) the slow ON-OFF response was originated in a small population of pyriform amacrines; (ii) the sustained ON response was recorded from a rare population of pyriform amacrines; (iii) most fusiform cells possessed long and fine peripheral processes in addition to the commonly described dendrites; and (iv) dye coupling took place between dendrites of similar-shaped amacrine cells.

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THE EFFECT OF POLARIZATION OF BIPOLAR CELLS ON AMACRINE CELLS IN THE CARP RETINA. KUJIRAOKA,T., SAITO,T. AND TOYODA,J-I.
Dept. of Physiol., St. Marianna Univ., Sch. of Med., 2095 Sugao, Miyamae-ku, Kawasaki 213.

To elucidate the synaptic mechanism between bipolar and amacrine cells, we examined the effect of polarizaiton of a bipolar on an amacrine cells by simultaneous intracellular recordings of both cells. When an ON-bipolar was dipolarized by a extrinsic current pulse, ON amacrine was depolarized in a sustained manner. This depolarizarion was blocked by a background light. It was enhanced as well as the light response when the ON amacrine was hyperpolarized by a steady current. On the other hand, an ON-OFF amacrine cell was stransiently depolarized at the depolarization of either ON- or OFF-bipolar cells polarized by the current pulse. These transient depolarizations were also blocked by a background light. These results suggest that the inputs of bipolar cells onto amacrine cells are excitatory, but their synaptic mechanisms are quite different between sustained and transient amacrine cells.

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SPECTRAL PROPERTIES AND RESPONSE SHAPE OF AMACRINE CELLS IN THE DACE RETINA. HASHIMOTO, Y. Dept. Physiol., Tokyo Women's Med. Coll., Shinjuku-ku, Tokyo 162

Response patterns of the amacrine cells to light stimulation are generally divided into two types, a sustained type and a transient type. The sustained cells responded with depolarization at light on (on-type) or with hyperpolarization at light on (off-type). The transient cells responded with transient depolarization at either on, or off, or on-off of light (on-type, off-type and on-off type, respectively). Spectral properties at center and at surround of their receptive field are quite variable. First group of cells show a similar behavior to both spectral properties and response shape at center and at surround. Second group of cells indicate an antagonistic spectral responses at center and at surround. Third group of cells show a very complex response shape and spectral properties, especially at surround. Spectral properties are either similar to those of horizontal cells, or an opposite polarity of horizontal cells. We are not recognized all spectral properties of bipolar cells yet, but these results suggest that the inputs to amacrine cells are very complicated, especially to the sustained amacrine cells.

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STUDIES ON THE RESPONSE OF THE FROG PHOTORECEPTOR WITH A WEAK LIGHT STIMULUS. HANAWA, I., TOBA, Y., and KATSUDA, S. Dept. of Physiol., Kobe Univ. Sch. Med., Kobe 650

Transretinal potential changes induced by a 0.1 sec exposure to a weak light were studied in the dark-adapted frog isolated retina. The retina was treated with aspartate to suppress the PII component of the electroretinogram. The response to the light stimuli was biphasic, which consisted of a vitreal positive wave followed by a vitreal negative one. A reduction in the extracellular Na^+ concentration led to a decrease in amplitudes of the positive and the negative waves. Decreasing the extracellular K^+ concentration, the amplitude of the positive wave was enhanced, whereas that of the negative one was decreased. A reduction in the Cl^- concentration caused the enhanced negative wave. The negative wave disappeared by the application of Ba^{2+} on the receptor side of the retina. The positive wave was not observed on the retina with pigment epithelium. From these findings, it was suggested that the development of the biphasic response was due to a light-induced decrease in $[\ K^+\ ]_o$ in the photoreceptor layer.

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REEXAMINATION OF SPECTRAL SENSITIVITIES OF PHOTORECEPTORS IN THE TURTLE RETINA. OHTSUKA, T. Dept. of Information Physiol., Natl Inst. Physiol. Sci., Okazaki

Spectral sensitivities of photoreceptors in the turtle (Geoclemys) retina were studied by intracellular recording, and each cell was filled with Lucifer yellow (LY). Photoreceptors were classified into seven morphological types: rod, four types of single cones, and two members of a double cone. Single cones contained one of four different oil droplets; red, pale-green, and orange colored, and also clear. Double cones consisted of two apposed cones; principal members contained yellow oil droplets, while accessory members contained no oil droplet. Spectral sensitivities recorded from these seven types of photoreceptors were classified into one type of rod and three chromatic types of cones. Rods (n=19) showed peak sensitivity at 520 nm. Single cones containing either a red (n=51) or a pale-green (n=9) oil droplet were red-sensitive (λmax at 620 nm). Single cones containing an orange oil droplet (n=14) were green-sensitive (λmax at 560 nm). Single cones containing a clear oil droplet (n=3) were blue-sensitive (λmax at 480 nm). Both members of the double cone, principal (n=22) and accessory (n=15), were red-sensitive (λmax at 620 nm). No diffusion of LY was detected between the apposed members of double cones.

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SYNAPTIC CONTACTS BETWEEN RED-SENSITIVE CONES AND TRIPHASIC C-TYPE HORIZONTAL CELLS IN THE TURTLE RETINA. KOUYAMA, N., OHTSUKA, T*. Dept. Physiol., Tokyo Women's Med. College, Shinjuku-ku, Tokyo 162 and *Dept. of Information Physiol., National Inst. Physiol. Sci., Okazaki 444

Synaptic contacts between photoreceptors and triphasic chromaticity type horizontal cells (THC) were studied by combined physiological and anatomical methods in two species of the turtle. After horizontal cells were injected intracellularly with horseradish peroxidase (HRP), the synaptic contacts were investigated with light and electron microscopy. The types of photoreceptors were determined by the color of the oil droplets in reference to our recent study.

Electron microscopic observation revealed that the THC received direct inputs from blue- and red-sensitive cones. In Geoclemys, one THC had ribbon synaptic contacts with 19 cones; 12 red cones, 6 blue cones and one green cone. In Pseudemys, one THC had ribbon synaptic contacts with 21 cones; 16 red cones and 5 blue cones. The dendrites of THC were found in the lateral processes of the ribbon synapses in both red and blue cones. Therefore, responses to red flashes of the THC appear to be generated through synapses from red cones, not with the feedback circuitry transmitted through the blue cones as believed previously.

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A MODELING STUDY ON THE REDUCTION MECHANISM OF THE RETINAL HORIZONTAL CELL RESPONSES.
USUI, S., MURASE, K. Toyohashi Univ. of Technology, Tempaku, Toyohashi 440 Japan.

We have been studying the L-type horizontal cell layer of the carp retina to understand the mechanisms underlying the spatio-temporal information processing. One of the most prominent properties of the responses is the off-dynamics to flash stimuli, and we have suggested that it might be due to a nonlinearity of the gap junctional conductance as we called "the reduction mechanism" (Usui et al., 1983, 1984). In this study, to test the hypothesis, we constructed a model of the layer and simulated the responses. First, a solitary horizontal cell model which is based upon all the ionic current components found in the cells was developed using the Hodgkin-Huxley formulae. The cell model mimiced well the actual horizontal cell at various conditions; a generation of Ca-spikes to current stimuli, an anomalous rectification at hyperpolarized levels, etc.. Then, a horizontal cell layer model was formed by connecting a number of the cell models with gap conductances in a similar manner to the morphology of the layer. In the gap conductance model, the parameters could be manipulated so that various voltage-current relationships could be obtained. Spatio-temporal responses of the layer model were thus examined at various possible conditions and parameters, and it was found that the spatio-temporal properties of the layer could be simulated well at which the gap conductance exhibits a certain nonlinearity, as we have suggested.

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LIGHT-SUPPRESSED K^+-CONDUCTANCE IN AN EXTRAOCULAR PHOTORECEPTOR IN THE ONCHIDIUM GANGLIA.
GOTOW, T. Dept. of Physiol., Kagoshima Univ. Sch. Med., Kagoshima 890

The light-induced membrane current of an extraocular photoreceptor, the photosensitive neuron (A-P-1), in the Onchidium ganglia was studied using the voltage clamp method and monochromatic (490 nm) light stimulation. At the resting potential light elicited slowly developing inward currents which peaked at 20-30 sec. It was possible to predict amplitudes of the current response produced by any intensity of light by using the modified Michaelis-Menten equation. The light-induced current was associated with a decrease in membrane conductance, and the reversal potential for the current was sensitive to external K concentration. The current-voltage relation for the steady-state light-induced currents was nonlinear, but that for the instantaneous currents was almost linear. These results suggest that light decreases the voltage- and time-dependent K^+ conductance in A-P-1.

This K conductance was unaffected by 10 mM TEA, 1 mM 4-aminopyridine and 10 mM Ba^{++}. Finally, in the unclamped A-P-1 the light-induced depolarizing voltage response which was associated with a decrease in membrane conductance could be mimicked by cyclic GMP, but not by cyclic AMP, 5-HT and muscarine.

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EFFECT OF CALCIUM IONS ON THE PHOTORECEPTOR CELL PROPERTIES OF SQUILLA ORATORIA.
NAGAI,Y., Dept. of Physiol., Tohoku Univ. Sch. of Med., Sendai 980

The input resistance to depolarizing currents in the retinular cells of mantis shrimp, was measured using double-barreled electrodes under several ionic environments. The depolarizing receptor potential elicited by high intensity stimuli (0.6 sec, 10 $\mu W/cm^2$) was composed of the initial transient phase and the sustained phase with reduced amplitude, and 20 μM TTX reversibly blocked only the transient phase. The input resistance in normal saline(10 mM Ca^{2+}) was 12±7.6 MΩ (n=43)in darkness. This value decreased during illumination and was lower at the transient phase than at the sustained phase. In Ca^{2+}-free saline and in Co^{2+} or Ba^{2+} saline(20 mM), the membrane depolarized, the input resistance increased and the amplitudes of the two phases increased, but the sustained phase in Ba^{2+} saline reduced in amplitude. In Ca^{2+} rich saline (20 mM), the membrane hyperpolarized and the input resistance decreased. When Ca^{2+} was injected by iontophoresis a similar effect was observed. The reversal voltages of the two phases were the same (22±6.5 mV, n=10). In Ca^{2+} rich saline, the amplitudes of both phases reduced and changes in the reversal voltages were negligible. The results suggest the existence of Ca^{2+}-activated K^+ current in the receptor cell. However, the two phases of the receptor potential are not attributable to this current.

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RESPONSE PROPERTIES OF OCELLAR INTERNEURONS OF THE COCKROACH.
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The dynamic responses of the second order neurons (L-neurons) of cockroach ocelli have been studied in light-adapted conditions. The L-neurons responded to light intensity changes with graded potentials and generated one or a few spikes when the light intensity was decreased. These responses to moderate intensity changes occured when the adapting light intensity was more than about 0.3×10^{-3} lux and the frequency of intensity changes was less than 30 Hz. The effects of adapting light intensity upon the frequency response were examined. With decreasing adapting intensity, frequency-amplitude of graded potentials curve and frequency-probability of generation of spikes curve shifted along the frequency axis toward the low frequency. With decreasing adapting intensity, the amplitude of graded responses to low frequency stimuli decreased slightly and that to high frequency stimuli decreased strongly, and the probability of generation of spiking responses to low frequency stimuli increased and that to high frequency stimuli decreased.

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ROD-MEDIATED INPUT AND CALCIUM RESPONSE IN TRANSIENT AMACRINE CELLS OF THE CARP RETINA. WATANABE, S.-I. and MURAKAMI, M., Dept. Physiol., Keio Univ. Sch. Med., Tokyo 160

Using low intensity monochromatic light stimuli, a rod-mediated input was revealed in the transient amacrine cells in addition to the established red cone-dominant input; when dark-adapted, the transient depolarizations showed maximum amplitude to 520 nm light and 680 nm light evoked no detectable response. The rod-mediated input may contribute to a detection of a slight intensity change even under very low illumination. In 55 % of the 91 cells tested, depolarizing current injection evoked a transient depolarization whose duration was more than 300 msec. This depolarization was suppressed by perfusion with a Co-containing solution and was not produced in a Ca-free solution, indicating that the evoked depolarization was a calcium response. In addition, a potassium current seemed to be activated by membrane depolarization; when depolarizing current was turned off, 70 % of the cells produced an after-hyperpolarization which was not affected by tetrodotoxin and Co application. Since the calcium response may be involved in the transient photoresponse, it is necessary to reconsider the conventional view that the photoresponses consist solely of EPSP's and sodium spikes.

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OSMOTIC EFFECT OF PERFUSING SOLUTION ON THE MORPHOLOGICAL STRUCTURE OF THE ISOLATED CHICK RETINA. SATO, T., YONEYAMA, T.*, TAIRA, K., OKUDA, K. AND SUZUKI, T.A. Dept. Physiol., Sch. Dent., *Dept. Ophthal., Sch. Med., Iwate Med. Univ., Morioka, Iwate 020.

In our recent research it was clarified that the humor collected from subchoroidal space of the light-adapted chick eyeball is hypertonic (480 mOsm) and that the isolated chick retina preserves the c-wave of ERGs for several hours in the hypertonic perfusing solution, whereas in the isotonic perfusing solution (350 mOsm) it is difficult to maintain the normal excitability and the retina swells markedly in external appearance. In the present paper we aimed to compare morphologically the retina soaked in the hypertonic solution for 2 hours with that in the solution isotonic to the body fluid.

Thickness of each retinal layer was measured at five places equally divided of a section. Data obtained from five sections were analyzed. Thickness of the whole retina in the hypertonic solution was 199.3 ± 7.3 μm (mean ± SD), whereas, that in the isotonic solution was 230.1 ± 18.6 μm. In addition, it was noticed that in the case of the retina kept in isotonic solution there were marked furrows on the inner surface of the retina and this caused a loss of regular laminar arrangement in thickness as indicated by a 2.5-fold increase in SD. Thus the chick retina seems to be surrounded physiologically by hypertonic humor than it was in the body fluid.

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SPATIO-TEMPORAL DYNAMICAL INTERACTIONS OF THE PHOTORESPONSES OF CATFISH RETINAL NEURONS. HIDA, E. AND NAKA, K.-I. National Institute for Basic Biology, Okazaki, 444

Spatio-temporal dynamical interactions of the photoresponses of catfish retinal neurons (horizontal cell soma, off-center bipolar cells, off-center ganglion cells) were studied by measuring spatio-temporal receptive fields for various sizes of random checker stimuli. In horizontal cells, temporal dynamics and spatial profiles of receptive fields were not changed irrespective of the checker size indicating that spatial dynamics and temporal dynamics within the receptive field behaved independently. In bipolar cells, temporal dynamics for stimulation of the receptive field surround by uniform binary noise became slower compared with the case of stimulation by steady uniform light. On the other hand, temporal dynamics of ganglion cells were quickened by coarsening the stimulus pattern without changing the spatial profile of the receptive field. These results suggested that spatio-temporal dynamical interactions occurred as a result of antagonistic organization of the receptive field and that different center-surround interactions were involved in bipolar cells and ganglion cells.

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COMPARISON OF THE RECEPTIVE FIELD IN THE SOMA AND THE AXON TERMINALS OF THE HORIZONTAL CELLS IN THE CARP RETINA. YAGI, T., WATANABE, S., MITARAI, G.* Dept. of Aerospace Physiol., Res. Inst. of Environ. Med., Nagoya Univ., Nagoya 464 and *Dept. of Physical Ed., Chukyo Univ., Toyoda 470-03

The spatial properties of the soma and the axon terminals of the horizontal cells were studied by using a slit shaped dim light stimulus in the carp retina. The slope of the decline of the response amplitudes along a transverse axis of the slit stimulus were steeper in the soma than in the axon terminals. The response amplitudes of the axon terminals showed a single exponential decay against the distance from the stimulus. But that of the soma showed more complicated decay, consisting of two exponential components, one of which was very close to that of the axon terminals. These findings strongly suggest that the soma and the axon terminals have an electrical communication and the axon terminals modify the receptive fields of the soma. This observation was examined quantitatively in terms of a resistive network model.

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SIMULTANEOUS CONTRAST OBSERVED WITH LIGHT IN THE DIRECTION OF EARTH MAGNETIC FIELD. KEI TSUKEDA, Dept. of Physiol., Faculty of Med., Univ. of Tokyo, Tokyo 113

Light from A standard illuminant, placed in parallel with permanent geomagnetic field, successively passed a scattering plate, a slit, a monochromatic filter and a small hole in a rotating disc in this order and then was received at a fixed position in a dark-adapted eye. In the rotating disc, on the radius, two adjacent small holes of an angle 1° were bored. One of the holes was fitted with a color filter and the other with a gray filter. They were to be watched by the subject for a predetermined period. The simultaneous contrast under this condition was compared with that on the plane. The experiment was also performed with linear polarized light. 1) On a plane, with incandescent light, some simultaneous contrast was observed. 2) On a plane, with linear polarized light, small simultaneous contrast, 3) With light in the direction of earth magnetic field, slight simultaneous contrast. 4) With linear polarized light in the direction of earth magnetic field, almost no simultaneous contrast. To white light, cones' long wave sensitivity and rods' short wave sensitivity were naturally present. However, these were unrelated to stimulating color.

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NONLINEARITY OF VISUAL EVOKED POTENTIALS IN CERVEAU ISOLE CATS IN RESPONSE TO DECREASING STIMULUS INTENSITY. SHIBAGAKI, M., KIYONO, S., KAWASHIMA, T.* and WATANABE, S.**
Dept. of Physiol., Inst. for Develop. Res., Aichi Prefect. Colony, Kasugai, Aichi 480-03, *Inst. of Equilibrium Res., Sch. Med. Gifu Univ., Gifu 500 and **The Res. Inst. of Environmental Med., Nagoya Univ. Nagoya, Aichi 464

Characteristics of visual evoked responses to the flickering flash stimulation were studied with decreasing stimulus intensities in the cerveau isolé cats. The flash stimulation frequency was changed stepwise between 1 and 30 Hz in increasing and decreasing order. Stimulus intensity was varied to 10^{-1} (F1), 10^{-2} (F2), 10^{-3} (F3), 10^{-4} (F4) and 10^{-5} times (F5) 0.72 J/sec (F0) by neutral density filter. In all cases of F0-F5, the amplitude of the P1-N1 component showed a peak in 4-9 Hz frequency bands. With F0 and F1, the counterclockwise hysteresis phenomenon was observed around the peak. The peak of power for the 1st harmonics was observed at 3-6 Hz (the first) and 13-19 Hz (the second) driving frequency bands, and that of the 2nd harmonics at 2-3 Hz (the first) and 6-10 Hz (the second). The first peaks of the 1st and 2nd harmonics were observed in any intensity, while the second peaks began to appear in F1; they tended to become larger with F2, F3 and F4 and smaller in F5 intensities. Findings suggested two resonance systems with different properties. The nature of this resonance suggests the existence of nonlinear resonance structure in the generation system of visual evoked response.

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CHANGES OF INTRACELLULAR ION CONCENTRATIONS OF VISUAL CELLS BY LIGHT ADAPTATION. NAKAGAKI, I., SASAKI, S., IMAI, Y., AZUMA, K.* IWASAKI, N.*, SALEHI, S. A**& TAKAGI, M**
Dept. of Physiol. & * Biol., Osaka Med. Coll., Takatsuki, Osaka 569, & ** Dept. of Biol., Coll. Gen. Educ., Osaka Univ., Toyonaka, Osaka 560, Japan.

Using freshly frozen dried ultrathin sections of octopus and frog retina both in the dark and light adaptations, electron probe X-ray microanalysis was performed to determine the intracellular elemental concentrations such as Na, K, Cl, Ca, Mg and S, and the three dimentional observation of octopus visual cells was performed using high voltage electron microscopy (Natl. Inst. Physiol. Sci.).

Electron probe X-ray microanalysis showed that by the light adaptations, the Na, Cl and Ca concentrations increased, and the K concentration decreased in octopus rod outer segment, while the Na, Cl and Ca concentrations decreased and the K concentration increased in frog rod outer segment. These findings were confirmed by flame photometry experiments on the whole retina and vitreous humor.

The three dimensional observation revealed that many microtubules were running in the longitudinal direction in the cytoplasm of octopus rod outer segments.

From above findings the localization of Ca regulated large Na, Cl and K channels on the rabdomeres of rod outer segments and the function of the microtubules in rod outer segments were discussed. Freeze fracture technique was performed to observe the channel.

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SEROTONIN IMMUNOREACTIVE CELLS IN THE CATFISH RETINA. HIDAKA, S.*, NAKA, K.-I. and NEGISHI, K. National Inst. for Basic Biol., Okazaki 444. Kanazawa Univ. Sch. of Med., Kanazawa 920

Serotonin (5-HT) is a neurotransmitter candidate for a population of amacrine cells in some nonmammalian retinas. In channel catfish retina a class of cells with endogenous serotonin (serotonergic cells) were found direct immunocytochemically (serotonin antiserum, monoclonal, sera-lab). After destruction of serotonergic cells by injection of neurotoxin, 5,6-dihydroxytryptamine (5,6-DHT), dopaminergic cells uptaked exogenously injected 5-HT. The immunocytochemical application of serotonin antiserum stained dopaminergic cells.

The processes of serotonergic cells were seen in the proximal part of IPL. Some of the cells were very similar morphologically to the type N (or sustained amacrine) cells found in functional study. Somal distribution of the cells was mosaic. Electron microscopic observation showed the serotonergic cells were postsynaptic to the ribbon synapse of bipolar cells which were probably off-center type, and were also presynaptic to the processes of bipolar and other amacrine cells. The processes of dopaminergic cells were seen in both OPL and IPL.

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SIGNAL ENCODING OF GANGLION CELLS IN THE CATFISH RETINA. SAKURANAGA, M*, ANDO, Y.-I., and NAKA, K.-I., National Institute for Basic Biology, Okazaki 444, *Dept. of Physiol., Nippon Medical School, Tokyo 113.

White-noise modulated field of light was applied to the catfish (Ictalurus punctatus) retina and resulting responses were intracellularly recorded from gnaglion cells in the retina. The responses were separated into two different types of signals by truncating action potentials at their stems and by detecting the timing of spike discharges: one is a graded undulation of membrane potentials and the other is a spike train. The dynamic responses were analyzed by crosscorrelating the two types (slow and spike) of signals against the modulated input. Both types of signals produced the first and second order kernels, and there were no large changes in the character of kernels between the slow and spike components. The linearity is very similar to that in the bipolar cells, while the nonlinearity is also similar to that in the type C (transient amacrine) cells. A spike discharge was also found to correspond to a slow potential template, and the observed undulation of slow component was reproduced by temporal superposition of the all-or-none template potentials with a reasonable degree of accuracy.

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WHITE-NOISE ANALYSIS OF GANGRION CELLS IN CATFISH RETINA. HOSOKAWA, Y., NAKA, K-I., *SAKURANAGA, M. NIBB Okazaki 444, *Physiol. Nippon Med. Uni. Tokyo 110

Ganglion cell dischagres evoked by either white-noise modulated photic or extrinsic current injected into horizontal cells were anlayzed by crosscorrelation technique. Discharge patterns were analyzed from the signature of the first and second order kernels. We discovered: 1) Peak response time of light evoked discharges became shorter with higher mean illuminance but not of current evoked discharges, 2) the response had two major nonlinearities which were similar to those in the types N and C amacrine cells, and 3) second order kernels changed their patterns with increase in the mean illuminance in about 50% of cells examined.
We conclude that some ganglion cells receive nonlinear inputs exclusively from one of two amacrine cells and some others from both cells.

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Permeability to sodium ions of the endolymph-perilymph barrier. Konishi, T. and Mori, H.

The endocochlear potential and Na^+ activity in the endolymph were simultaneously measured with a double-barreled Na^+ selective liquid membrane electrode. The modified Na^+ conductance and permeability coefficient of the endolymph-perilymph barrier were calculated from the rate of change in the endolymph Na^+ concentration relative to the Na^+ electrochemical gradient across the barrier during permanent anoxia. In normal guinea pigs the Na^+ conductance of the endolymph-perilymph barrier is approximately 5 times less than its K^+ conductance. Exposure to noise or treatment with kanamycin suppresses the cochlear microphonics but does not result in significant alterations in the Na^+ permeability of the endolymph-perilymph barrier. It is likely that the Na^+ permeability of the endolymph-perilymph barrier is not involved in the physiological processes which lead to the cochlear damage produced by noise or kanamycin.

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VOLTAGE CLAMP OF SCALA MEDIA OF GUINEA PIG COCHLEA. HASHIMOTO, T., TANIGUCHI, I. and YAMAGISHI, S. Inst. Med. Dent. Eng., Tokyo Med. Dent. Univ., Tokyo 101, Med. Res. Inst., Tokyo Med. Dent. Univ., Tokyo 101, Natl. Inst. for Physiol. Sci., Aichi 444.

The cochlear microphonic current flow across the basilar membrane was measured by applying the voltage clamp method to the scala media of the cochlea under the sodium pentobarbiturate anesthesia. The tips of a pair of enamel-coated wire electrodes were inserted into the scala media through small holes drilled on the basal turn of the cochlea. One was used for measuring the potential in the scala media and the other for passing the feedback current through a voltage control amplifier to clamp the potential to a fixed level. In response to tonal stimulation , a stimulus-associated current of several hundreds nanoamperes was observed. By a simple electrical circuit model following Thevenin theorem, the magnitude of the resistance change that should occur at the basilar membrane, perhaps in the hair cells, was estimated from the experimental data of the cochlear microphonic current and the potential.

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A TRIAL FOR POTENTIAL RECORDINGS FROM MAMMARIAN VESTIBULAR ORGAN IN SITU. SATOW, Y., WATANABE, S.*, KAWASHIMA, T.** Gifu Coll. Med. Tech., Seki-shi 501-32, *Res. Inst. Environmental Med., Nagoya Univ., Nagoya 464, **Inst. Equilibrium Res., Gifu Univ. Sch. Med., Gifu 500.

Extracellular potential recordings from the ampulla or the macula of the vestibular organ in a hamster or a cat were performed under an anaesthetized condition, after locations of the receptors were confirmed. The external auditory maetus, the tympanic membrane and the small bones were removed and a part of bulla was broken, in order to expose the oval and round windows. The superior and lateral ampullae were located in the thin bone near the oval window. The saccule and utricle maculae were seen through the windows by taking off the stapes rested in the oval window and the round window membrane, respectively. The ampullae and maculae were removed and were dissected under a microscope. Hair cells were observed through water immersion lens. Spontaneous potentials were sometimes recorded, when a microelectrode filled with 1M NaCl was inserted into the ampulla or macula region. Spontaneous discharges recorded from the ampulla region were suppressed by pressing on membranous canal near the recording electrode with a capillary. It is not certain whether these potentials show the receptor activities in the vestibular organ. However, it is important to identify each of receptor activities in living animals.

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Ca-DEPENDENT K CHANNELS IN GOLDFISH SACCULAR HAIR CELLS
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Goldfish saccular hair cells were isolated with enzymatic treatment and studied with patch clamp techniques. Current records under whole cell clamp mode suggested the presence of Ca-dependent K channels as well as other channels such as Ca, A-current K, and anomalous rectifier K channels. Single channel currents of Ca-dependent K channels were successfully isolated with cell-attached and inside-out modes of patches. With inside-out patch, they were found to be large conductance channels of 300 pS whose open-state probability was changed depending on potential and on internal Ca concentration in a similar fashion as reported in other preparations. However, relaxation to potential changes of the channel was much faster in the present preparation. The open-state probability after application of depolarizing steps reached the plateau level within a period as short as 10 ms, in contrast to 75 ms reported of chromaffin cells. But, it is not yet clear whether there are any substantial differences in relaxation rate among hair cells of different origins.

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SLOW DEPOLARIZING RESPONSE FROM SUPPORTING CELLS IN THE GOLDFISH SACCULE.
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Potentials were recorded intra- and extracellularly from the saccular macula of anaesthetized goldfish. Sound-evoked responses recorded intracellularly consisted of positive microphonic potentials, nerve responses and a slow depolarizing potential. Of these three potentials, only the slow depolarization was peculiar to intracellular records. It rose and fell exponentially with time constants of about 30 and 200 msec, respectively. The amplitude of slow depolarization never exceeded 5-6 mV, even when a loud sound was applied. It is argued that the impaled cell was supporting cell and the slow depolartization presumably reflected a rise of extracellular K^+ concentration, during sound stimulation. It was also shown that this nerve response which was resistant to the action of tetrodotoxin probably represented the excitatory postsynaptic current that flows into the dendrites of afferent fibers. Efferent stimulation suppressed the nerve response.

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GENERATOR POTENTIALS AND IMPULSE INITIATION OF THE AUDITORY FIBERS WITH SPONTANEOUS FIRINGS IN THE GOLDFISH.
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Responses were recorded from single auditory fibers (S2 fibers) in the goldfish to study relationships between the generator potentials and firings. In cases where a clear generator potential could be recorded in response to each sound wave, slow spontaneous potential changes which were irregular in amplitude and in time course, were also observed without apparent sound stimuli, and contained much slower ones than the sound-evoked generator potentials. The latter potentials in S2 fibers showed a delay following each sound wave and time courses comparable to the sound-evoked EPSPs of large S1 fibers. They also shared other properties such as adaptive decline in amplitude and off-suppression. The threshold level of generator potentials showed almost no apparent difference between spontaneous and sound-evoked firings. We concluded that generator potnetials which underlie the spontaneous and sound-evoked firings of S2 fibers are produced through the activities of afferent synapses of hair cells and that even the release of afferent transmitter in silence is possibly triggered by some form of hair cell activities.

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TWO TYPES OF Ca CURRENTS CONTRIBUTE TO THE PHASIC AND TONIC ACTIVITIES OF THE SENSORY EPITHELIUM IN PLOTOSUS ELECTRORECEPTORS. SUGAWARA, Y. and OBARA, S., Dept. Physiol., Teikyo Univ. Sch. of Med., Tokyo 173

On constant current stimuli, the Plotosus electroreceptor in situ shows graded and bi-directional responses in the ampulla (sensory epithelium), and similar PSPs in the afferent nerve, both consisting of phasic and tonic components. V-clamp analysis of isolated ampullae revealed Ca currents superimposed with a transient K current. In the presence of K-blockers, the Ca current declined exponentially, with two time constants successively. Double pulse experiments showed I-dependent inactivation for the peak Ca current. Na reduction from 170 to 80 mM abolished this initial component, and revealed a late less Na-sensitive Ca current which declined extremely slowly, and was unaffected by pre-pulses. Both components reversed their sign at a potential level much lower than the PSP suppression level, which suggests less ionic selectivity. Experiments in normal saline further revealed that the transient K current and PSPs were similarly depressed by pre-pulses, also I-dependently. These data suggest that the initial inactivating Ca current is involved in the oscillatory phasic response in situ, by inducing the K transient and the transmitter release, while the late non-inactivating current is responsible for the tonic response and the maintenance of the steady epithelial current.

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FREQUENCY THRESHOLD CURVES OF RAT COCHLEAR NERVE FIBERS
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Frequency threshold curves (FTCs) from 500 cochlear nerve fibers were measured from 81 albino rats under Nembutal anesthesia. The best frequencies (BFs) were distributed between 0.50 kHz to 62.6 kHz and those of the most sensitive fibers with threshold of about 5 dB SPL were between 20 and 40 kHz. Mean and variance of Q(10) to Q(50) increased with BF increase, where Q(N) is the ratio of BF to band width at N dB above the BF threshold. Normalized regression lines of their distributions almost agreed and could be expressed as $q(N)\times N=31\log_{10}f+29$, where q denotes a theoretical value corresponding to Q and f denotes frequency in kHz. H(N) and L(N) are the ratios of BF to the divided band widths which are taken to the higher- and lower-sides respectively from BF. The lines for L(N) changed with N gradually. When N were 10 and 50, they were expressed as $\ell(10)\times10=65\log_{10}f+56$, $\ell(50)\times50=20\log_{10}f+64$. The lines for H(N) were unstable. The expression for H(10) was $h(10)\times10=243\log_{10}f+42$.

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EFFECTS OF DRUGS ON THE ACTIVITIES OF FROG EIGHTH NERVE FIBERS. YANO, J., OOYAMA, H., SUGAI, T. AND SUGITANI, M. Dept. of Physiol., Kanazawa Med. Univ., Uchinada, Ishikawa

In the bullfrog, the occipital artery anastomoses with the carotid artery, which supplies the vessel to the inner ear, via the pharyngo-maxillary artery. Glutamate (Glu) (50 mM) or GABA (50 or 100 mM) in Ringer's solution was injected into the cannulated occipital artery and thier effects on the afferent discharges of the eighth nerve fibers were studied. At the injection rate of 4 µl/sec, Glu and GABA (0.24-0.48 ml) did not affect the firing rate. Injection of Glu (0.5-0.8 ml) at 30-50 µl/sec, however, increased the firing rate in the fibers with high characteristic frequencies (CF's) (1800-1900 Hz), supposedly the basilar papilla (BP) fibers, and decreased the rate in the fibers with the low CF's (20-60 Hz), supposedly the amphibian papilla (AP) or the saccular fibers. At similar injection rate, the firing rate did not change with injection of GABA (0.5-0.7 ml) or Ringer's solution (0.5 ml). Responses to Glu, therefore, do not seem to reflect any mechanical artifact due to the injection. The facilitatory effect of Glu on the BP fibers suggests that Glu could be a putative transmitter released from the hair cells as the BP has no efferent innervation. The inhibitory effect of Glu on the AP and the saccular fibers might be mediated through the efferent innervations to these organs.

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CENTRAL PROJECTION AND COURSE WITHIN THE MEDULLA OF GOLDFISH PRIMARY AUDITORY FIBERS AS REVEALED BY INTRAAXONAL INJECTION OF LUCIFER YELLOW

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Intra-axonal injection of Lucifer Yellow was performed on large and small afferent fibers (S1 & S2 fibers) to study their course within the medulla. Upon entering the medulla, S1 fibers proceeded mediorostrally toward the area where lateral dendrite of Mauthner cell was located. Main bundle of S2 fibers was found to run mediocaudally to end a little short of the midline. Very often they showed many fine arborizations near their terminals. The course of S2 fibers were also traced by recording field potentials with KCl-filled microelectrode. The potentials were evoked at twice the frequency of sound, but, relative size of deflections corresponding to different sound phases varied with minute changes in the recording site indicating that fibers responding to different phases of sound formed separate bundles which ran parallel each other. Field potentials were negative along most of medio-caudal course of S2 fibers, but the polarity was turned to positive near their terminal region, perhaps representing a conduction block.

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AN ELECTROPHYSIOLOGICAL STUDY OF THE AUDITORY EFFERENT FIBER BIFURCATING IN TFH EIGHTH NERVE OF THE TOAD.

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The presence of neural connections between the amphibian papilla (AP) and the vestibular nerves was studied in the isolated head preparation of toad. The nerve branches to the vestibular organs and the AP were cut and the central ends were sucked into two glass pipettes, acting either as stimulating or recording electrodes. Electrical stimulation of the AP nerve elicited compound action potentials in saccular, lagenar and three ampullar nerves and the shape of action potentials were similar when the stimulating and the recording electrodes were exchanged between each pair of branches. Thus neural links are present between the AP and the vestibular nerves. The conduction velocities were estimated approximately 2 to 3 m/s at 15°C, and correspond to the fiber diameters of 2 to 3 μm. When the eighth nerve was sectioned two weeks before, no potentials could be elicited between any pair of the eighth nerve branches, though good responses were obtained in afferent nerves. Results indicate that the auditory and the vestibular organs are connected by efferent fibers bifurcating in the eighth nerve. Single unit studies revealed that the stimulation of the bifurcated efferent nerve to the AP could elicit facilitation.

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DIFFERENCE BETWEEN NEURONAL RESPONSES IN THE ISOFREQUENCY PLANE OF THE RAT'S INFERIOR COLLICULUS.

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In the isofrequency plane of the inferior colliculus (IC) of anesthetized albino rats, responses of the single neurons to monaural and binaural tone-burst stimulations were studied with a tungusten microelectrode. Locations of neurons were determined from the lesions made by an electric current. Neurons excited only by the contralateral stimulation and those excited by the bilateral stimulation tended to be located caudally in the central nucleus of the IC. Neurons excited contralaterally and inhibited ipsilaterally were located in the middle and rostral portions of the nucleus. Along the dorsocaudal-to-ventrorostral penetrations of the electrode which were almost parallel to the isofrequency planes, the latency of the excitatory response gradually shortened from about 20 ms to 4.0 ms with increase in the depth of the neurons in the central nucleus. Thus it is concluded that the function of neurons are different according to their location in the isofrequency plane of the rat's IC.

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THE PROCESSING OF ULTRASOUNDS IN THE CEREBRAL CORTEX OF MONGOLIAN GERBILS. KAMADA, T. AND KAMEDA, K. Dept. Oral Physiol., Hokkaido Univ. School of Dent., Sapporo 060

Ultrasound signals in rodents have specific role and are differentiated from their audible signals. Ultrasonic sensitivity of 8th nerve and inferior colliculus of Mongolian gerbils (*Meriones unguiculatus*) was reported previously (1981, 1984). The present communication deals with responses of cerebral cortex to ultrasounds and is a preliminary study of how ultrasound signal is processed suitably to their biological significance. Two bolts were put on the skull by dental cement under Nembutal anesthesia. Overnight after surgery a glass micropippette filled with 3 M KCl or a glass-coated platinum-iridium electrode was inserted to the exposed cortex. A pure tone of 1 - 120 kHz was presented to the animal in open field from a condenser speaker with a 5.5 cm diameter. The response of a single unit was recorded in the area between middle cerebral artery and rhinal sulcus. Best frequency of the units ranged from 1.036 to 41.098 kHz with the thresholds of 20 - 40 dB SPL. Units with broad and sharp type tuning curves were observed. Q_{10} dB was 3.18 in 1 - 7 kHz and 3.69 in 33 - 41 kHz, respectively, while it was 14.07 in 8 - 20 kHz at average. Latencies of one group of units were 7 - 13 msec while those of the other group were 17 - 62 msec. Slow latency units might be the secondary auditory neurons. The results indicate the possibility that the gerbils can receive and process the ultrasounds in the auditory cortex.

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STUDY ON THE FUNCTION OF BAND OF NOISE UNITS IN THE AUDITORY CORTEX. MARUYAMA, N., SAITOH, K., KUDOH, M. AND FURUKAWARA, M. Dept. of Neurophysiol., Brain Res. Inst., Niigata Univ., Niigata 951

In the primary auditory cortex of the cat, about half of the units responded best to band of noise (BN). These BN units were divided into 3 types: N, M and W type, based upon the best band width. The BN units responded also to saw-tooth AM tones (STAMs). The best modulation frequencies correlated with the best band width. In the present experiment, we examined the responses of BN units to saw-tooth FM tones (STMFs) and synthesized formants generated by the terminal analog method (TFs). The best modulation frequencies for STAM and STFM and the best pitch frequency for TF were the same in each unit. In many of M type units, STAMs and TFs were more effective than STFMs. Usually the BN unit had a wide effective range for center frequency of BN, and in some units the effective ranges were separated into several patches of frequencies. We found a few exceptional cases in which the special combination of 2BNs were slightly more effective than single BNs. But in most cases the combined BNs were not more effective than single BNs.

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AUDITORY RESPONSE OF VOCO-AUDITORY ASSOCIATION AREA IN CANARY. MAEKAWA,M. Dept. of physiol., Dokkyo Univ., Med. Sch., Mibu, Tochigi 321-02. Japan

The voco-auditory association of passerine is well developed and necessarily referred to the song learning. Canary cerebral vocalization centers, HVc and RA are each connected with auditory center, field L by interneuronal nuclei(association area), "SH" and "SA" which are connected with the HVc and the RA, respectively. Most of auditory unit responses from the "SH" were of "on type" (70%), while all unit responses from the "SA" were of "sustained type". The auditory response of "on type" in the "SH" tended to be enforced by conditioning stimulation of the afferent from vocal motoneuron nucleus(nXIIts). Intracellular recording revealed slow spike potentials of which depolarization elicited fast spikes of "on type" burst discharge and were followed by after-hyperpolarization. This slow spike was of intrinsic electrogenesis rather than of synaptic component, and supposed as originated from dendritic portions. The data imply to suggest that this slow spike attributes to the trigger mechanism capable of burst disharge and tuning the latency to the sound.

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SPEECH-PHYSIOLOGICAL STUDIES ON THE "S","T" AND "R" SERIES IN JAPANESE LANGUAGE. KASAHARA, T., OHKUBO, S., Dept. of Physiol, Dental Clinic in the House of Representatives., 2-2-1, Nagatacho Chiyoda-ku Tokyo

We have made uninterrupted observation on six Tokyo-accent children without any defects in their speech-organ at their period of changing dentition from shedding of milk central incisors to eruption of lateral incisors by recording on Palatogram and Sonagram.

We have compared the results of the observation with those on pronunciation of "S", "T" and "R" series by children and adults.

The comparison showed that in the Palatogram of children at the period of changing dentition, the pronunciation of "TA","TE" and "TO" was complated in their Palatogram at a comparatively early stage.

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COMPARISON BETWEEN ACID-INDUCED RESPONSE OBTAINED FROM THE DORSAL LINGUAL EPITHELIUM AND THAT FROM OTHER EPITHELIA IN THE BULLFROG

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After adaptation to Ringer's solution, stimulation of mucosal surface with various acids produced a slow change in transepithelial potential difference between both surfaces of the isolated dorsal epithelium and other epithelia in bullfrog. The response obtained from the dorsal epithelium was negative on the mucosal side and its electrophysiological property was in many respects to that of the receptor potential in taste cells, while the response from other tissues was not always similar to that of taste cells. Ouabain had no significant effect on the response of the dorsal and ventral epithelia, and the palatal membrane, while it markedly depressed the response of the skin and bladder membrane. Effects of local anesthetics on the response were dorsal epithelium > ventral epithelium = palatal membrane > skin = bladder membrane, in the strong order. In the dorsal epithelium, the response magnitude to organic acids was higher than that to inorganic acids, even when stimulus acids were equivalent in pH. The results suggest that the response to acids obtained from the dorsal epithelium may have some influence on the process of frog taste reception.

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ACTION OF INTRACELLULAR TRANSMITTER ON RECEPTOR POTENTIAL OF FROG TASTE CELL ELICITED BY BITTER STIMULUS.

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Our previous experiments suggest that quinine-induced receptor potential in a frog taste cell may be related mainly to Na^+ influx through the basolateral membrane. In the present experiments, the action of cyclic nucleotides on the receptor potential of bullfrog taste cells in response to quinine-HCl was studied. The magnitude of the receptor potential was elevated by 50% by interstitial 10^{-3} M theophylline and this effect disappeared when interstitial Na^+ was eliminated. Interstitial 10^{-3} M vanadate decreased the receptor potential to 50% of the control. 10^{-4} M ouabain in the interstitial fluid did not change the receptor potential. When cyclic GMP or cyclic AMP was electrophoretically injected into a taste cell by a negative current, a depolarization was initiated. The depolarization amplitude increased with increasing current intensity and duration for injection. At present we can not find a positive correlation between cyclic nucleotide-induced depolarization and quinine-induced depolarization. It is possible that cyclic nucleotides do not act as a second messenger, but as a modulator.

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RELATIONSHIP BETWEEN GUSTATORY NERVE SIGNALS AND REFLEX ACTIVITY IN THE HYPOGLOSSAL NERVE.

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Gustatory input-motor output relationship in the linguo-hypoglossal reflex arc was studied, using immobilized bullfrogs. Afferent impulses in the glossopharyngeal nerve produced by varying concentrations of taste stimuli and consequent reflex discharges in the hypoglossal nerve were recorded, simultaneously. A restricted area of each response integrated was expressed relative to that of 1 M NaCl response in the respective nerve. The concentration-response relationships obtained showed that, compared with relatively small amount of afferent response to acetic acid, much greater amount of hypoglossal response with a factor of, for example, 8 at 10 mM, was obtained. Similar enhancement was not observed in the relationship for quinine but for warmed saline, suggesting the existence of central neurons highly sensitive to particular inputs. Taste responsiveness of solitary tract (NTS) and hypoglossal neurons so far studied showed that, at NTS, change in temporal pattern occurred only for acid signals, whereas, a large amplification for the same signals was seen to occur between NTS and hypoglossal neurons.

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THE INHIBITORY EFFECTS OF Ca ON THE Mg RESPONSE AND THE Na RESPONSE OF WATER FIBERS IN THE FROG GLOSSOPHARYNGEAL NERVE. KITADA, Y. Dept. of Physiol., Okayama Univ. Dent. Sch., Okayama 700

Neural responses were recorded from single water fibers of the frog glossopharyngeal nerve during stimulation of the tongue with $MgCl_2$ and NaCl. Thresholds for $MgCl_2$ and NaCl were around 10 mM and around 100 mM, respectively. Ca antagonized the effects of Mg and Na. To explain the inhibition by Ca, competitive inhibition by Ca was assumed. The assumption was supported by double-reciprocal plot analysis of the data. In Mg-Ca antagonism, the apparent dissociation constants for Mg and Ca were 7.0×10^{-2} M and 6.3×10^{-4}M, respectively. In Na-Ca antagonism, the apparent dissociation constants for Na and Ca were 1.0 M and 1.2×10^{-3} M, respectively.

To learn whether or not Mg and Na react with the different receptor sites, a 0.1% pronase was topically applied to the tongue dorsum. After pronase treatment, responses from the whole nerve to 0.5 M NaCl remained unchanged, but the magnitude of the response to 0.1 M $MgCl_2$ was markedly decreased. The results indicate that a Mg receptor site is different from a Na receptor site.

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INPUT-OUTPUT RELATIONS IN THE CHEMORECEPTIVE REFLEXES OF THE FROG. NOMURA, H., KUMAI, T. AND SUZUKI, H. Dept. of Oral Physiol., Matsumoto Dental Coll., Shiojiri 399-07

Input-output relations were compared between nostril-closing (linguo-trigeminal) and tongue-folding (linguo-hypoglossal) reflexes in the frog, as input-output relation is known to depend on the number of steps of the neuron nets in reflex pathways. Rana catesbeiana and Rana nigromaculata were used in the studies of both the reflexes, respectively. 0.5mM $CaCl_2$ dissolved in 0-50mM NaCl solutions and 0.005-0.5mM QHCl dissolved in 0.1M NaCl solutions containing 5mM HEPES (pH 7.2) were used as stimuli. The magnitude of afferent and reflex responses were obtained from the records of unitary discharges except for integrated reflex response in the tongue-folding reflex. Motor units in the nostril-closing reflex were divided into tonic, phasic and intermediate types, while those in the tongue-folding reflex were not. The curves of input-output relations of the tonic and intermediate motor units in the former reflex were steeper than that in the latter reflex, suggesting that the pathway of the former reflex is more complicated than that of the latter one.

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QUALITATIVE DISCRIMINATION AMONG AMINO ACIDS, 5'-NUCLEOTIDES AND THE FOUR BASIC TASTE SUBSTANCES IN MICE. NINOMIYA, Y. AND FUNAKOSHI, M. Dept. of Oral Physiol., Gifu College of Dentistry, Gifu 501-02

Qualitative discrimination among amino acids, 5'-nucleotides and the four basic taste substances(sucrose, NaCl, HCl and quinine-HCl) were studied by examining the generalization patterns of a conditioned aversion from single chemicals to the other compounds in mice. Among amino acids and 5'-nucleotides tested, Na L-glutamate, Na L-aspartate, 5'-IMP and 5'-GMP (MSG group) did not strongly generalized to any of the four basic taste substances, but L-glutamine and glycine did to sugars and Na saccharin. Differences in generalization patterns between the MSG group and NaCl were, however, largely reduced after section of bilateral glossopharyngeal nerve, although such reduction was not evident after section of bilateral chorda tympani nerve. These results suggest the possibilities that 1) mice can qualitatively discriminate between the MSG group and the four basic taste substances, and 2) for this discrimination the information conveyed by the glossopharyngeal nerve is relatively more important among peripheral taste inputs.

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Gustatory receptors to amino acids and betaine in the puffer.
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The characteristics of the gustatory receptors to amino acids(Ala, Gly, Pro) and betaine were examined by recording the whole nerve or single fiber responses from the palatine nerve of the puffer, Fugu pardalis. Single fiber analyses showed that the gustatory units could be divided into two groups; 1) those responding well to Ala, Gly and Pro, 2) those responding well to Bet. The extent of cross-adaptation between two stimuli of the above four solutions were examined in the whole nerve preparation. The combination of Ala-Gly showed no appreciable second peaks. The combination of Ala-Pro, Gly-Pro, or Pro-Bet showed a distinct second peak. In the combination of Ala-Bet or Gly-Bet, the second response was greatly enhanced by the application of the first stimulus. Cross adaptation experiments were also performed in the amino acid or betaine sensitive fibers. The results confirmed the above findings and showed that the enhancement of second response occurred in the amino acid sensitive fibers. These results indicate that the gustatory receptors of the puffer contain three different receptors for Ala and Gly, Pro, and Bet.

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PHOTOAFFINITY LABELLING STUDY OF SWEET RECEPTOR PROTEIN IN MONKEY CIRCUMVALLATE PAPILLAE.
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Presence of proteins specifically binding to thaumatin(sweet-tasting protein) in circumvallate papillae of the crab-eating monkey was studied by using photoaffinity labelling method with ^{3}H-azidobenzoate-thaumatin. SDS-polyacrylamide gel electrophoresis of a 0.1M Na phosphate buffer extract from ^{3}H-thaumatin-crosslinked papillae indicate a radioactive band at MW of 70K. The amount of the crosslinked protein was reduced by the presence of unlabelled thaumatin, but not affected by acetylated thaumatin, which does not taste sweet. The 70K peak was detected nither in other fractions from ^{3}H-thaumatin-crosslinked papillae, nor in fractions from papillae without taste buds. The results indicate that circumvallate taste buds in the monkey specifically contain a thaumatin-binding protein having a MW of 50K. It is postulated that the protein is concerned with recgnition of sweet stimuli at the taste cell receptor membrane.

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TASTE PREFERENCES TO SOME SWEETENERS IN VARIOUS STRAINS OF MICE.
IWASAKI, K. and SATO, M. Tokyo Metropol. Instit. for Neurosci., Fuchu, Tokyo 183

Preference behavior for sucrose, Na saccharin, D-Phe, D-Trp, Gly and L-Ala in 6 strains of mice (C3H/He(H), BALB/c(B), DBA/2(D), C57BL/6(C), BDF_1(F) and ddy(y)) were studied by using the two-bottle choice method. The prefer thresholds for sucrose in 6 strains were C=y<F<H<B<D, and a significant correlation found between log sucrose threshold concentration and saccharin threshold, but not Gly. C showed most high sensitivity to D-Phe and D-Trp, but B and D did not prefer them, while H showed most high sensitivity to Gly and L-Ala. Principal component analysis of data revealed that component scores for these sweeteners were clearly divided into two groups, Gly and L-Ala belonging to a group and sucrose, Na saccharin, D-Phe and D-Trp in another group. These results indicate there are differential taste mechanisms in two groups, and that preferences for Gly and L-Ala are influenced by different genes from those related to other 4 sweeteners.

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IMMUNOHISTOLOGICAL STUDIES ON SUBDIVISION OF THE RABBIT PYRIFORM CORTEX. ONODA, N., IMAMURA, K., MORI, K., and FUJITA, S. C. Depts. Physiol. and Pharmacol., Gunma Univ. Sch. Med., Maebashi 371.

The layers of the pyriform cortex (PC) were studied immunohistologically with monoclonal antibodies (MAbs) produced against the rabbit olfactory bulb. MAb 11-4F2 stained intensely layer Ia deep to the lateral olfactory tract (LOT), whereas MAb 11-7H5 stained intensely the whole Ib layer. According to different staining patterns for MAb 11-4F2, the adult PC could be divided into two subdivisions: dorsal and ventral PC; corresponding to results from cytological studies by M. Rose (1931) in the rabbit. On the other hand, in the postnatal rabbit, MAb 11-7H5 stained intensely layers Ib and III, but layer II was not stained at all. However, in layer II, fiber bridges were found which connected between layers Ib and III. Thus, the anterior PC might be divided into several regions by the bridges.

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MONOCLONAL ANTIBODIES RECOGNIZE SUBCLASSES OF VOMERONASAL NERVE FIBERS WITH SEGREGATED TERMINATIONS IN THE ACCESSORY OLFACTORY BULB. IMAMURA, K., MORI, K., FUJITA*, S. C., and OBATA, K. Depts. Physiol. and Pharmacol., Gunma Univ. Sch. Med., Maebashi, Gunma 371.

Organization of the projections of vomeronasal nerve (VNN) fibers to the accessory olfactory bulb was studied in the rabbit using monoclonal antibodies (MAbs) which recognize all or subclasses of the VNN fibers. One MAb (R2D5) labeled all VNN fibers. Another MAb (R4B12) recognized a subclass of the VNN fibers, and the third MAb (R5A10) the complementary subclass. The two subclasses of the VNN fibers were intermingled with each other in the VNN bundles and showed sharply segregated terminations in the glomerular layer of the accessory olfactory bulb.

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CHANGES IN MEMBRANE FLUIDITY AND MEMBRANE POTENTIAL INDUCED BY ODORANTS IN PORCINE OLFACTORY EPITHELIAL CELLS. KASHIWAYANAGI, M., SAI, K. AND KURIHARA, K. Fac. Pharm. Sci., Hokkaido Univ., Sapporo 060

Porcine olfactory epithelial cells containing 20-40 % olfactory cells were used in the present study. The changes in the membrane potential and the membrane fluidity were measured with fluorescence probes. The results obtained are as follows. 1) The membrane potential of porcine olfactory cells was depolarized by an increase of KCl-concentration. 2) Various odorants depolarized the porcine olfactory epithelial cell dose-dependently. 3) The magnitude of the depolarization induced by odorants was not appreciably changed by the elimination of Na^+, Ca^{++} and Cl^- in the stimulating solution. 4) For respective odorants, the membrane fluidity was changed at similar concentrations where the membrane potential was changed. 5) Changes in the membrane fluidity in response to odorants were measured with ANS, 7-AS, 12-AS, 12-AO and DPH which monitor the membrane fluidity at different sites in the membrane. The results obtained suggested that the odorants which have different odor are adsorbed at different sites in the membrane.

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RESPONSE PROPERTIES OF HUMAN CUTANEOUS MECHANORECEPTORS TO RANDOM VIBRATORY STIMULI. TOMA, S., NAKAJIMA, Y. and HOMMA, S. Dept. Physiol., Sch. Med., Chiba Univ., Chiba 280

To estimate the time course of generator potentials of human cutaneous mechanoreceptors, we investigated the response properties of skin receptors to vibratory stimuli. Single afferent impulses were recorded with a tungsten microelectrode inserted percutaneously and manually into the median, radial and peroneal nerves of sixteen awake subjects. The skin receptors were identified into 4 types according to their receptive fields and adaptations. Vibratory stimuli with a triangular waveform were applied to the center of the receptive field at random interstimulus intervals. Cross-correlograms between the vibratory stimuli and the single afferent impulses revealed a peak, which is composed of time-locked impulses to the vibratory stimuli. Fourty nine units (41 from the glabrous and 8 from hairy skin) were analyzed. From the correlograms, mean values of the conduction velocity and the width of the peak were measured. The width of the peak of RA units was significantly shorter than that of SAI units ($p<0.05$). But no significant differences were obtained between the other units.

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ULTRASTRUCTURE OF THE CAPSULAR CELLS IN THE FROG MUSCLE SPINDLE. FUNAHASHI,A., HANAICHI,T., FUJITSUKA,N. and ITO,F. Dept. Physiol. Nagoya Univ. Sch. Med., Nagoya 466

Ultrastructure of capsular cells of the muscle spindle isolated from semitendinosus muscles of the frog (Rana catesbeiana) has been studied using a deep etching technique as well as ultra-thin section and freeze fracture methods.

The outer capsule was identified by existence of many vacuoles in its cytoplasm which were shown in the three kinds of preparations.

Deep etching specimens revealed numbers of filaments with differnt diameters (4, 6, 10 and 15 nm), making a web-like cytoskeletal network. This network in the outer capsule seems to play a role for maintaining an elastic property of the muscle spindle to passive stretch or muscle contraction.

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MOVABILITY AND VISCO-ELASTIC CHARACTERISTICS OF THE CRAYFISH MECHANOSENSORY SETAE. CHICHIBU, S., CHIBA, A. and YAGINUMA, S. Dept. Physiol., Kinki Univ. Sch. Med., Sayama, Osaka 589

Visco-elastic properties of the hair socket and the pit of the sensory setae on the antennas of P. clarkii were studied. The development of the stress in response to the applied mechanical force was analyzed in relation to the amplitudes of the displacement. The general pattern of the stress developed to the saw-tooth shaped mechanical force was the combination of the initial delay and the following gradual stress increase. In the L-type hair, the stress to the downward displacement was larger than that to the upward one. The magnitude of the stress was in the range of $0.1 - 0.3 \times 10^{-6}$ N/ 10 deg. In the S-type hair, the relation was reverse. The stress developed was $0.8 - 2.3 \times 10^{-6}$ N/ 10 deg. for the downward, and $1.1 - 7. \times 10^{-6}$ N/10 deg., respectively. The modulus of rigidity of the hair pit structure on the plane parallel to the antennal axis was calculated. The value of the modulus in the L-type hair was nearly zero in the vicinity of the resting position, but it increased up to $1 - 3 \times 10^{-6}$ N/ rad for the shear angle of 2 - 5 degrees. Because of the structural limitations, the S-type hair showed a distinctive difference in the modulus values between the up- and downward shear angles. The value for the upward displacement ranged $20 - 50 \times 10^{-6}$ N/ rad while that for the downward one was less than 5×10^{-6} N/ rad.

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DIRECTIONAL SENSITIVITY OF THE PERIODONTAL MECHANORECEPTORS IN THE MAXILLARY CANINE OF THE CAT. KARITA, K. AND TABATA, T. Dept. of Physiol., Tohoku Univ. Sch. Dent., Sendai 980

Directional sensitivity of the periodontal mechanoreceptors using unitary responses from a superior alveolar nerve was studied in the cat. The canine tooth was stimulated in the twenty four directions by a specially designed stimulator on the horizontal plane. Responses of 328 slowly adapting units observed were affected by the direction of stimulus. These units were classified into three groups by the dynamic range of the response field: a heart-shaped type(more than 180°), a round type(90-180°) and an oval type(less than 90°). They were 27 units(8.2%), 284 units(86.6%) and 17 units(5.2%), respectively. Each response field of them showed little change of the dynamic range with the increase of the stimulus intensity. Off response was often observed in the heart shaped units when stimulated in the directions at the both edges of the response field. Every response field investigated showed a unimodal distribution. This result was different from that by Mei et al.(1975) who described that the response fields of units recorded from a gasserian ganglion generally consisted of two parts.

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PROJECTION PATHWAY FROM TOOTH PULP TO IPSILATERAL SOMATOSENSORY CORTEX (SI) OF THE CAT. MATSUMOTO, N., SATO, T. and SUZUKI, T.A. Dept. Oral Physiol., Sch. Dent., Iwate Med. Univ., Morioka, Iwate 020.

It has been known that the majority of neurons in the first somatosensory cortex (SI) receives projection from the contralateral side of the body. However, 40% of the tooth pulp-driven (TPD) neurons in the oral area of SI received input from the ipsilateral tooth pulp. This ipsilateral input pathway was explored in the cat anesthetized with nitrous oxide.

HRP study indicated that cells giving axon to the oral area were located mainly in layer III of the homotopic area in the contralateral cortex and medially in the ipsilateral VPM (nucleus ventralis posteromedialis) proper. In 6 cats, cooling experiments which kept temperature of the cortex beneath the chamber at 28.1 °C were performed. The cooling of the contralateral homotopic area caused little change in the firing rate of TPD neurons for ipsilateral pulp stimulation, whereas microinjection (1-2 μl) of 1% lidocaine into ipsilateral VPM caused diminution in the rate. Moreover, 32% of TPD neurons (n=19) which are distributed in the shell region of VPM proper responded to the ipsilateral pulp stimulation. These results suggest that ipsilateral pulp input to TPD neurons in the oral area is carried via projection fibers from the ipsilateral VPM but not via commissural ones from the contralateral oral area.

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THE EFFECT OF TEMPERATURE ON THE FAST- AND SLOW-ADAPTING RESPONSES OF THE PACINIAN CORPUSCLE IN THE CAT'S MESENTERY. SAKADA, S., TAZAKI, M., KATOGI, K., SHIOZAKI, K., SAITO, T., Dept. of Physiol., Tokyo Dent. Coll., CHIBA 260

Pacinian corpuscles are not only rapidly adapting mechanoreceptors that discharge on- and off-responses to displacement stimuli, but also vibration receptors that respond to high-frequency vibration. In the past, their electrophysiological properties have been believed to be caused by a mechanical high-pass filter action of non-nervous capsular tissue and by fast accommodation of the sensory nerve fiber. In vivo electrophysiological investigations have shown Pacinian corpuscles from cat planta and mesentery to be slow-adapting mechanoreceptors. This report describes electrophysiological experiments planned to demonstrate that isolated Pacinian corpuscles elicit both fast-adapting and slow-adapting responses to displacement stimuli and to low-frequency vibration at higher temperature whereas they elicit only fast-adapting responses at lower temperature. This work has been done in the hope of suggesting a new hypothesis to resolve previous discrepant interpretations of Pacinian corpuscles' responses to displacement s timuli.

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POLYMODAL RECEPTORS AND RAPIDLY ADAPTING MECHANORECEPTORS RECORDED FROM THE SUPERIOR SPERMATIC NERVE OF DOGS. MIZUMURA, K., SATO, J. AND KUMAZAWA, T. Dept. Nervous & Sensory Functions, The Research Institute of Environmental Medicine, Nagoya Univ., Furo-cho, 464 Nagoya

Using the testis-superior spermatic nerve preparations excised from anesthetized dogs, afferent nerve activities were recorded in vitro. More than 90 % of recorded units was polymodal type, while about 3 % (24 units) responded differntly.: the latter showed rapid adapting responses often followed by off-response to a steady mechanical stimulation, and seldom responded to other types of stimulus. Thus they are called rapidly adapting mechanoreceptors (RA). They seemed to respond better to moving mechanical stimuli. Some RA had several receptive fields about 2-3 mm in diameter. None showed spontaneous discharges. While almost all of the polymodal units responded to heat and algesic chemicals, RA never responded to heat and seldom to chemicals.:only 8 out of 20 units tested responded to 616 mM NaCl solution with significantly lower discharge rate than that of polymodal units, and 4 out of 10 to 60 mM K solution. Remarkably 14 out of 15 units did not respond to bradykinin (9×10^{-8} M). Cold stimuli were also ineffective (3 units). The conduction velosity (31.9±4.4 m/sec, n=7) of RA was significantly faster than that of polymodals.

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DISTRIBUTION PATTERNS OF SUBSTANCE P AND MET-ENKEPHALIN IN THE TRIGEMINAL NUCLEUS IN CROTALINE SNAKES. TERASHIMA, S. Dept. of Physiol., Ryukyu Univ. Sch. of Med., Nishihara-cho, Okinawa 903-01

In crotaline snakes there are dual nuclei in the trigeminal sensory system, a common descending nucleus (DV) and a lateral descending nucleus (DLV). They have the different functions of a common skin sensations and an infrared sensation, respectively. The present experiment was done to examine whether these functional differences are reflected in the distribution of some of the neuroreactive peptides: substance P (SP), Met-enkephalin (Met-ENK), cholecystokinin (CCK-8) and somatostatin (SRIF).

The SP fibers in the marginal zone of the DLV seem to come from vagal ganglion cells. This is also true of the DV and solitary nucleus. These fibers are not finly distributed in these nuclei.

The Met-ENK fibers are found in the reticular formation and also in the DV, but far less are found in the DLV.

Almost no CCK-8 fibers and very few SRIF fibers are found in both trigeminal nuclei, but found in the reticular formation.

In conclusion SP and Met-ENK show the possibility of a close relationship in the DV, but not in the DLV. The CCK-8 and SRIF do not seem to have much relationship to the trigeminal sensory nuclei function.

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CEREBRAL EVOKED RESPONSES ELICITED BY DIRECT STIMULATION OF LATERAL SPINOTHALAMIC TRACT IN HUMAN. TAIRA,T., AMANO,K., KAWAMURA,H., TANIKAWA,T., KITAMURA,K. Dept.Neurosurg. Tokyo Women's Medical College, Shinjuku-Ku, Tokyo 162

Electrically stimulated signals in peripheral nerve are believed to conduct mostly through posterior column of spinal cord and medial lemniscus system, while signals of noxious stimuli being in lateral spinothalamic tract(LST) at the level of spinal cord. The authors recorded cerebral evoked responses elicited by direct stimulation of human LST during percutaneous cordotomy for pain relief for investigation of central conduction of noxious stimuli. These responses consisted of four negative potentials , peak latency being 3.8, 8.4, 12.2 and 21.7msec respectively. These potentials were named as N1, N2, N3 and N4. N1 showed wide distribution over the scalp and so it was considered to be of subcortical origin. N2-N4 distributed both temporal and central area. Different distribution pattern of N2-N4 from conventional somatosensory evoked potential indicates different projection of LST from medial lemniscus system.

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THE EFFECTS OF HALOTHANE ON SENSORY EVOKED POTENTIALS. Miyamoto,T., Ryu,H., Yokoyama,T., Uemura,K., Nakajima,S. Dept. of Neurosurgery, Hamamatsu University,School of Medicine,Hamamatsu 431-31

The effects of halothane anesthesia on sensory evoked potentials were studied to know their usefulness as intoraoperative monitoring. Eight adult cats (2.6-4.0kg) were anesthetized with thiopental and immobilized. They were artificially ventilated with mixture of oxygen, nitrous oxide and halothane. The concentration of halothane increased up to 4.0% by 0.5%. Short latency somatosensory evoked potentials (SL-SEP) and auditory brain stem responses (ABR) were recorded at each concentration. The peak amplitude of each wave decreased gradually as halothane concentration increased, and finally disappeared from those with longer peak latency in order. These changes were more prominent in ABR. Halothane, however, did not produce any statistically significant changes on the latencies of sensory evoked potentials below 3.0%.

In conclusion, sensory evoked potentials were fairly constantly recorded during experiment under the practical halothane concentrations. The recording of sensory evoked potentials will be quite usefull to monitor the condition of CNS of the patients during surgery.

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EFFECTS OF ELECTROACUPUNCTURE, MORPHINE AND NALOXONE ON THE TAIL FLICK REFLEX IN RAT. TSURUOKA, M., MATSUI, A., MATSUI, Y. and ICHIOKA, M. Dept.of Physiology, Showa University School of Dentistry, Tokyo 142

Effects of electroacupuncture stimulation (EAS) on the tail flick muscle activity evoked by noxious heat stimulation to the tail skin were investigated in lightly anesthetized rats. EAS (50Hz, 5ms, 200μA, 30min) was applied to two meridian points, the Hoku and the Zusanli, for comparison of inhibitory effects on the muscle activity. EAS of the Zusanli markedly suppressed the muscle activity, but no changes of the tail flick latency and the threshold temperature producing the tail flick reflex were observed. These effects were not antagonized by naloxone. On the other hand, EAS of the Hoku produced a suppressive effect on the muscle activity, the rise of threshold temperature and the prolongation of the tail flick latency. These effects were antagonized by naloxone. Effects of morphine injected intraperitoneally were very similar to those of the Hoku stimulation. The results indicate that effects of EAS on the Zusanli are mediated with a non-opiate system, and effects of the Hoku stimulation are related to naloxone-reversible endogenous opiate mechanism in the central nervous system.

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D-PHENYLALANINE ANTAGONISM OF INHIBITION OF EVOKED POTENTIALS BY ANALGESIA INHIBITORY SYSTEM. HISHIDA, F., LUO, C.P., TANAKA, M. and TAKESHIGE,C. Dept. of Physiol., Sch. of Medicine, Showa Univ., Shinagawa-ku, Tokyo 142

Inhibition of evoked potentials (EP) in the lateral periaqueductal central gray (lPAG) by stimulation of the analgesia inhibitory system (AIS), both induced by acupuncture point (AP) or non-point (NAP) stimulation, was investigated. The tibialis and abdominal muscles were chosen as AP and NAP, respectively. EPs were recorded from AIS, lateral centromedian nucleus of the thalamus (lCM) and the posterior hypothalamus (PH), by AP or NAP stimulation. The threshold for EP stimulation was lower in NAP than in AP. Inhibition of lPAG EP by lCM or PH stimulation was antagonized by d-phenyl-alanine (DPA). The site of inhibition in the lPAG, localized by evoked potential recording, was in the rostral lPAG. The relation between PH and lCM in AIS was determined when lCM lesion abolished inhibition of lPAG EPs by PH stimulation. EPs in the lPAG, but not in the dorsal PAG were depressed gradually by repetitive AP and NAP stimulation. This depression was also antagonized by DPA. From these results: 1) Inhibition started from acupuncture or non-point, passed through PH and lCM in AIS, and extended to lPAG. 2) DPA antagonized AIS inhibition of EPs caused by analgesia inhibitory system stimulation.

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RELATION OF HYPOPHYSIS AND ADRENAL GLAND IN ACUPUNCTURE ANALGESIA. OKUBO, K., TANAKA, M., MURAI. M. and TAKESHIGE, C. Dept. of Physiol., Sch. of Medicine, Showa University, Shinagawa-ku, Tokyo 142

It was found previously that hypophysectomy abolished acupuncture analgesia (AA) caused by low frequency stimulation of acupuncture point. The relation between hypophysis and adrenal gland to cause AA was investigated in rats by measuring tail-flick latency. Antiserum of BETA-endorphin abolished AA and analgesia produced by stimulation of dorsal periaqueductal central gray and anterior hypothalamus, which had been identified as acupuncture afferent pathway from acupuncture point to hypophysis. Electrical lesion of stalk of hypophysis did not abolish AA until after 3 days. AA was augemented for six hours after hypophysectomy (HE) then reduced gradually and disappeared within 12 hours. Similar results were observed after adrenalectomy (AE). Neither naloxone nor dexamethasone abolished analgesia after HE or AE. Acupuncture non-point stimulation produced analgesia (ANA) after inhibitory system lesion, and electrical stress shock-induced analgesia were abolished until 3 days after HE and AE. From these results, AA, ANA and SIA were abolished by hypophysectomy as well as adrenalectomy, but analgesia having a nature different from AA appeared for 12 hours after AE and HE.

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LOCALIZATION OF ELECTRIC GENERATORS WITHIN THE BRAIN BY A BOUNDARY ELEMENT METHOD. NAKAJIMA, Y., HOMMA, S., MUSHA, T*. and OKAMOTO, Y*. Dept. Physiol., Sch. Med., Chiba Univ., Chiba 280, *Dept. appl. Electr., Tokyo Inst. Technol., Yokohama 227

This study deals with an inverse problem, in which location of electric generators within the brain was solved as a dipole from the potential distribution recorded with surface electrodes over the head. At first, we assumed the head to be a ball. Human somatosensory potentials (SEPs) were solved. The computed results of the each wave showed the relative location within the brain, but lacked the anatomical informations. To access to correlation of generator location with the brain structures, in the next, geometry of the head was exactly measured. A boundary element method was used in numerical calculation. The validity of this model was examined in experiments on the cat, in which error in estimation of the location of a dipole was within 1 mm. Using this method, locations of electric generators of SEPs were solved in man. We obtained a preliminary result that the location of the dipole of P_{24} wave was in the somatosensory cortex.

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SUPPRESSION OF SALT RESPONSES BY APPLICATION OF AMILORIDE TO THE TONGUE. FUNAKOSHI, M AND NINOMIYA, Y. Dept. of Oral Physiol., Gifu College of Dentistry, Hozumi, Motosu, Gifu 501-02

Effects of amiloride on taste sensitivity were studied by examining rat single chorda tympani responses and human taste thresholds to ionic taste stimuli and electrical stimulations. Application of amiloride reduced the responses to NaCl used as a chemical stimulus and a bathing solution for electrical stimulation in a population of rat chorda tympani fibers which were highly sensitive to NaCl. Whereas no such suppression was observed in the other fibers which responded strongly to all ionic taste stimuli used. Results of cross-adaptation studies between NaCl and KCl indicate that amiloride affects at least the single receptor mechanism which is highly sensitive to NaCl and also to some extent to KCl at its higher concentrations. Human taste thresholds for NaCl and electrical stimulation significantly increased after application of amiloride. Since the recovery time of threshold for NaCl, however, was shorter than that for electrical stimulation, it is possible that amiloride affects not only the mechanism highly sensitive to NaCl but also the other one activated by electrical stimulations.

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EFFECT OF AROUSAL STATE ON NEURONAL ACTIVITIES IN THE TRIGEMINAL MAIN SENSORY NUCLEUS IN RATS. KANOSUE, K., SHEN, Z.W., and NAKAYAMA, T. Dept. of Physiol., Osaka Univ. Med. Sch., Osaka 530

Somatosensory relay neurons in the ventrobasal complex (VB) of rat thalamus respond stronger to stimulations of their receptive field when EEG exhibits desynchronized pattern. To make clear the mechanism of this augmented response during arousal state, activities were examined for the neurons of the trigeminal main sensory nucleus (MSN) which responded to tactile stimulations and projected to the VB. The neurons were identified with antidromic response to electrical stimulation of the contralateral medial lemniscus. EEG activities were monitored from the occipital cortex. In the present anesthetic condition (urethane 1.2 g/Kg), EEG was usally in synchronized pattern. EEG desynchronization was produced by warming the scrotum from 30 to 40°C (Brain Res. 328, 1985). MSN neuronal responses to receptive field stimulation were not influenced by EEG activity. Thus, augmented response to receptive field stimuli during EEG arousal observed in VB neurons should be brought about by the direct influence on the VB neuron from "activating system".

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LOCALIZATION OF CORTICAL TASTE AREA IN HAMSTERS. YAMAMOTO, T., ASAI, K., MATSUO, R. AND KAWAMURA, Y. Dept. of Oral Physiol. Osaka Univ. Faculty of Dentistry, 1-8 Yamadaoka, Suita, Osaka 565

The cortical taste area in hamsters was delineated by the evoked potential and multi-unit recording methods and by the behavioral study using a conditioned taste aversion technique. The chorda tympani (CT) projection area was just dorsal to the rhinal sulcus and anterior to the middle cerebral artery. The projection area for the trigeminal component of the lingual nerve (LN) was anterior to the CT area, and the glossopharyngeal nerve (GN) area posterior to the CT area. The mean latency was 7.5 ms, 12.4 ms and 16.5 ms for LN, CT and GN responses, respectively. Stimulation of the tongue with an anodal D.C. current (20 μA) or taste solutions elicited impulse discharges in the cortical neurons located within the CT area and the ventral part of the GN area. This taste area, which is about 1 mm x 1 mm in size, was within the agranular insular cortex (Rose 1928). Bilateral ablations of the taste area attenuated or abolished, depending on the size and location of the ablations, the conditioned taste aversion (to 0.005M Na-saccharin) that had been acquired before ablations. The present results corresponded very well to those obtained in rats (Yamamoto et al. 1980).

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INFLUENCE OF EXPERIENCE OF SUCROSE INTAKE UPON GUSTATORY NERVE RESPONSES OF DEVELOPING MOUSE. YAMADA, T., UMEZU, M., ENDO, M.*, HOKIGUCHI, T.* and Sato, K.*, Physiol. Lab., Japan Women's Univ., Bunkyo-ku, Tokyo

A filter paper soaked with 40% sucrose solution was applied to the tongue of one half of a litter of mice during the period 2-35 days after birth. The other control half was given only water by the same method. The influence of experience of sucrose on the 38-45 day-old mice was studied by recording the integrated response of chorda tympani nerve to NaCl, HCl, sucrose and other sugars. The value of the responses to HCl of both two groups being taken as 100 each, the mean relative magnitude of responses to sucrose and NaCl became larger in the test group than in the control group. When the value of the responses to sucrose was taken as 100, the mean relative magnitude of responses to HCl and lactose became smaller in the test group than in the control group. The influence of experience of sucrose on developing mice was similar in gustatory nerve response to suclose and NaCl.

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THE ROLE OF MUCOSAL INFORMATION IN THE PERCEPTION OF MANDIBULAR POSITION
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The functional role of the mechanoreceptors of the denture-supporting mucosa in the perception of mandibular position was studied with eight edentulous subjects by examining the mandibular position (MP) as determined by the adjustable hydraulic jacks (Hydraulic Vertical Displaciometer) method. The mandibular position which the subjects began to perceive as being high (HMP) was shifted toward the closed position when the distribution rate of bite pressure against the incisal region was increased. This implied that the perception of MP was modulated by the information regarding the distribution rate of bite pressure. Furthermore, the HMP was also shifted toward the closed position when the stimulation of the incisal palatal region of the denture supporting mucosa by a dotted stimulus plate was greater than that of the molar palatal regions in spite of the constant rate of distribution of bite force. And it was shifted maximally toward the closed position when the distance between dotted stimulus points was in the range of 3 mm to 5 mm which was very close to the threshold of the two-point discrimination at the incisal palatal mucosa. The present findings suggest that the mechanoreceptors in the mucosa play an important role in the perception of MP by providing precise information as to the distribution of bite pressure.

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EFFECTS OF NORADRENERGIC NUCLEI STIMULATION ON THE ACUPUNCTURE-INDUCED NEURONAL ACTIVITY OF RAPHE MAGNUS CELLS IN THE RAT.
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Single unit recording was made from neurons extracellularly in the nucleus raphe magnus (NRM),which received excitatory inputs from the periaqueductal gray matter and projected to the spinal cord. The spontaneous firing rate increased after acupuncture stimulation of Ho-Ku in about 70% of the recorded cells, suggesting that these NRM cells are concerned with the descending analgesic system. After electrical stimulation of the A_1 nucleus, acupuncture-induced excitation was strongly suppressed in about 70% of the cells. This suppressive effect was antagonized by iontophoretically applied an alpha-antagonist, piperoxane. While, a beta-antagonist, sotalol scarcely blocked this suppression. In case of A_6 stimulation, the suppression was also observed in about 45% of the acupuncture-excited cells. However, neither piperoxane nor sotalol antagonized the A_6-induced suppression. Following microinjection of piperoxane into the 4th ventricle, the suppression induced by either A_1 or A_6 stimulation was rarely observed. The results suggest that there are a direct noradrenergic inhibitory pathway from A_1 and an indirect one from A_6 to the NRM cells. Therefore, it may be assumed that noradrenergic actions originated from A_1 and A_6 nuclei could be suppressed by acupuncture stimulation.

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ANALYSIS OF ACUPUNCTURE AFFERENT AND EFFERENT PATHWAYS IN THE BRAINSTEM TO CAUSE ACUPUNCTURE ANALGESIA.
SATO, T., JAUWHIE, J. and TAKESHIGE, C. Dept. of Physiol., Sch. of Med., Showa Univ., Shinagawa-ku, Tokyo 142

The acupuncture analgesia (AA) caused by low frequency stimulation of acupuncture point was measured by tail-flick test in rats. Animals were classified into acupuncture effective (EA) and non-effective animals (NEA). Analgesia caused by stimulation (SPA) of the raphe magnus (RM), reticulogigantocellular nucleus (NRGC) or reticuloparagigantocellular nucleus(NRPG) was different in EA and NEA. SPA lasted after termination of the stimulation in EA, while it appeared during stimulation in NEA. SPA after termination of stimulation was abolished by naloxone (1mg/Kg i.p.) or by lesion of the dorsal periaqueductal central gray, which was identified as the part of the acupuncture afferent pathway from acupuncture point to the hypophysis. RM-SPA was abolished by intraperitoneal methysergide (2mg/Kg), while NRGC-SPA and NRPG-SPA were abolished by intrathecal phentolamine (20μg). It was concluded that both acupuncture afferent pathway and acupuncture efferent pathway, which is the descending pain inhibitory system, exist around the RM, NRPG and NRGC.

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EFFECTS OF DIVALENT CATIONS ON THE EXCITATION-CONTRACTION COUPLING IN SKELETAL MUSCLE FIBERS. HATAE, J. and KAWATA, H. Dept. Physiol., Fukuoka Univ. Sch. Med., Fukuoka 814-01

Effects of divalent cations, Ca, Mg, Ni and Ba on the contraction threshold, twitch tension and tetanus were examined using single twitch muscle fibers of the frog. When divalent cations were added to normal Ringer solution, contraction threshold was increased but it was not changed with equimolar substitution of divalent ions except Ni. Tetanic tension was decreased by an elevation of the divalent ion concentration. Twitch tension was potentiated with equimolar substitution of divalent ions. Post-tetanic potentiation was observed in the presence of Ca, Mg and Ni, but it was largely suppressed by Ba. In a high Ca solution, the resting potential was hyperpolarized and the after-potential was increased although the late after-potential in tetanic stimulation was reduced. From these results, it seems that the differences in the response tested by single or tetanic stimulation among various divalent cations reflect not only their effects on the surface charge of membrane but also on some step of the excitation-contraction coupling.

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PROPERTIES OF THE ELECTRICALLY INDUCED SUSTAINED CONTRACTURE OF THE SKELETAL MUSCLE. KAWATA, H. and HATAE, J. Dept. Physiol., Fukuoka Univ. Sch. Med., Fukuoka 814-01

The sustained contracture induced by applying a prolonged depolarizing pulse through the sucrose-gap was investigated on the small bundle of bullfrog's toe muscle. The contracture induced in TTX-containing solution consists of fast and slow components, both depended on the extent and duration of depolarization. Raising or lowering the external Ca concentration respectively suppressed or increased both components, which could be attributed to an effect on the surface potential, although a prolonged perfusion with low Ca resulted in a gradual decline of tension. Conditioning depolarization potentiated the contracture while hyperpolarization exerted an opposite effect. These effects were not dependent on the changes in membrane potential. The inhibitory effect was more marked in the fast component, just as observed when the interval of pulsing was increased. Caffeine (1 mM) suppressed the post-anodal inhibition, whereas depolarizing and hyperpolarizing pulses had no effect on the caffeine (5 mM) contracture. A possible role of Ca ion in the vicinity of the T-SR junctional membrane was postulated.

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INTRACELLULAR Ca ION MOVEMENT IN THE SKELETAL MUSCLE FIBERS. OCHI, K., MATSUMURA, M. AND TOYOTA, H. Dept. of Physiol., Kawasaki Med. Sch., Kurashiki 701-01

Ca-transients were recorded in the *Xenopus* skeletal muscle fibers into which the Ca sensitive dye arsenazo III (AZ III) was injected. The computer simulation was carried out to estimate the concentration changes in Ca-troponin, Ca-parvalbumin and Mg-parvalbumin following the action potentials. The assumptions were that the time course of the AZ III signals was equal to that of free Ca ion concentrations and it was approximated by 6th degree polynomials, and that the concentrations of myoplasmic Ca buffer proteins in *Xenopus* muscle were the same as those in rabbit skeletal muscle. In standard Ringer solution, the concentration changes in Ca-troponin complex following a single action potential reached its peak of the 64% maximum saturation within 8-9 msec at 22°C. In NO_3-Ringer solution, Ca-troponin complex curve was greatly delayed in the falling phase, although it was increased in peak amplitude only slightly. After repetitive stimulation of 9 pulses at the frequency of 30/sec, the falling phase of Ca-troponin concentration was also markedly prolonged accompanied with only 20% increase in peak. Marked prolongation but relatively slight increase in peak of the Ca-troponin concentration curve in NO_3 solution or during repetitive stimulation may provide the basis of A.V. Hill's active state theory.

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Ca^{2+} SENSITIVITY AND CONTRACTILE REGULATORY PROTEINS OF GLYCERINATED MUSCLE FIBER

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Ca^{2+}-sensitive fiber(CaS-fiber) of glycerinated rabbit psoas muscle lost Ca^{2+}-sensitivity by digestion of a part of TN-complex by trypsin or intrinsic protease(ex. CANP). Especially, TN-T was removed completely from the bands of SDS polyacrylamide gel electrophoresis by these factors. However, the addition of TN-T, isolated from TN-complex through the column using SP Sephadex C-50 according to the method of Wakabayashi & Ebashi (1968), did not bring about the recovery of Ca^{2+}-sensitivity, but TN-I & -C were fainted from the gel. Same result was also obtained from the experiment of CaS-fiber. Then, we checked if the added TN-T behaved itself as an extruding factor of TN-complex using fluorescence analysis. And, it was found that they were extruded into the extra-fluid.

On the other hand, CaS-fiber lost Ca^{2+}-sensitivity transiently when the fiber was immersed in the conditions of high ionic strength and pH 4.5 at 25°C for 2-3 min. But, the treated fiber did not lose any TN-complex on the gel. In this case, Ca^{2+}-desensitization was caused by the functional modification of all contractile proteins. So, we examined the effect of NEM(N-ethylmaleimide), by the fluorescence analysis using DACM (a maleimide delivative), and it was suggested that CaS-fiber lost Ca^{2+}-sensitivity also by the block of SH-groups in another contractile proteins.

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INACTIVATION OF POTASSIUM CONTRACTURE AND THE INCREASE OF AMOUNT OF Ca EXTRACTED FROM FROG SKELETAL MUSCLE.

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We examined whether the amount of Ca extracted in a 0 mM Ca and 1 mM Sr-choline Ringer solution (Sr-Ringer) is increased during inactivation of potassium (K) contracture of whole sartorius muscle of the frog. Extraction of calcium from the sartorius muscle into a Sr-Ringer was performed according to the procedure of Bianchi and Narayan (1982). Calcium ions were measured by Ca-selective electrode (Radiometer, F2110 Ca). Amount of Ca extracted was increased at spontaneously relaxed state of 190, 120 and 80 mM K contractures and during conditioning depolarization with 20 mM K. These changes were enhanced by lowering $[Ca^{++}]_o$(0.18 mM) and reduced by high $[Ca^{++}]_o$(10 mM). Effect of dantrolene (2.5 - 10 μM) was also examined. Extractable Ca which had increased during 190 mM K contracture was removed by recovering sartorius muscle in a 0 mM Ca and 0.7 mM Ni^{++}-choline Ringer from the full inactivation. These results suggest that the inactivation of K contracture may be due to the increase, during depolarization, of the amount of Ca extracted.

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MUSCLE FATIGUE AND Ca ACCUMULATION IN FROG SKELETAL MUSCLE.

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In order to clarify the mechanism of excitation-contraction uncoupling on muscle fatigue, we measured the amount of Ca extracted from the fatigued frog sartorius muscle induced by repetitive stimulation at 2 Hz for 5 min and examined the effect of a twitch potentiator (No_3^-) on the time course of fatigue and the Ca extraction. Following results were obtained. 1) After 5 min at 2 Hz, the twitch tension was inhibited by 21% of the maximum tension in normal Ringer solution. This inhibition was significantly ($p<0.01$) enhanced in No_3^- Ringer solution. 2) The amount of Ca extracted from the fatigued muscle induced by stimulation for 5 min was significantly ($p<0.01$) increased in normal and No_3^- Ringer solution. 3) The amount of the extracted Ca was more largely increased in No_3^- Ringer than in normal Ringer. These results suggest that muscle fatigue is due to the increase of mechanical threshold which can be considered to be caused by the accumulation of Ca in transverse tubular lumen.

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IS THE Ag-INDUCED TENSION DEVELOPMENT ATTRIBUTED TO MEMBRANE DEPOLARIZATION ?
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To elucidate the mechanism by which Ag develops spontaneous contraction in intact muscle fibers, we measured the continuous change in membrane potential together with that in tension development following Ag administration. The effect of Ag on contraction in fibers in which the membrane potential had been depolarized by elevating external K concentration was also examined. The decrease in membrane potential at the onset of tension development was only 1.6 mV from -87.8 mV before 5 μM Ag exposure to fibers. When external K concentration was rised to 10 mM (an subthreshold concentration for mechanical activation and equivalent to -69.5 mV), the fiber could respond to Ag and show magnitude and pattern of contraction similar to controls observed in 2.5 mM K solution. 20 or 50 μM Hg caused no contraction, although the muscle membrane was depolarized to the same extent as 5 μM Ag did. We concluded from these data that a slight depolarization of membrane induced by Ag was not trigger for the contraction.

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REGULATION OF Ca^{2+}-INDUCED Ca^{2+} RELEASE FROM SARCOPLASMIC RETICULUM.
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Activation mechanisms of Ca efflux from sarcoplasmic reticulum(SR) by caffeine and AMP were studied. SR was isolated from leg muscle of bullfrog. ^{45}Ca uptake was started by adding acetyl phosphate into a reaction solution, and ^{45}Ca efflux was induced by 20-fold dilution. ^{45}Ca influx was measured by dilution of ^{40}Ca uptake solution with ^{45}Ca containing solution. It was found that ^{45}Ca influx was negligible in the presense of 0.05mM Mg, therefore ^{45}Ca efflux was considered as net Ca efflux except when ATP or ADP was present in the dilution solution. Study on time course of ^{45}Ca efflux revealed that ^{45}Ca efflux consists of fast and slow components. The amount of fast efflux was enhanced by caffeine and AMP. The maximum value of fast efflux in the presence of caffeine was obtained in lower Ca concentration than that in control. Procaine counteracted the effect of caffeine on the fast efflux. On the other hand, procaine inhibited the fast efflux Ca independently in the presence of AMP. Caffeine increased further the amount of fast efflux enhanced by AMP.These results suggest that caffeine and AMP regulate Ca release in different manner.

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ON THE MECHANISM OF DECOUPLING OF E-C COUPLING IN SKELETAL MUSCLE FIBRES.
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In order to measure the threshold potential of local contraction induced by extracellular electrical stimulation in the presence of Mn ions in the external solution, it was tried to measure the membrane potential by the methods reported by A. L. Hodgkin & W. A. H. Rushton (1946, 1947) and B. Katz(1948).

As it was found difficult to transfer single skeletal muscle fibres from Ringer's solution into the liquid paraffin layer without overstretching the single muscle fibres, some procedures were needed to prevent this stretching. The author devised an exchange trough which consisted of two rotatory teflon palisades, but the effectiveness of this exchange trough is now under examination.

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TWITCH POTENTIATING MECHANISM OF CAFFEINE IN SINGLE FROG SKELETAL MUSCLE FIBRES. KURIHARA, S., KONISHI, M., KAWAMURA, T., SAKAI, T. Dept. of Physiol., The Jikei Univ. Sch. of Med., Nishishinbashi, Minato-ku, Tokyo 105

Caffeine has been known as a twitch potentiator of skeletal muscle fibres without changing membrane properties, and this effect is considered to be due to an increase of released calcium from sarcoplasmic reticulum. We measured intracellular free calcium concentration in single frog skeletal muscle fibres with aequorin to elucidate the mechanism of twitch potentiation by caffeine. Low concentrations of caffeine (0.2-0.6 mM) increased or sometimes decreased a peak of light signal of aequorin with prolonged time course when twitch was potentiated. Increase of caffeine concentration further enhanced twitch tension but rather diminished the peak of light signal. Caffeine (higher than 0.4 mM) increased resting light level which changes in parallel with twitch tension. These results suggest that twitch potentiation by caffeine can be interpreted by an increase of intracellular free calcium concentration before stimulation.

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EFFECTS OF ENFLURANE ON EXCITATION-CONTRACTION COUPLING IN FROG SKELETAL MUSCLE FIBERS. KONISHI, M., KURIHARA, S., MIYAGISHIMA, T. and SAKAI, T. Dept. of Physiol., The Jikei Univ. Sch. of Med., Nishishinbashi, Minato-ku, Tokyo 105.

The effect of enflurane on the E-C coupling of frog skeletal muscle was studied using Ca sensitive photo-protein, aequorin. High concentration (108.52±4.52mg%) of enflurane markedly reduced the peaks of both tension and aequorin light signal during twitch. It reduced the peak of action potential, but prolonged the duration measured at 50% peak height. The elevation of light level induced by low concentration (e.g. 0.2 mM) of caffeine was enhanced by enflurane, sometimes accompanying tension development. The light and tension during rapid cooling contracture were also potentiated in the presence of enflurane. In glycerinated muscle fibers, enflurane shifted pCa-tension relation to the left in high pCa range and suppressed maximally activated tension in low pCa. These results suggest that in frog skeletal muscle, 1: Enflurane inhibits E-C coupling process without abolition of action potential. 2: Direct Ca release from s.r. induced by caffeine was enhanced and Ca sensitivity of contractile element was increased in the presence of enflurane.

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CHANGES OF CA UPTAKE ACTIVITY ON FSR FROM DENERVATED MUSCLE. NISHIJIMA, H., TAKAHASHI, A., SAKAI, T. Dept. of Physiol., The Jikei Univ. Sch. of Med., Nishishinbashi, Minato-ku, Tokyo 105

It was known that the Ca-uptake activity on fragmented sarcoplasmic reticulum (FSR) from skeletal muscle immediatly after birth was differ from FSR in skeletal muscle of adult rate. It was generally assumed that the muscle in adult rat changed to infant direction by denervation. In this experiment, the maximum value of Ca-uptake on FSR from muscle of adult rat was 240 x 10^{-9} moles/mg protein /30 sec in EDL and 180 x 10^{-9} moles/mg protein/45 sec in M. soleus. On the FSR from muscle with the advance a period of denervation, the maximum value and the activity of Ca-uptake on FSR from their muscle were lowered. And the inhibition of Ca-uptake activity in initial phase was strongly indicated more than the inhibition of maximum value. Otherhand the Ca-release from FSR by caffeine was lowered with a period of denervation. From these results, the Ca-uptake activity on FSR from denervated muscle indicated the change in infant direction, but the sensitivity for caffeine on FSR was differ from the FSR in the infant muscle.

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CALCIUM-INDUCED BIPHASIC CONTRACTION OF THE GLYCERINATED RABBIT PSOAS MUSCLE FIBER AND THE MODIFICATION OF THIOL-GROUP. TSUJIMOTO, T. AND NONAKA, Y. Dept. of Physiol., Wakayama Med. Coll., Wakayama 640

The effect of SH-group modifier on the previously found phenomena was studied: "Mg-ATP-induced tension in pCa>8"; "Ca-induced biphasic contraction". Tension mechanogram and ATPase activity or pCa were recorded at pH 7.0, 12.5°C. MgATP (0.74 mM) induced a contraction-relaxation cycle without Ca^{++}: medium pCa was higher than 8. Thimerosal (3.77 μmoles/mg protein) greatly augumented the MgATP-tension. Dithiothreitol (DTT) reversed the elevation. The above results suggested a loss of Ca-control on the contractile system as a cause of MgATP-tension. Addition of Ca^{++} (pCa, 6.28) following MgATP tension caused the biphasic contraction: initial fast (Ca_f); secondary slow (Ca_s). The Ca_f was the highest with 135nmoles of p-hydroxymercuribenzoate (pMB)/mg protein, while the Ca_s was the highest with 1.25 μmoles of pMB/mg protein: Sensitivity of the Ca_f to the modifier was higher than that of the Ca_s. Ethacrynate, the most slow-reactive one of the reagents tested, clearly separated the Ca_f and the Ca_s. The above results suggested the coexistence of fast and slow-reactive contractile element in the psoas muscle as a cause of the biphasic contraction. Action of N-ethylmaleimide on SH was fortified in the presence of MgATP, and an interaction was suggested between the SH-group of myosin and the substrate binding site.

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RADIAL AND LONGITUDINAL STIFFNESS IN RADIALLY COMPRESSED FROG SKINNED MUSCLE FIBERS. UMAZUME,Y., Dept. Physiol., Jikei Univ. Sch. Med., Minatoku Tokyo 105

The radial stiffness (K) and the longitudinal stiffness (γ), and 1,0 lattice spacing (d) at the relaxing condition of mechanically skinned single fibers of semitendinosus muscle were measured at various concentrations of polyvinylpyrrolidone (PVP, K-30, Mr = 40,000) from 0 to 10 % at 20°C. In a standard relaxing solution (MgATP 4, Mg^{2+} 1, EGTA 4, ionic strength 150 mM and pH 7), d was 41.3 ± 0.75 (mean ± SD) nm and decreased exponentially as the PVP concentration increased, and at 10 % PVP, d was 29.5 ± 0.27 nm. The K increased considerably in a relaxing solution (1 mM Mg^{2+}) at $d \lesssim 35$ nm and in 30 mM Mg^{2+} at $d \lesssim 32$ nm. The γ measured by appling sinusoidal stretching (0.2 % Lo) was also increased at $d \lesssim 35$ nm in both 1 and 30 mM Mg^{2+}. The relation between γ and the frequency indicates that the increase of γ may reflect an interaction between myosin heads and thin filaments. The effect of Mg^{2+} was not shown in γ but in K. This indicates that K reflects not only the number of attached cross-bridges but the elastic property of the higes between S1 and S2 and/or S2 and LMM.

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CALCIUM ION INDUCED DETERIORATION OF RIGOR SKINNED MUSCLE FIBERS. KASUGA,N. and UMAZUME,Y. Dept. of Physiol.,Jikeikai Univ. Sch. of Med.

The tension developed in the rigor (no Mg-ATP contained) solution decreased irreversibly by adding Ca^{2+} at room temperature. The cause of the decrease (ΔP) was investigated. The ΔP was observed at pCa 7.2 and reached to maximum at pCa 4.8. The ΔP was suppressed by lowering the temperature and $Q_{10} \sim 4$. The pH optimum for ΔP was around 7 and little ΔP was observed at 5.5 and 8.5. Above characteristics were also observed in the fiber stretched to sarcomere length of 5 μm. The SDS gel electrophoresis indicated that the 90k dalton component (α-actinin) was released from the fiber in Ca^{2+} rigor solution. The electron microscopic picture showed that the Z-lines disappeared in the Ca^{2+} rigor solution. The calcium activated neutral protease (CANP) inhibitor (leupeptin,E-64) blocked the deterioration. We consider that CANP still exists in the skinned fibers and it is activated by extremely low calcium ion concentration compared with other results. The deterioration of the fiber contracted by Ca^{2+} in the Mg-ATP containing solution was inhibited by adding 0.5 mM leupeptin.

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THERAPEUTIC EFFECTS OF ARPHAMENINE A AND B, MICROBIAL PROTEASE INHIBITORS, ON MURINE MUSCULAR DYSTROPHY. KUSUMOTO, S., DOI, Y., TSUJI, S., SAWADA, H. and MATSUSHITA, H. Dept. Physiol., Wakayama Medical College, 9-bancho, Wakayama-shi, Wakayama 640

Dystrophic mice were treated with subcutaneous injection of saline solution containing microbial protease inhibitors Arphamenine A and B at 5 weeks of age. Treatment with the protease inhibitors at this age had no restrating effect on the clinical symptoms but had distinct weight gain in the treated dystrophic mice. After a periods of 4-12 weeks with continuous administration, a marked increase of CPK, LDH and PK activities which are so called as marker enzymes for the disease progress, in skeletal muscle coupled with a significant decrease of these enzyme activities in serum was observed in the treated dystrophic mice. Proteolytic activities at neutral and alkaline pH conditions which are involved in the degenerative process of muscular dystrophy, also decreased extremely in the treated dystrophic mice. Creatine secretion and creatine to creatinine ratio in urine improved to normal level in the treated dystrophic mice.

Moreover, continuous administration of Arphamenine B significantly prolonged the life span of the treated dystrophic mice.

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DENERVATION EFFECTS ON MUSCLE ELECTROLYTE AND WEIGHT OF THE RATS. NAGAOKA, R., YAMASHITA, S. AND AKAIKE, N. Dept. Physical Education and Biological Inst., College of Liberal Arts, Kagoshima Univ., Kagoshima 890, and Dept. Physiol., Fac. Med., Kyushu Univ., Fukuoka 812

The experiments were carried out on the soleus (SOL) and extensor digitrum longus (EDL) muscles of male Wistar strain rats aged 9 and 52 weeks. The distal denervation of these muscles was accomplished unilaterally by exposing and sectioning the sciatic nerve. The contralateral nerve was sham-operated, and the contralateral companion muscles served as each control (innervated muscle). These innervated and denervated muscles were excised and prepared for Na^+ and K^+ analyses within a week after the surgical procedure.

The muscle weight decreased day by day after the denervation, but the decrease was significantly greater in 9-week-old rat muscles than in 52-week-old rats. The weight changes were faster in SOL than in EDL. In contrast, the accumulation of intracellular muscle Na^+ and the loss of K^+ were greater in EDL than in SOL. In both muscles, the changes of electrolyte were greater in the younger rats. The pretreatment of actinomycin-D failed to decrease the differences in the muscle weight and electrolyte between younger and old rats after the denervation.

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MECHANICAL ACTIONS OF PROSTAGLANDINS ON THE SARTORIUS MUSCLE OF FROG.

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The mechanical actions of PGE_2 and PGF_{2a} were examined in the sartorius muscle of frog(Rana Japonica). PGE_2 and F_{2a}(1 - 100 μM) had no effect of their own on the muscle tone. However,these PGs augmented and prolonged the twitch response stimulated directly. The augmentation of twitch was enhanced in the presence of caffeine(1 mM) or theophylline(2 mM). Lanthanum(1 - 10 mM) did not affect the enhancement action by PGs. Caffeine(1 - 3 mM)-induced contracture in the normal and Ca(-) K-depolarized preparations was enhanced by PGs(10 mM). After treatment with glycerol(400 mM)-containing Ringer solution for 1 hour, Caffeine(3 mM)-induced contracture was also enhanced by PGs(10 μM) although the twitch responses were abolished.

The results indicate that PGE_2 and F_{2a} may act mainly on the Ca store sites, largely on the sarcoplasmic reticulum(SR).

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COMPOSITION AND VOLUME OF THE SARCOPLASMIC RETICULUM IN QUININE-TREATED SKELETAL MUSCLE. YOSHIOKA,T.,*NAKAHARA,Y., NARUSAWA,M., & NAKANO,S. Dept. Physiol., Tokai Univ. Sch. Med., Kanagawa, * Fac. Educ., Mie Univ., Mie.

The effects of quinine on the composition and volume of the terminal cisternae(TC) of the sarcoplasmic reticulum (SR) in frog skeletal muscle (Rana pipiens) were determined with rapid freezing, electron microscopy, electron probe analysis, and stereological measurement. Quinine (5mM) released > 70% of the Ca content of the TC in 3 min. The release of Ca from the TC was associated with a highly significant increase in its Mg content. On the basis of the increase in Mg content during quinine contracture, caffeine contracture(Yoshioka and Somlyo,1984,J.Cell Biol., 99:558-568), and during tetanus (Somlyo,et al., 1981,J.Cell Biol., 90:577-594), we suggest that the mechanisms of Ca release are associated with an increase in the Ca and Mg permeability (possibly through a common channel) of the SR membrane. The TC were not swollen in rapidly frozen, quinine-treated muscles (1.8% of fiber volume), in contrast to the swelling of the TC observed in conventionally fixed preparation (2.6%). The swelling of the SR is not main factor of Ca releasing.

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SUPPRESSION OF ACTION OF SOME EXCITATION-CONTRACTION (E-C) UNCOUPLING AGENTS AND MECHANISM IN E-C COUPLING IN SINGLE SKELETAL MUSCLE FIBERS OF FROG, R. JAPONICA. M. FUJINO, T. ARIMA, Y, SATO, H. TAKAI. 1.Dept of Physiol., Natl. Def. Med. Coll., Tokorozawa.

1. Inhibitory action of acrolein on E-C coupling is weakened in presence of sulfhydryl reagents tested (mercaptoethanol, cysteine,thioglycolate, and thiomalate). 2. Acrolein and the reagents (mercaptoethanol, thioglycolate, and thiomalate) were shown spectrophotometrically to from a complex when they were mixed in aqueous solutions. 5 mM. acrolein-mercaptethanol complex isolated can abolish the inhibitory action of acrolein. These findings would indicate that acrolein-thioglycolate and -thiomalate complexes have the similar suppressing effect to acrolein-mercaptoethanol complex on acrolein-action, suggesting that the inhibitory action of acrolein is brought about through an extracellular process. 3. Inhibitory action of phenylglyoxal (PGO) on E-C coupling is also abolished by acrolein-mercaptoethanol complex. 4. Uncoupling action of dantrolene sodium (DaNa) was not influenced by the complex. 5. Preliminary observations showed that DaNa seemed to interact with amino-group or a similar group to it of not protein but a kind of carbohydrate. 6. These results would suggest that the strategic site responsible for E-C coupling seems to consist of two sites of amino-group or a similar structure, one of which is of protein in nature.

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NEW OPTICAL SIGNALS IN NK2935(A POTENTIAL PROBE)-INJECTED MUSCLE FIBERS OF THE FROG, RANA JAPONICA. M. FUJINO, Y. SATO, T. ARIMA. 1. Dept. of Physiol., Natl. Def. Med. Coll., Tokorozawa.

An impermeant dye, NK2935 (WW781) was injected ionophoretically into single fibers of reduced movement, through microelectrodes. The injected dye-related absorbance changes (ΔAi) after single electric shock were recorded with monochromatic (570, 630 nm) or band-passed (550-580, 620-650 nm) filters. Signal-averaged ΔAi appeared about 0.5 msec of time lag (25°C) from onset of action potential (AP). The lag was influenced by D_2O-Ringer, reduced (Na)o, low temperature, and formamide treatment. ΔAi in fibers treated with formamide (0.6 M, 10 min), was almost a duplicate of AP in time-course and behaved very similarly to the case (a control experiment) in intact nerve fibers of crayfish, Procambarus clarkii. Simultaneous records of Ca-transient (arsenazo III) with wavelength, 590 nm, developed 1.2 msec after ΔAi-onset at 26°C. ΔAi were characterized as follows: (a) The lags from AP-onset were five- to ten-fold those of ΔA in external application (ΔAo). (b) ΔAi consisted of only a spike.(c) Wavelength dependences of ΔAo and ΔAi were of same relation.(d)Time required for maximum response after probe-application was larger than ΔAo-case. These results suggested that the major part of ΔAi originates in bound dye molecules to T-side foot-end or junctional sheet so as to direct same as in case of outside surface of T-tubule membrane.

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DINAMIC VISCO-ELASTIC PROPERTIES OF A SKINNED FIBER.
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The dynamic visco-elastic properties of a frog skinned fiber were investigated in a relaxing solution at sarcomere length (L) of 2.5-5.0 μm. The length of the fiber is oscillated sinusoidally and the complex modulus (E) and the tangent of an angle of phase shift (tanθ) were measured. At about 15 min after the fiber was stretched, E increased with the increase of frequency (0.1-100 Hz). On the other hand, with the increase of frequency tanθ increased at $L \leqq 3.0$ μm, was almost constant at L=3.3 μm , and decreased and then increased at $L \geqq 3.5$ μm. The changes of E and tanθ can be explained by the generalized Maxwell model. After the fiber was stretched to $L \geqq 3.0$ μm, the resting tension and E (30 Hz) decreased exponentially but tanθ hardly changed. It is suggested that an exponential decrease of the resting tension is mainly caused by the decrease of elastic modulus. We suggest that the visco-elastic properties of the skinned fiber obtained in the present study reflect the properties of the elastic components bound to an A-band because the large part of the resting tension is generated by that.

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CHANGES IN THE X-RAY DIFFRACTION PATTERN FROM TETANIZED FROG SKELETAL MUSCLE DURING SINUSOIDAL LENGTH CHANGES.
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To study the dynamic behaviour of the cross-bridges, sinusoidal length changes (10 Hz, 1% of Lo) were applied to isometrically contracting frog sartorius muscle, and the resulting changes in the X-ray diffraction pattern were examined at 10 msec intervals by use of synchrotron radiation. Both the equatorial and meridional reflections exhibited distinct periodic changes in response to the applied length changes. The equatorial 1,0 and 1,1 intensities changed nearly in phase and in antiphase with the length changes respectively. The meridional 143 Å intensity changed in a very characteristic manner; it decreased during both the stretch and release phases of the sinusoidal length perturbation, and edhibited a maximum at each boundary between the stretch and release phases. The above behaviour of the meridional 143 Å intensity suggests that, during the applied sinusoidal length perturbation, the position of the cross-bridges remains virtually unchanged except for their distal region. These experiments were performed at the Photon Factory in colaboration with Dr. Y. Amemiya.

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TENSION AND STIFFNESS CHANGES DURING RAMP LENGTH CHANGE IN FROG MUSCLE FIBERS.
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Mechanically skinned fibers from frog sartorius or semitendinosus muscle were reactivated in ATP salt solution containing 10 uM Ca. After development of isometric contraction, fibers were released at constant speed (0.03-2.4 muscle length (l_o) s^{-1}). During ramp shortening, stiffness was determined from the slope of the tension-length diagram obtained during superimposed quick stretches. Both force and stiffness decreased, as the ramp shortening proceeded and approached a steady value after 60 ms. An increse in speed of shortening caused a decrease in tension and stiffness and an increse in the ratio of stiffness to tension, suggesting a decrease in both the number of attached crossbridge and in the average force per crossbridge.

Stiffness was also measured in living single muscle fibers from frog tibialis anterior muscle during and after ramp lengthening (0.05-0.06 l_o, 0.02-0.1 l_o s^{-1}) by superimposing sinusoidal vibration (1 kHz, 0.001 l_o). Stiffness initially increased and then decreased linearly during lengthening and decreased exponentially after lengthening.

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CHANGES IN TENSION AND SARCOMERE LENGTH DURING CONSTANT-VELOCITY STRETCH IN FROG SKELETAL MUSCLE FIBERS. IWAMOTO, H. and °SUGI, H. Zool. Inst., Fac. Sci., Univ. Tokyo, Bunkyo-ku, Tokyo 113, °Dept. Physiol., Sch. Med., Teikyo Univ., Itabashi-ku, Tokyo 173.

Ramped stretches of various speeds (ranging over several orders) were applied to the frog skeletal muscle fibers during tetanic contraction or in rigor state, and the changes in tension and sarcomere length during the stretches were recorded. The changes in the sarcomere length at the middle of the fiber were almost proportional to the applied length changes. In the contracting fibers, the dependence of tension responses on the speed of stretch showed at least three phases. In the first and the third, the magnitude of the responses increased with the speed of stretch, whereas in the second, it remained virtually constant. The speeds of stretches at the lower and the upper limits of the second phase increased by factors of 1.6 and 2.7, respectively, when the temperature was raised from 0°C to 10°C. Thus, the phases may reflect the elementary rate processes involved in actin-myosin interaction which have different temperature coefficients. On the other hand, the tension responses of the fibers in rigor state showed only a small, monotonic dependence on the speed of stretch. This may indicate that the actin-myosin interaction in the rigor fiber is largely static.

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THE EFFECT OF MYOSIN SULPHYDRYL MODIFICATION WITH P-PHENYLENEDIMALEIMIDE ON THE MECHANICAL RESPONSE OF GLYCERINATED RABBIT PSOAS MUSCLE FIBERS. CHAEN, S., SHIMADA, M., AND SUGI, H. Dept. Physiol. Sch. Med., Teikyo Univ., Itabashi-ku, Tokyo 173

It has been reported that p-phenylenedimaleimide(p-PDM) selectively reacts with the sulphydryls on the myosin head to result in the loss of its ability to combine with actin and hydrolyze ATP. As an attempt to address the question of physiological role of myosin two heads, we examined the contraction characteristics of partially p-PDM modified glycerinated rabbit psoas muscle fibers. The magnitude of Ca-activated isometric tension of the modified muscle fibers(relative to the control value) was found to be proportional to the square of the ATPase activity(also relative to the control value). It was also proportional to the square of the muscle fiber stiffness (relative to the control value). If it is assumed that the probability to be modified with p-PDM is the same between the two heads of each myosin molecule, the above relation among the isometric tension, ATPase activity, and muscle fiber stiffness strongly suggests that (1) a crossbridge can exert force only when both heads are not modified, (2) the stiffness represents the total number of unmodified myosin heads, and (3) there is no cooperative interaction between heads of each myosin molecule in catalyzing ATP splitting.

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UNIFORMITY OF LOCAL HEAT PRODUCTION IN FROG SKELETAL MUSCLE DURING ISOMETRIC CONTRACTION WITH AN INFRARED THERMOGRAPHY. KOBAYASHI, T., KAMIYAMA, A. AND SUGI, H. Dept. Physiol., Sch. Med., Teikyo Univ., Itabashi-ku, Tokyo 173

In the experiments of myothermic measurement, there is a basic assumption that all the elementary segments of muscle respond in the same manner. In order to examine the validity of this assumption, we measured local heat production along the entire length of bullfrog (Rana catesbeiana) sartorius muscle by means of an infrared thermography (JEOL, JTG-MG) with a resolution of 20 m°C. The muscle was divided into several segment by fine metal markers attached to the muscle surface, and tetanized isometrically for 5sec. The length and temperature changes in every segment were simulteneously recorded with the thermography by repeated scanning along the long axis of muscle, and displayed on acolor TV screen. During an isometric tetanus, there was always a non-uniformity in both length and temperature changes; in general, the segments near the pelvic end tended to shorten by stretching those near the tibial end, and the amount of heat produced was larger in the former than in the latter. These results indicate that the amount of heat production is not uniform, but differs from segment to segment along the muscle length, and that the infrared thermography is useful in the field of muscle energetics.

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THE EFFECT OF LOWERING THE INTRACELLULAR PH ON THE MAINTENANCE HEAT PRODUCTION OF FROG SKELETAL MUSCLES. KITANO,T.,KOMETANI,K.,TANOKURA,M. AND YAMADA,K.
Dept. of Physiol. Medical College of Oita, Oita 879-56

We studied the effect of lowering the intracellular pH (pH_i) on the maintenance heat production of frog sartorius muscles (Rana japonica). The pH_i was lowered by increasing the CO_2 partial pressure in Ringer solution, which contained 18mM bicarbonate ions, and was measured using ^{31}P-NMR from the chemical shift of inorganic phosphate peaks. The heat and tension were measured during 5s isometric tetanus at 4°C. As the pH_i was lowered the maximal tension was reduced and the relaxation phase was strikingly prolonged. The total heat production consists of two parts,the labile and stable heats. Both total and stable heats were reduced as pH_i was lowered. But labile heat did not change significantly within the pH_i range studied. The results obtained in this study are summerized in the table below.

Force and heat;Mean(S.E.) in 6 muscles

pCO_2	pHo	pH_i	Po(N/cm^2)	labile heat(mJ/g)	stable heat(mJ/g s)
5%	7.0	7.2	26.2(.47)	25.4(1.77)	25.6(1.12)
10%	6.7	6.9	23.5(.47)	21.8(1.15)	22.5(1.05)
20%	6.4	6.7	18.7(.57)	25.6(1.88)	16.8(0.87)
45%	6.1	6.4	12.6(.59)	21.5(2.42)	9.57(0.77)

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THE EFFECT OF 2,3-BUTANEDIONE MONOXIME ON THE INITIAL HEAT PRODUCTION OF FROG SKELETAL MUSCLE. KOMETANI, K., Dept. of Physiol. Medical College of Oita, Oita

It has been shown that 2,3-butanedione monoxime(BDM) has negative inotropic effects on heart muscles. These effects have been suggested to be mainly due to the inhibition of cross-bridge cycling(Mulieri et al, 1984).

We studied the effect of BDM on the initial heat production of frog skeletal muscles at 5°C. BDM reduced both the tension and the heat production. With 1mM BDM twitch tension(Pt) was reduced to 30% of control. The relation between heat and Pt was linear for 0-2mM BDM. With BDM concentration where Pt approaches to zero, the initial twitch heat is about 30% of control, which is nearly equal to the activation heat obtained by reducing the filament overlap in normal Ringer solution.

BDM reduced not only the amplitude of tetanus tension but also the rate of tension development. Times to half maximal tension were 0.04, 0.17 and 0.4 s at 0,1 and 2mM BDM, respectively. Total heat production can be divided into labile and stable parts based on its time course. Since the time course of heat production may be affected by the time course of tension development, the labile heat was not studied. The relation between the stable heat(h_b) and the plateau tension was linear with 0-3mM BDM. Linear regression with tension indicated that at zero tension h_b would be reduced to 24% of control. The corresponding value obtained by changing sarcomere length was 28%.

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ULTRASTRUCTURAL CHANGES WITHIN TRIADS CAUSED BY SEVERAL CONDITIONS APPLIED EXTRACELLULARLY, USING SINGLE MUSCLE FIBERS OF FROG, R. JAPONICA. M. FUJINO, Y. SATO, T. ARIMA, S. TAKAMATA, H. TAKAI. 1. Dept. of Physiol., Natl. Def. Med. Coll., Tokorozawa.

1. In longitudinal sections of fibers, no special ultrastructural features are almost recognizable within terminal cisternae (tc), except that one electron-less-dense portion (ld-portion) occurs near triadic junction in low percent (about 20% of total triads observed). 2. Dantrolene sodium (DaNa) increases the percentage depending on its concentrartion, of which increase is completed already 10 sec after DaNa-immersion (10 sec was the shortest time capable of experimenting), suggesting an immediate occurrence after the immersion. 3. Phenylglyoxal (PGO), K-depolarization(K-Dp), and hypotonicity behave similarly to DaNa-case, and the occurrence is also fast. In case of K-Dp, normal percentage is restored fast after fibers reover from depolarization 4. Hypertonicity causes no increase but rather a decrease in percentage. 5. Tangential sections of tc along central axis of tc-tubes show that ld-portions are nearly an elliptic form of about 100 - 200 A in shorter diameter and increase in number (not in diameter) along the axis depending on condition-intensity. 6. Ruthenium red stains the contents in tc except ld-portion. From these: (a) ld-portion seems to behave as an intracellular component, which has a relation to E-C coupling;(b) if the portion consists of materials, it is of no negative charge.

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HEAT OF CALCIUM-BINDING BY WHEAT GERM AND BOVINE BRAIN CALMODULINS.

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Calorimetric titrations were carried out at pH 7.0 and at 25°C in order to study the enthalpy (ΔH) and entropy changes (ΔS) of the Ca^{2+}-binding to plant (wheat germ) calmodulin (wCaM) and to compare with those for bovine brain calmodulin (bCaM) as reported previously (J.Biochem.(1983)94,607; ibid.(1984)95,643). In the absence of Mg^{2+}, the enthalpy titration profile of wCaM can be interpreted in terms of the four equivalent Ca^{2+}-binding sites, in contrast to bCaM, which has two low-affinity and two high-affinity sites. The Ca^{2+}-binding reaction of wCaM (binding constant, 10^5 M^{-1}) was endothermic (ΔH=3.8 kJ/mol site), and hence was driven only by large entropy change (ΔS= 108 J/mol/K). Such unusual thermodynamic features of Ca^{2+}-binding are the same as those for bCaM, and distinctly different from the exothermic Ca^{2+}-binding reaction of other Ca^{2+}-binding proteins such as troponin C and parvalbumin. When 10mM Mg^{2+} was present, the Ca^{2+}-binding reaction of wCaM was converted to the exothermic reaction (ΔH=-16.5 kJ/mol site), in agreement with the endothermic reaction of Mg^{2+}-binding. As a whole, the thermodynamic characteristics were very similar between wCaM and bCaM, and would reflect the unique conformational changes in calmodulin molecule accompanying the Ca^{2+}-binding distinctly different from other Ca^{2+}-binding proteins.

We wish to thank Nisshin Flour Milling Co., Ltd. for generous supplies of wheat germ.

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TIME-RESOLVED ^{31}P NMR STUDIES OF SKELETAL MUSCLE CONTRACTION

YAMADA, K., TANOKURA, M. AND KITANO, T. Dept. of Physiol., Medical College of Oita, Oita.

The course of changes of creatine phosphate (PCr) and inorganic phosphate (P_i) after brief isometric tetanic contractions was studied by using ^{31}P NMR. Eight pairs of bullfrog sartorius muscles (~10g) were used, and free induction decays (FIDs) phased with the stimulus were accumulated during repeated contractions of up to 64. Each spectrum thus obtained has a time-resolution of~10ms (spin-spin relaxation time, T_2, of phosphoruses in muscle cells being ca. 10ms). Observation intervals were either 2 or 4 s. The results indicated a post-contractile PCr(or ATP) utilization; the level of PCr continues to decrease for a few min after the muscle has relaxed from tetanic contraction, while the level of P_i is maximal at the end of contraction. The post-contractile PCr utilization was not affected by the intervals of observations; this rules out any artefact due to the changes in T_1, the spin-lattice relaxation time. Furthermore the post-contractile PCr utilization was strongly dependent on temperature; at 10°C the course becomes almost undetectably fast with the present system of ^{31}P NMR measurements.

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INTERCONNECTIONS BETWEEN THICK FILAMENTS IN STRETCHED SKELETAL MUSCLE FIBERS.

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The ultrastructure of frog semitendinosus muscle fibers was studied using the freeze-fracture, deep-etch, rotary-shadowing technique in order to observe the interconnections between thick filaments. Mechanically skinned fibers were stretched in relaxing solution to decrease or eliminate the overlap of thick and thin filaments prior to rapid freezing with liquid propane. In relaxed, contracting and rigor fibers, a significant number of bridge like interconnections, distinct from those observed in the M-region, were observed between adjacent thick filaments in the non-overlap region. The interconnections were rod-shaped, 5.0 - 6.5 nm in diameter and 24.0 - 33.0 nm in length. Their diameter and half-length corresponded approximately to the known dimensions of the cross-bridge (or myosin S-1). Further, a fine stripe of low electron density could sometimes be observed midway along the interconnection. These results suggest that the interconnection may be formed by the binding of two opposed cross-bridges projecting from adjacent thick filaments. The interconnections may play a role in stabilizing the thick filament lattice in highly stretched sarcomeres. Further experiments will be needed to make clear the physiological role of interconnections in muscle contraction.

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TIME RESOLVED X-RAY DIFFRACTION STUDIES OF THE FROG SKELETAL MUSCLE ISOMETRICALLY TWITCHED BY TWO SUCCESSIVE STIMULI USING SYNCHROTRON RADIATION. TANAKA, H., KOBAYASHI, T., SUGI, H. Physiol., Teikyo Univ. Sch. Med., Tokyo 173

The muscle was stimulated by two successive stimuli at an interval at which the second twitch started on the first one so that the peak tension of the summated response was about 1.5 times greater than that of the first one. On contraction the equatorial intensity of I(1,0) decreased and that of I(1,1) increased, and further decrease in I(1,0) and increase in I(1,1) were seen in response to the summated tension response. The intensity of 42.9 nm off-meridional myosin layer line decreased to about 1/4 of the resting value by the first stimulus, but the second stimulus did not cause further decrease in the 42.9 nm intensity. This may be taken to indicate that, in spite of difinite differences both in peak tension and in equatorial intensities between the first and the summated mechanical response, the proportion of myosin heads out of the regular helical arrangement around the thick filaments are the same in both cases. These experiments were performed at the Photon Factory in collaboration with Dr. Y. Amemiya.

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REPRIMING OF THE UNEXPLAINED ENTHALPY PRODUCTION IN ISOMETRIC TETANUS OF FROG SKELETAL MUSCLE. YAMADA, T. AND HOMSHER, E.* Dept. of Physiol., Sch. of Med., Teikyo Univ., Itabashi-ku, Tokyo 173 and *Dept. of Physiol., Sch. of Med., Univ. Calif. at Los Angeles, CA 90024, U.S.A.

In an isometric tetanus frog skeletal muscle produces about 40 mJ/g of unexplained enthalpy, i.e., the total enthalpy produced minus the enthalpy to be produced from the calculation of the high energy phosphates split during the tetanus. In the present study we gave two successive tetani with various intervals to R. temporaria sartorius muscles at 0°C, and studied the energy liberation and the splitting of high energy phosphates. The total energy liberation was analysed by assuming $H(t)=HL\cdot[1-\exp(-k\cdot t)]+HM\cdot t$, where HL and HM represent the labile heat and the stable heat rate, respectively. The amount of the stable heat rate was the same between the first and the second tetani, but the labile heat decreased to 30% of the first tetanic value in the 6-sec-interval second tetanus and reprimed with two rates of 0.1 and 10 min^{-1}. The unexplained enthalpy also decreased to about 38% in the 6-sec-interval second tetanus and reprimed with a rate of about 0.6-1.6 min^{-1}. The present results are consistent with that the dominant part of the unexplained enthalpy is produced by the binding of released Ca^{2+} with intracellular protein(s), and also suggest that the significant parts of the unexplained enthalpy and the labile heat are produced by the same biochemical reaction(s).

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IS STEPWISE SARCOMERE SHORTENING POSSIBLE ? TAMEYASU, T. Dept. of Physiol., St.Marianna Univ. Sch. of Med., Kawasaki 213.

I examined if a crossbridge model capable of stepwise sarcomere shortening explained major energetical and mechanical properties of skeletal muscle contraction. Essential points of the model are:(i) the crossbridge has three states, i.e.(1) detached state, (2) attached state with force generation and (3) attached, final state, (ii) the reaction (2)->(3), which causes 10 nm sliding between the thick and thin filaments, occurs when sum of the potential energy of state (2) bridges in a half sarcomere exceeds the load on it, (iii) the thick and thin filaments and the crossbridge are so rigid that the crossbridges in a half sarcomere cannot complete their cycle independently each other, and (iv) the rate constant k_{12} is the function of the position of the crossbridge, while k_{23} and k_{31} are the functions of load on the muscle. Based on this model, computer simulation was made on energy consumption during isotonic contraction, load-velocity relation, isotonic velocity transients, isometric transients and so on. The simulated energetical and mechanical properties closely resembled the observed ones both qualitatively and quantitatively. Furthermore, this model could relate the constant a of the heat of shortening with the constant a in the load-velocity relation.

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INTRACELLULAR CALCIUM LOCALIZATION IN SINGLE SMOOTH MUSCLE CELLS ISOLATED FROM GUINEA PIG TAENIA COLI. OOTA, I. and YABU, H. Dept. of Physiol., Sapporo Med. College, Sapporo 060.

The intracellular localization of Ca as a pyroantimonate precipitate was studied in the intact and the chemically skinned cells isolated from guinea pig taenia coli. The chemically skinned cells were prepared by treating with saponin (7μg/ml, 10 min). In the chemically skinned cells at relaxed state, the pyroantimonate precipitates were mainly localized along the inner surface of the plasma membrane, and also at the sarcoplasmic reticulum, the mitochondria and the nucleus. A similar distribution pattern of the precipitate was seen in all intact cells examined. Electron probe X-ray microanalysis showed that the precipitate within these cells containes Ca. These results indicate that the chemically skinned cells retain the intracellular stored Ca and the intracellular localization of Ca is essentially unchanged from that in the intact isolated single cells.

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LOCAL CHARACTERIZATION OF Ca^{2+} TRANSPORT IN GUINEA-PIG STOMACH SMOOTH MUSCLE. SAKAI, Y., ISOBE, A. and ICHIKAWA, S. Dept. of Physiol., Showa Univ. School of Med., Shinagawa-ku, Tokyo 142

Microsomal fractions were isolated from gastric antrum and fundus smooth muscle of guinea-pigs. Ca^{2+} uptake into, and Ca^{2+} release from the membrane vesicles (MV) were studied by a rapid filtration method. Differences between Ca^{2+} transport properties of the different regions of the stomach were compared. ATP-dependent Ca^{2+} uptake was similar in microsomes isolated from both regions. This uptake was increased by oxalate and was not affected by NaN_3. Oxalate affected Ca^{2+} permeability of both antrum and fundus MV similarly. Fundus MV preincubated with 100 mM NaCl and diluted 20 times in Na^+-free media had significantly higher ATP-independent Ca^{2+} uptake than vesicles preincubated by 100 mM KCl. This was not true for antrum MV. Monensin abolished Na^+-dependent Ca^{2+} uptake. NaCl enhanced Ca^{2+} efflux from fundus MV actively loaded with ^{45}Ca. Plasma membrane isolated from fundus preparation by sucrose gradient centrifugation also showed Na^+-dependent Ca^{2+} uptake and release by NaCl. Monensin and gramicidine inhibited these Na^+-dependent Ca^{2+} movements. Results suggest a Na^+-Ca^{2+} exchange in fundus preparations.

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SINGLE CA CHANNEL CURRENTS IN SMOOTH MUSCLE CELLS OF THE GUINEA-PIG TAENIA COLI. YOSHINO, M. AND YABU, H. Dept. of Physiol., Sapporo Med. College, Sapporo 060.

Recordings of single Ca channel currents in mammalian visceral smooth muscle cells were obtained using patch clamp techniques. Smooth muscle cells from guinea-pig taenia coli were prepared by enzymatic dispersion using 0.3 % collagenase (Obara, 1984). The recordings were obtained from cell-attached membrane patches of isolated cells with a pipette filled with isotonic Ba^{++}. When the membrane patch was depolarized, brief inward current pulses of unitary size and small amplitude were observed. The amplitude of these single channel currents decreased linearly with increasing depolarization in a voltage range from -20 mV to +50 mV about the resting potential. The slope conductance was about 30 pS. The mean current reconstructed by averaging individual current responses showed kinetic behaviour with a rapid activation and a slower deactivation process similar to the macroscopic Ca current observed in strips of guinea-pig taenia coli. The present study suggested that the inward current pulses of unitary size induced by voltage-clamp pulses were due to Ba ions passing through a single voltage dependent Ca channels.

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Ca PATHWAYS INVOLVED IN CARBACHOL RESPONSE IN GUINEA-PIG TRACHEAL MUSCLE. BABA, K., HONDA, K., TAKAI, A. AND TOMITA, T. The 2nd Dept. Int. Med. and Dept. Physiol., Sch. Med., Nagoya Univ., Nagoya 466

Effects of verapamil on concentration-response (CR) curves for Ca and Ba in the presence of 40 mM K and /or carbachol (30 μM) were analyzed in the guinea-pig tracheal muscle. The ED_{50} was smaller and the Hill coefficient for Ca was larger in the presence than in the absence of carbachol. On the other hand, the ED_{50} and the Hill coefficient for Ba were not significantly influenced by carbachol. Verapamil (0.1-3.0 μM) displaced the CR curves to the right in parallel without suppressing the maximum response, so that pA_2 values could be determined. The pA_2 of verapamil for Ca was 6.5-6.6 with and 7.0-7.1 without carbachol. The value for Ba was 7.0-7.1 independent of carbachol. These results are difficult to explain by the prevailing assumption of two different pathways: voltage-dependent and receptor-operated Ca channels. It may be that the occupation of muscarinic receptors by carbachol may modify the properties of Ca channels and that Ca is involved in this modification.

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ELECTRICAL AND MECHANICAL ACTIVITIES IN THE GUINEA-PIG TRACHEAL MUSCLE HONDA, K., BABA, K., TOKUNO, H. AND TOMITA, T. The 2nd Dept. Int. Med. and Dept. Physiol., Sch. Med., Nagoya Univ., Nagoya 466

The effects of indomethacin, prostaglandins, isoprenaline and forskolin on the membrane potential and the muscle tone were studied in the guinea-pig tracheal muscle. The membrane potential was -50.6 ± 0.6 mV (n = 200). Spontaneous slow oscillation of membrane potential (slow wave) was often observed. There was a close correlation between the amplitude of slow waves and the membrane potential. The maximum amplitude and frequency of the slow wave were 30 mV and 55/min, and their mean values were 11.9 mV and 43.4/min, respectively. Indomethacin (1 μM) slowly abolished the slow wave and muscle tone, without affecting the membrane potential significantly. A partial recovery was observed with prostaglandins $F_{2\alpha}$ and E_2 (about 0.1 μM). Both isoprenaline and forskolin produced relaxation and blocked the slow wave accompanied by hyperpolarization (the mean value: 10 mV), but the potency of forskolin was 10 times less than isoprenaline, the ED_{50} of which being 0.01 μM.

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MEMBRANE IONIC CURRENTS IN RESPONSE TO ACh IN THE GUINEA-PIG VAS DEFERENS SMOOTH MUSCLE. INOMATA, H. AND WAKUI, M. Dept. of Applied Physiol., Tohoku Univ. Sch. of Med., Sendai 980

Double sucrose gap voltage and current clamp techniques were used to study acetylcholine (ACh) induced responses in longitudinal smooth muscle of the guinea-pig vas deferens. In normal Krebs medium containg adrenergic blocking agent such as guanethidine, ACh (5×10^{-5} - 1×10^{-3} g/ml) causes a single slow depolarization (ACh induced depolarization). This depolarization is associated with a conductance decrease and reversed polarity at a membrane potential close to the K ion equilibrium potential(E_K). On the electrically evoked action potential, ACh slows the rising phase, decreases the peak amplitude and delays the repolarization. ACh application to voltage clamped cells confirmed the conductance decrease. On initial current due to Ca ions, ACh decreases the maximum Ca current ($\bar{I}_{Ca}$) and shifts appreciably its reversal potential (E_a) towards negative voltage without affecting the chord conductance ($\bar{g}_{Ca}$). The activation is slowed slightly, but the inactivation is slowed markedly. On outward current due to K ion, ACh suppresses the fast outward current without affecting the late current. The former maximum conductance ($\bar{g}_K$) is reduced markedly, but the reversal potential (E_K) are not altered.

It seems likely that ACh induced depolarization may be closely related to intracellular Ca concentration.

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EFFECTS OF DIVALENT CATIONS ON THE NEUROMUSCULAR TRANSMISSION IN THE GUINEA-PIG MESENTERIC ARTERY. YAMAMOTO, Y. and HOTTA, K. Dept. Physiol., Nagoya City Univ. Med. Sch., Mizuho-ku, Nagoya 467.

Electrophysiological experiments were carried out on the guinea-pig mesenteric arteries in an attempt to elucidate the effects of changing the external magnesium concentration ($[Mg]_o$) and calcium concentration ($[Ca]_o$) on the excitatory junction potentials (e.j.p.s) due to noradrenaline (NA) release. Increasing the $[Mg]_o$ decreased the e.j.p. amplitude over the entire range examined (0.2-10.0 mM). This did not necessarily mean that Mg inhibited NA release, because the properties of the postsynaptic membrane were also modified: the membrane depolarized by a 5 mV and the membrane resistance increased as the $[Mg]_o$ was increased from 0.2 to 10.0 mM. When the $[Ca]_o$ was varied in the range from 1.25 to 5.0 mM, the e.j.p. amplitude did not change as expected from the Ca-dependent nature of the transmitter release. The variation of $[Ca]_o$ in this range did not alter the membrane potential nor the membrane resistance significantly. NA release in this artery may be less dependent on the external divalent cations than those in other smooth muscles.

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Na-PUMP ACTIVATION AND ENERGY METABOLISM IN THE GUINEA-PIG TAENIA COLI TAKAI, A., TOKUNO, H., UNO, Y. AND TOMITA, T. Dept. Physiol., Sch. Med., Nagoya Univ., Nagoya 466

The guinea-pig taenia coli was exposed to K-free solution for 1 hr in order to reduce the intracellular K concentration. The mechanical activity was transiently increased on removal of K, but complete relaxation occurred within 20 min. When 5.9 mM K was readmitted, the K content of the tissue was quickly recovered at a rate of 1.3 μmoles/min/g wet weight, accompanied by an immediate increase in the rate of oxygen consumption by 0.11 ± 0.07 μmoles/min/g and also of lactate production by 0.04 ± 0.04 ($n = 12$). Since the mechanical activity started about 30 min after K readmission, the increase in oxygen consumption is likely to be related to the Na-pump activation. The rate of recovery in the K content was very close to the value estimated from the increase in oxygen consumption and lactate production, assuming an ATP:K coupling ratio of 1:2 for the pump. This suggests a strong correlation between the Na-K pump activity and intracellular energy metabolism.

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MEMBRANE POTENTIAL OF GUIGNEA-PIG 'TAENIA COLI DURING PROLONGED EXPOSURE TO K-FREE SOLUTION TOKUNO, H., TAKAI, A., NAKAYAMA, S. AND TOMITA, T. Dept. Physiol., Sch. Med., Nagoya Univ., Nagoya 466

In the guinea-pig taenia coli, the membrane is depolarized by 5-10 mV by removing the external K and it is transiently hyperpolarized by 10-20 mV by readmitting K. Since this hyperpolarization is blocked by ouabain, it is considered that the electrogenic Na-pump is responsible for the hyperpolarization. When the removal of K was prolonged, the membrane started to be slowly hyperpolarized between 40 and 50 min after K removal and the potential reached to more than -80 mV, probably due to an increase in K conductance. After the membrane had been hyperpolarized, K readmission produced depolarization. The peak of depolarization caused by K readmission became progressively less negative with the time of exposure to K-free solution. In glycogen-depleted preparations, the electrogenic activity of Na-pump could not be demonstrated without applying either glucose or β-hydroxybutyrate, but the slow hyperpolarization during a prolonged exposure to K-free solution was not much affected by glycogen depletion.

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THE LONG-LASTING CONTRACTION PRODUCED BY TRANSMURAL STIMULATION IN THE GUINEA-PIG LONGITUDINAL STOMACH MUSCLE. SAKAMOTO, Y. Dept. of Physiol., Fukuoka Univ., Sch. of Med., Fukuoka 814-01

The transmural stimulation induced triphasic responses; an initial cholinergic contraction, a subsequent relaxation and a long-lasting contraction. Exogeneous noradrenaline and ATP also caused the initial relaxation followed by the long-lasting contraction. The long-lasting contractions were not blocked by atropine and phentolamine. Furthermore most of these contractions(23/33) were not inhibited by TTX, but some of those(10/33) were reduced to 59 % of the control response. However, all of the long-lasting contractions were completely inhibited by pretreatment with indomethacin. Sympathetic nerve destraction drugs, guanethidine and 6-hydroxydopamine, partly inhibited the long-lasting contraction. Thus it is suggested that the transmural stimulation, is likely to release noradrenaline and ATP simultaneously from the sympathetic nerve terminal and then these transmitters release prostaglandin from smooth muscle or some cell, inducing one part of the long-lasting contraction, or directly affects an unknown some cell, inducing the other part of the long-lasting contraction.

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CHANGES IN THE PROPERTIES OF THE CIRCULAR MUSCLE OF POSTPARTUM RAT UTERUS. MARUTA, K., OGASAWARA, T. and OSA, T. Dept. of Physiol., Yamaguchi Univ. Sch. of Med., Ube 755

Changes in the membrane activity and ß-adrenergic effects were studied for the circular muscle of postpartum rat uteri. Spike potential dominated during the period between 0 and 15 hr after the delivery of the first newborn. During the postpartum period between 20 and 48 hr, the plateau potential was dominant. Application of 10^{-9}M isoprenaline strongly depressed the contraction during early postpartum period (0-15 hr), and the depressant effect was much smaller thereafter. Administration of estradiol-17ß (50 µg) or progesterone (50 µg) to postparturient rats for 2 days did not alter the postpartum changes in membrane activity, nor the effect of isoprenaline. The postpartum changes in muscle properties mentioned above were prevented to occur in the distended portion of uterus, when several fetuses and placentas were artificially kept inside the uterus for 2 days, while other fetuses were delivered out.

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THE CONTRACTION INDUCED BY DOPAMINE IN THE RABBIT AORTA. MURAKAMI, H., ISHIKAWA, T.* Dept. of Physiol. and Biol.*, Nihon Univ. Sch. of Dent. at Matsudo, Matsudo, Chiba 271

In an attempt to elucidate the site of action of dopamine (DA) in the rabbit aorta, the effects of alpha-adrenergic and some DA antagonists on the contraction by DA and phenylephrine (PHE) were investigated in the isolated aortic strips. The dose-response curves of DA and PHE were shifted to the right by prazosine, haloperidol and butaclamol in a competitive manner and further the former was shifted to the right by yohinbine in the same manner. The pA_2 values of prazosine against DA and PHE were 8.04 and 8.06. Those of haloperidol against DA and PHE were 6.76 and 6.91. Those of butaclamol against DA and PHE were 6.86 and 6.53. The pA_2 value of yohinbine against DA was 5.56. Bufetolol and sulpiride did not possess the antagonistic action on the contraction by DA.

These findings indicate a possibility that the contractions by DA and PHE antagonized by haloperidol and butaclamol are mediated through the dopamine receptors, and that both the contractions antagonized by prazosine and yohinbine are mediated through alpha-adrenoceptors.

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IONIC MECHANISM OF "REBOUND CONTRACTION" INDUCED BY HOMOPANTOTHENIC ACID, A DERIVATIVE OF γ-AMINOBUTYRIC ACID, IN THE INTESTINAL SMOOTH MUSCLE OF THE GUINEA-PIG. FUKUSHI,Y. AND WAKUI,M. Dept. Applied Physiology, Tohoku University School of Mediceine,Sendai 980

Homopantothenic acid (HOPA) produced an initial relaxation which was followed by a contraction (rebound contraction) in both preparations of taenia coli and ileum when they had moderate tonus. The HOPA-induced relaxation was associated with a depolarization which accompanied by an increase in the size of electrotonic potentials of the membrane. Furthermore, the relaxation induced by HOPA was not abolished by treatments with TEA, 4-aminopyridine or a K-free solution, by which the relaxation induced by noradrenaline was completely inhibited. The HOPA-induced contraction was synchronized only to a development of Ca^{2+}-spike potentials activated by the preceding depolarization, which was supported by the finding that HOPA could not evoke a contraction in a solution containing D600 or in a Ca-free solution containing 1 mM EGTA, although acetylcholine could evoke the contraction in these solutions. It is suggested that the relaxation of the intestinal smooth muscle induced by HOPA was not resulted from a change in membrane potential but from other mechanisms which are not clear at present. While, the contraction induced by HOPA was mediated by Ca-entry into the cytoplasm through voltage-sensitive Ca channels activated by a depolarization, due mainly to a decrease in membrane conductance to potassium ions.

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ENERGY DEPENDENCY OF K CONTRACTURE IN SMOOTH MUSCLES ITO, M., BABA, K., TAKAI, A. AND TOMITA, T. The 2nd Dept. Int. Med. and Dept. Physiol., Sch. Med., Nagoya Univ., 466 Nagoya

In the tracheal muscle and taenia coli of guinea-pig, the lactate production rate (μmoles/g/min) increased from 0.17 ± 0.16 to 0.51 ± 0.32 and from 0.11 ± 0.05 to 0.37 ± 0.17, respectively, during K contracture. Similarly, the oxygen consumption rate (μmoles/g/min) increased by 0.14 ± 0.06 and by 0.31 ± 0.11, respectively. When oxidative phosphorylation was inhibited by 1 mM NaCN or by anoxia during K contracture, the tension decreased by 15% in the trachea but by 90% in the taenia, accompanied with an increase in lactate production to 1.6 ± 0.7 and to 1.1 ± 0.2, respectively. In glucose-free, excess (40 mM) K solution, the contracture produced by Ca readmission gradually decreased with time in both tissues, the rate being much slower in the trachea. The glycogen content (μmole glucose unit/g) was higher in the trachea (8.2 ± 1.1) than in the taenia (3.9 ± 1.1). It was concluded that the tracheal muscle is less dependent on the oxidative process than the taenia coli to maintain tension.

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CA^{2+}-INDUCED CA^{2+} RELEASE MECHANISM IN SAPONIN-TREATED SINGLE SMOOTH MUSCLE CELLS. OBARA, K., ITO, Y., KUNIMOTO, M. and YABU, H. Dept. of Physiol., Sapporo Med. College, Sappro 060.

The Ca^{2+} release from intracellular Ca^{2+} storage sites of saponin-treated single smooth muscle cells was studied by the filtration method using ^{45}Ca. The single cells were isolated from guinea pig taenia caeci by digestion with 0.3% collagenase and treated with saponin (7 μg/ml) in a relaxing solution for 10 min at 35°C. In the presence of ATP, NaN_3 and 10^{-8} M Ca^{2+}, saponin-treated single cells could accumulate 0.2 nmol of Ca^{2+} per mg protein. The Ca^{2+} release from intracellular Ca^{2+} storage sites of saponin-treated single cells was enhanced by the presence of submicromolar concentrations of Ca^{2+} in the solution. The Ca^{2+} release was enhanced by caffeine and adenine, and suppressed by Mg^{2+} and procaine. These results suggest that the Ca^{2+}-induced Ca^{2+} release mechanism may play a role in the release of Ca^{2+} from intracellular storage sites of guinea pig taenia caeci smooth muscle cells. The threshold concentration of Ca^{2+} for the Ca^{2+}-induced Ca^{2+} release was approximately 2×10^{-8} M in saponin-treated single cells.

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EXTRACELLULAR CALCIUM-DEPENDENCE OF THE CONTRACTURE INDUCED BY SIMULTANEOUS APPLICATION OF CALCIUM WITH HIGH K TO CALCIUM FREE SOLUTION IN STOMACH SMOOTH MUSCLE. KOSAKA, I., YABU, H., Dept. Physiol., Sapporo Med. Col., Sapporo 060 Japan

Thin bundle (less than 100 μm in diameter) of stomach circular muscle of the newt, Cynops pyrrhogaster, responded to the simultaneous application of Ca with high K (113.5 mM K) to EGTA Ringer solution (Ca-free + 1 mM EGTA). In the muscle of which carbachol (CCh) sensitive Ca store had been depleted by the treatment with 10^{-5} M CCh, the curve relating Ca concentration to Ca contracture tension started to sharply rise between 2.8 X 10^{-6} M and 5.5 X 10^{-6} M Ca to about 50% of its maximum level. Then the curve showed plateau to 10^{-4} M Ca and again rise, reaching 100% at 1.8 mM Ca. CCh contracture in EGTA high K solution after 90 sec loading of Ca could be induced 90% of its maximum level even at 1.3 X 10^{-6} M Ca by which Ca contracture was little induced. In Ca depleted muscle, when Ca contracture was interrupted by Ca removal with EGTA after the application of 1.8 mM Ca, Ca contracture was observed to be maximum at 5 - 10 sec after the application of Ca. In spite of this Ca loading, CCh contracture could be induced only about 40% of the Ca contracture and followed by slow increase up to 70% in about 30 sec after Ca application when Ca contracture was almost completely relaxed. The curve relating Ca contracture tension to K concentration showed to be sigmoid, while that relating the CCh contracture tension to K concentration was linear.

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TRANSIENT MOVEMENT OF INTRACELLULAR FREE CALCIUM ON ELECTRICAL STIMULUS MONITORED BY FLUORECENT DYE. HOTTA, K., OBA, T., and YAMAMOTO, Y. Dept. Physiol., Nagoya City Univ. Med. Sch., Mizuho-ku, Nagoya 467.

The movement of intracellular free-Ca concentration([Ca]i) of the smooth muscle was studied using Quin 2 in the guinea-pig jejunum. Single electrical pulse(10 ms) increased [Ca]i in two phases: a rapid phase and a following larger one during which [Ca]i reached several μM. Tetanus stimulation induced a continuous increase of [Ca]i. Mechanical response appeared about 400 ms after the stimulation. Caffeine(2mM) facilitated the second phase of [Ca]i-change and this was reduced by procaine(1mM). Verapamil inhibited the first phase and there was no occurrence of the following large [Ca]i-increase. Both verapamil and procaine inhibited the contraction strongly. It is presumed that the first [Ca]i-increase reflects Ca influx upon depolarization of the membrane and that this Ca triggers the release of intracellulary-stored Ca. Thus, Ca-induced Ca-release may be physiologically important for the mechanical activation in the smooth muscle cells.

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COMPARATIVE STUDIES ON RESPONSE TO AND RELEASE OF CATECHOLAMINE IN EU, HYPO AND HYPER-THYROID RAT ATRIUM. MIYASE, T., IMANAGA, I., INATOMI, K. Dept. of Physiol., Fukuoka Univ., Sch. of Med., Fukuoka 814-01

After the sino-atrial muscle with spontaneous activity is stimulated by sine wave of 50-60 Hz, it shows transient potentiation (AP) following initial inhibition (AI) of contraction. AI is completely abolished by atropine, AP, by β-receptor blocker, reserpinization and both AI and AP, by TTX. These suggest AI is caused by acetylcholine and AP, by catecholamine released from the autonomic nerve terminals. The AP is increased as stimulation intensity or duration is raised. The AP in hyperthyroid state is larger than that in eu- or hypothyroid state in all ranges of the intensity or the duration. By the duration more than 5 sec, time to peak AP from the end of stimulation (t) is shortened showing maximal response of AP in the presence of atropine. The shortening of t may indicate amount of catecholamine release. But the shortening of t is not significant in three thyroid states. On the other hand, dose-response relation curve between twitch contraction of atrial muscle (driven with 1 Hz) and noradrenaline shifts to the left in hyper- and to the right in hypothyroid state. Moreover, in hyper-thyroid state, arrhythmias with extrasystoles are observed more often. These results suggest that in hyperthyroid state, response of the myocardial cell to catecholamine is augmented rather than release of catecholamine from the sympathetic nerve terminal.

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EFFECTS OF Mg^{2+} AND MgATP ON THE MAXIMUM ISOMETRIC TENSION AND ATPase ACTIVITY IN GLYCERINATED CAT PAPILLARY MUSCLE. KATO, C., SAEKI, Y. AND YANAGISAWA, K. Dept. Physiol., Tsurumi Univ. Sch. Dent. Med., Yokohama

Effects of Mg^{2+}(0.3mM and 10mM) and MgATP(50μM and 10mM) on the maximum isometric tension, ATPase activity and the transient tension response to step length stretch(less than 1% of Lo within 2msec) have been studied in saturating Ca^{2+} or Ba^{2+}-activated glycerinated cat right ventricular papillary muscles(2-3mm long, 175-300μm in diameter) at ionic strength 0.2M, pH 6.8 and 25°C. Increasing MgATP from 50μM to 10mM at constant Mg^{2+} induced the significant reduction of maximum tension and the ATPase activity in both Ca^{2+} and Ba^{2+}-activated muscles. When Mg^{2+} increased from 0.3mM to 10mM at constant MgATP, the maximum tension showed a considerable amount of reduction but the ATPase activity decreased only slightly in both Ca^{2+} and Ba^{2+}-activated preparations. The transient tension change, the profile of which was almost the same in Ca^{2+} and Ba^{2+}-activated preparations, became faster with increasing MgATP but was not influenced with increasing Mg^{2+}. Although the maximum tension of Ba^{2+}-activated muscle was always greater than that of Ca^{2+}-activated muscle, ATPase activity in Ba^{2+}-activated muscle was lower than that in Ca^{2+}-activated muscle. In the absence of Ca^{2+}, neither ATPase activity nor tension development were observed. In the absence of MgATP, remarkable tension development was observed but the ATPase activity was extremely low.

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EFFECT OF INOTROPIC STATES ON THE SHAPE OF RAT CARDIAC SARCOMERE LENGTH-TENSION RELATION.

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The sarcomere length-tension relation was determined in rat right ventricular muscle. Sarcomere length was measured by laser diffraction. At temperature of 26°C, the shape of length-tension relation changed from rectilinear to convex when extracellular [Ca^{2+}] was elevated (1.9→3.8 mM). Conversely, the shape changed to concave with decrease in [Ca^{2+}] of the perfusate (1.9→1.0 mM). The same effect occurred by raising stimulus frequency from 12 to 48 /min. At 37°C, an increase in stimulus rate decreased tension development until the rate reached 50-100 /min ; pronounced positive staircase occurred with further increase in stimulus frequency. Stimulation at 300 /min increased tension 2-3 times and, similar to the elevation of [Ca^{2+}], rates of tension development and relaxation increased. Despite the significant increase in tension development, the shape of the sarcomere length-tension relation was not affected by the stimulus frequency (24-300 /min) at physiological temperature. Unlike prior observations at non-physiological stimulus conditions, changes in contractility do not necessarily affect the shape of sarcomere length-tension relationship.

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PRESSURE-VELOCITY RELATION DURING CONTRACTION IN THE ISOLATED RABBIT LEFT VENTRICLE

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The relation between pressure (P) and volume-velocity ($\dot{V}$) during relaxation was determined in coronary perfused rabbit left ventricle by isovelocity method. The measuring system consists of a pulse motor, a ball gear and an acrylic syringe. The ball gear was connected to the pulse motor, so that volume of the syringe could be changed linearly and easily with corresponding pulses. The P-$\dot{V}$ relation during relaxation was well approximated by a hyperbolic equation similar to that during contraction, $(P+A)(\dot{V}+B)=B(P_o+A)$, where A, B and P_o are constants. These constants depend on ventricular inner volume or heart rate, if the other conditions are equal. The effect of volume on P-$\dot{V}$ relation is well explained from the dynamic constants of wall muscle (Hill constants a and b) using the thick-walled spheric ventricle model. When the initial volume increased, both A and B decreased, but the ratio of B to A ($=\dot{V}_{max}/P_o$) was not changed. This result suggests that during relaxation as well as contraction the dynamic constants of wall muscle are maintained constant in spite of initial volume change. When the heart rate increased, the value of $\dot{V}_{max}/P_o$ also increased. Therefore, this value could be the index of contractility during relaxation as well as contraction.

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EFFECTS OF THYROXINE ON SINUS-ATRIUM PREPARATION.

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Prolongation of cardiac interval(+20%) in acute L-thyroxine(T.) application(40uM, 50 min) was observed in an everted sinus-atrium preparation of adult bullfrog. This prolongation was consisted of two processes. They were the initial(0-10 min) and the delayed(10-50 min) prolongation. The initial one was accompanied with slight and transient increase in contractility but the delayed one was accompanied with decrease in contractility to the control level. The initial prolongation was suppressed by 15mM TEA pretreatment and antagonized by 10uM Cd^{2+}. On the other hand, the delayed one was enhanced by 15mM TEA pretreatment and suppressed by the metabolic inhibitors such as 0.5mM NaCN, 10μM DNP. Both the decrease in contractility and increase in cardiac interval induced by the delayed effects of T_4 was partially restored by Ca^{2+} ionophore(3μM A23187). These results suggested that the initial effects of T_4 were accompanied with transient increase in intracellular Ca^{2+} by which hormonal actions might be mediated at the cellular level and the delayed effects of T_4 were accompanied with the acceleration of metabolism.

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OPTICAL RECORDING OF RHYTHMIC ACTIVITY IN EARLY EMBRYONIC CHICK DOUBLE-HEART.

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Double-hearted embryos were produced by whole embryo-culture of chick embryos which were microsurgically cut through the tissue of the anterior intestinal portal at the 1-6 somite developmental stage, at the time when the cardiac primordia have not yet fused in the bulboventricular region. The cultured embryos were removed from an incubator usually at the 7-10 somite stages of development, and then spontaneous electrical action potentials and/or contractions were optically recorded simultaneously from both the right and left half-hearts, using a 10×10-element photodiode matrix array together with a voltage-sensitive merocyanine-rhodanine dye (NK 2761). At the 7-8 somite stages, spontaneous action potentials were detected from bilateral prebeating half-hearts or sometimes from one half-heart. In each half-heart, the first spontaneous beating was often observed in the half-heart of the 9 somite embryos. In the beating half-hearts regular activity was always observed, while in the prebeating half-hearts at the 7-8 somite stages, both the regular and irregular rhythms of action potentials were detected, and the incidence of occurrence of regular activity significantly outnumbered that of the irregular rhythm. The heart rate in the left half-heart was faster than that in the right half-heart in the great majority of the prebeating and beating double-hearted embryos.

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TETANIC TENSION OF FROG VENTRICCULAR MUSCLE AND Ca^{2+} SOURCES. TANAKA, S., NISHIYE, H. and MASHIMA, H. Dept. of Physiol., Sch. of Med., Juntendo Univ., Hongo, Tokyo 113

Ca^{2+} sources of tension developments were suspected to be different in each of twitch and tetanic tensions. Tetanic tension was induced by stimulation with alternating current pulses(10Hz to 15Hz, 1sec or 2sec duration). In 1.8mM Ca^{2+} containing Ringer's solution, Co^{2+} gradually decreased twitch tension. Twitch tension was depressed to ca. 15% of the control in 20min by application of 3mM Co^{2+}. Whereas, tetanic tension almostly was not depressed even by application, of 6mM or 12mM Co^{2+}. With application of TTX(10^{-6}M - 5 x 10^{-6}M), tetanic tension was depressed to 50% - 65% of the control. And the twitch tension promptly was depressed by application of 10^{-6}M TTX. Under the coexistence of Tb^{3+}, TTX depressed tetanic tension as similar as that without Tb^{3+}. It seems that TTX and Tb^{3+}, have simple additive effect, presumably independent effect against tetanic tension development. Tetanic tension was almostly depressed by TTX and Tb^{3+}, whereas Co^{2+} almostly not depressed tetanic tension.

It is suggested that Ca^{2+} entry through Ca channel of cardiac plasma membrane has only very small contribution to development of tetanic tension in frog's ventricular muscle and that depolarization dependent Ca^{2+} entry and/or Ca^{2+} entry through Na^{+}-Ca^{2+} exchange system mostly contribute to development of tetanic tension.

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THE REPLACEMENT OF POTASIUM BY CESIUM IN THE RABBIT SINOATRIAL NODE CELLS.
SUDO, S., GOTO, K., TAKAHASHI, T., MIYAMAE, S., NAKANO, S., MATSUDA, T. AND NAKANO, M. Dept. of Physiol., Kanazawa Med. Univ., Uchinada, Ishikawa 920-02

Small specimens of tissue (0.5 mm x 0.5 mm) were isolated from the rabbit sinoatrial node, and superfused with Tyrode solution (5.4 mM-K, pH 7.4) at 37°C. When a specimen was exposed to a modified solution (20 mM-Cs, K-free), an electrogenic automaticity disappeared within 15 min and thereafter, restored within 30 min (Cs-automatism). An arrythmia appeared during the later phase of disappearance. These reactions appeared when 20 mM-Cs was perfused after the automaticity was stopped by K-free solution. And these reactions also appeared when Cs concentration was reduced to 6 mM, but in the lower concentration of Cs, these reactions were obscured. The appearance of Cs-automatism was depended on external K concentration. The limitting K concentration seems to be less than 1 mM. The arrythmia which appeared during the disappearance phase was suppressed by lidocaine (0.3 mM), propranolol (6.6 x 10^{-6} M) and phentolamine (10^{-5} M). Only lidocaine suppressed the Cs-automatism. This arrythmia, therefore, might not be essential to the Cs-automatism. Phentolamine (10^{-5} M) suppressed normal automatism but not Cs-automatism. These results suggest that the Cs-automatism may be operated by other mechanisms.

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EFFECT OF D_2O ON THE ACTION POTENTIAL AND CONTRACTION IN GUINEA-PIG PAPILLARY MUSCLE.
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The effect of D_2O on the action potential and isometric tension development was studied on the papillary muscle isolated from the heart of guinea-pig. In D_2O solution, the magnitude of contraction first decreased by about 90%, and then recovered to reach a constant level which amounted about 30% of that in normal Krebs solution. D_2O prolonged the duration of action potential by about 50% without affecting resting membrane potential. If the muscle was returned to normal Krebs solution after a prolonged immersion in D_2O solution (more than 30min), the contraction showed a marked transient potentiation before returning to the initial steady level. The potentiation was maximum at 1.5-3min after returning the muscle to normal Krebs solution, the magnitude of the potentiated contraction being about two times the steady value in normal Krebs solution. In both normal and D_2O solutions, a reduction of $[Ca]_o$ decreased the magnitude of contraction and prolonged the duration of action potential, while an increase in $[Ca]_o$ increased the magnitude of contraction and decreased the duration of action potential, indicating that the effect of $[Ca]_o$ is qualitatively the same in both cases. These results suggest that D_2O primarily act on the Ca-channels through which Ca ions enter into the muscle cell.

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EFFECTS OF REDUCED $[Ca]_o$ ON THE OUABAIN-TREATED SINO-ATRIAL NODE CELLS.
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Oscillatory potentials induced by removal of $[Ca]_o$ were observed in rabbit sino-atrial node cells superfused with 10^{-5} M ouabain. The oscillations increased from spontaneous voltage fluctuations of less than 2 mV to a peak amplitude of 46 ± 3 mV(n=10). Similarly, oscillations developed in 2 mM EGTA solutions after pretreatment with Ca-free solutions or 0.5 mM Co solutions. The amplitude of the oscillations evoked by superfusing with 2 mM EGTA solutions after pretreatment with Ca-free solutions became 35 % ($P<0.001$) smaller from the application of caffeine. The amplitude of the oscillations also became 46 %($P<0.001$) smaller in the 6 mM EGTA solutions than in the 2 mM EGTA solutions. The amplitude of the oscillations induced by superfusing with 6 mM EGTA solutions was 80 %($P<0.01$) larger after pretreatment with the Ca-free solutions than with the 2.5 mM Ca solutions. The time to initiation of the oscillations measured as the interval from the point of removing $[Ca]_o$ to that of the initiation of the oscillations became 68 % ($P<0.001$) and 76 %($P<0.001$) shorter in 6 mM EGTA solutions after pretreatment with Ca-free and 2.5 mM Ca solutions than those evoked by exposure to 2 mM EGTA solutions. It is concluded that reduction in calcium overload induced by ouabain lead to an increase in the amplitude of the oscillations.

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EFFECTS OF ACETYLCHOLINE ON ACONITINE-INDUCED DELAYED AFTERDEPOLARIZATION IN THE FROG ATRIUM AND VENTRICLE.
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Delayed afterdepolarization (DAD) and triggered activity (TA) was induced by aconitine (10^{-5} g/ml) under the Ca^{2+}-free condition. Effects of acetylcholine (Ach) (10^{-7} g/ml) on aconitine-induced DAD was studied in frog atrial and ventricular preparations. In atrial preparation, Ach significantly ($P < 0.001$) depressed the amplitude of DAD within 3 min of the application with later recovery. The amplitude of DAD after washout of Ach showed more than control value in 7 out of 13 preparations and developed to triggered activity. Resting potential significantly hyperpolarized (6-10 mV) at early time of Ach application with later depolarization. The time course of this change was correlated with the change of DAD. Potentiating effect of DAD after washout did not follow the change of resting potential. In ventricular preparation, amplitude of DAD and resting potential were not affected by application of Ach, while shortening of action potential duration was less compared with that in atrial preparation. Atropine (10^{-6} g/ml) completely inhibited the effect of Ach, but propranolol (10^{-6} g/ml) did not. These results indicate that Ach showed transient inhibition of DAD through hyperpolarization of membrane potential in atrial preparation. Desensitization of muscarinic receptor is considered as a mechanism of the transient inhibition of DAD by Ach.

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EFFECTS OF LYSOPHOSPHATIDYLCHOLINE ON IONIC CURRENTS OF GUINEA PIG VENTRICULAR CELLS.
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The effects of lysophosphatidylcholine (LPC), an ischemic catabolite of membrane phosholipids, on the ionic currents of single ventricular cells were studied using a 'giga-ohm seal' patch electrode. LPC (10-50 μM) added to the external solution decreased resting potential and induced spontaneous, repetitive action potentials with early and delayed afterdepolarizations. LPC (20 μM) decreased the conductance near the resting potential (from 26 ±8 nS, control to 16 ±3 nS, mean and SD, n=4) within 10 min and occasionally induced transient inward currents after prolonged administration (>12 min). LPC decreased the resting potential and induced sustained depolarization even in the presence of Cd (0.2 mM) or in the presence of Ca-free solution containing EGTA (0.2 mM) in which possible Ca accumulation in the cell have been prevented. From these findings, we concluded that the main ionic current which is suppressed by LPC and responsible for the LPC-induced depolarization is the "inwardly rectifying potassium current" (I_{k1}).

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THE PACE MAKER POTENTIAL PICKED UP FROM THE CULTURED CHICK EMBRYONIC HEART CELL BY ALTERED CONCENTRATIONS OF Na and Ca IONS.
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The action potential of cultured chick embryonic heart cell was recorded with the microelectrode method. The increase in external Ca ion shortened the duration of action potential in initial repolarization phase, in later phase, however, prolongation was produced. Such effects of Ca ion were not removed with previous application of TEA. The increased Ca ion with decreased Na ion produced the successive spontaeneous potential like sine curve occasionally superimposed with spike-like potential. This seemed to be still keeped pace maker potential. It seems to be possible to explain the results as follows: With the altered concentrations of Ca and Na ions, the pace maker potential got larger and longer, while the initial spike shortened and reduced. The sum of two potentials made the pace maker potential more manifest in comparison with usually observed action potentials of heart muscle.

Single-channel currents were recorded with the cell-attached patch-clamp technique from the embryonic chick heart cell, exposed to high K ion solution in the pipette, but with normal extracellular solution in the bath.

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Na-H EXCHANGE MECHANISM IN ISOLATED SINGLE VENTRICULAR CELLS OF GUINEA-PIG.
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Effects of depleting Na from the Tyrode solution or adding amiloride to the Tyrode solution on the amplitude and the time course of the inactivation of the i_{Ca} were examined in the single ventricular cells of the guinea- pig. The amplitude of i_{Ca} decreased when the pH of the pipette solution was lowered to pH=5, but at pH=6 no significant change was observed. With the pipette solution of pH=7.2, superfusion of Na-free Tyrode or 1 mM amiloride gave no significant effect on i_{Ca}, but with the acidic pipette solution application of Na-free or amiloride gave a prominent decrease in the amplitude and the time course of inactivation of i_{Ca}. Even using such a slight acidic solution as pH=6.8 in the pipette, the i_{Ca} markedly decreased in the Na-free Tyrode or amiloride. This observation suggests that when the proton is applied from the tight sealed pipette, it quickly extruded by the Na-H exchange mechanism and the intracellular pH was not sufficiently lowered. This experiment also suggests that the proton may act on the i_{Ca} from the inside site of the cell, since the superfusion of pH=6.8 Tyrode solution gave no appreciable change in i_{Ca}. This notion was proved by the fact that when the pH of the pipette solution is 8.0, the amplitude of the i_{Ca} did not show an appreciable change in response to lowering the external pH to 6.0.

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SODIUM CONDUCTANCE IN CALCIUM CHANNELS OF GUINEA-PIG VENTRICULAR CELLS INDUCED BY REMOVAL OF EXTERNAL CALCIUM IONS.
MATSUDA, H. and NOMA, A.
Natl. Inst. Physiol. Sci., Okazaki, 444

Na conductance in Ca channels of single guinea-pig ventricular cells was studied using a 'giga-ohm seal' method for whole cell and single channel current recordings. An inward Na current characterized by a slow inactivation, was induced when the extracellular Ca concentration was reduced by EGTA. This current decreased in amplitude as the concentration of external divalent cations was elevated. Blocking the Na current by divalent cations could be approximated by a bimolecular interaction between divalent cation and channel, with a dissociation constant of 1.2 μM for Ca and 80 μM for Mg. The unit amplitude of the Na current was calculated from the variance-to-mean current relationship at around -40 mV. When the extracellular Ca concentration (pCa) was reduced from 5.5 to 7.5, it was increased from 1.1 to 2.7 pA, but the number of functional channels of ∿4500 was unchanged. Single channel current recordings in the cell-attached configuration gave results consistent with those of ensemble fluctuation analysis.

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TRANSIENT OUTWARD CURRENT CARRIED BY K AND Na IN QUIESCENT ATRIOVENTRICULAR (A-V) NODE CELL OF RABBIT. NAKAYAMA, T and IRISAWA, H. Nat. Inst. Physiol. Sci. Okazaki

About 20% of the rabbit single A-V node cells prepared by treating with collagenase became rounded in the Tyrode solution and about 20% of them showed spontaneous activity while the rest remained quiescent. With the whole cell voltage clamp, depolarizing clamp pulses from the holding potential of -83 mV induced an outward current decaying rapidly with the time course similar to that of the transient outward current of other cell. The current amplitude became larger when progressively more positive clamp pulses were given from -83 mV. Two exponential inactivation time courses were found and the recovery from inactivation was slow. Unit channel recordings revealed a class of channels which activated more frequently during the initial part of the test pulses. Averaging of the unitary currents well reproduced activation and inactivation time courses of the macroscopic current, suggesting that this channel corresponds to the transient outward current. The I-V relation of the channel was linear with the slope conductance of 19.9$\pm$ 1.8 pS (n=7) and the reversal potential was near the resting potential of the A-V node cell with 5.4 mM KCl and 134.6 mM NaCl in the pipette. K and Na ions were passing through this channel: pNa/pK was about 1/5. This channel may play a role to keep the constant resting potential of the follower cells.

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MECHANISM OF SINGLE Ca CHANNEL BLOCK BY Co^{2+} AND Mg^{2+} IN GUINEA-PIG VENTRICULAR CELLS. HINO, N., *HSU, T., **YOSHINO, M. AND OCHI, R. Dept. of Physiol., Sch. of Med., Juntendo Univ., Hongo, Tokyo 113, *Cardiovasc. Div., Chang-Gung Memorial Hosp., Taipei, Taiwan, R.O.C. and **Dept. of Physiol., Sapporo Med. College, Sapporo 060

The blocking actions of Co^{2+} and Mg^{2+} on single Ca channels were studied using cell-attached patch recordings from single ventricular myocytes of guinea-pig. The Ca agonist BAY K 8644(1μM) was applied to the superfusate to prolong the open state of Ca channels. The patch pipettes contained 50mM Ba^{2+} as charge carriers. When Co^{2+}(0.025-0.2mM) was added to the pipette solution, only bursts of short openings appeared instead of the long-lasting openings. With raising concentrations of Co^{2+} the openings were shortened by breif gaps occured more frequently but burst duration was not varied. Such changes in the currents were not produced by Co^{2+}(2mM) in the superfusate. The histograms of the open times(τ_o) and blocked times(τ_b) within burst distributed with single-exponential functions; at 80mV depolarization from the resting potential τ_o and τ_b for 0.1mM were 1.16ms and 0.60ms. Blocking rate(τ_o^{-1}) altered linearly with while unblocking rate(τ_b^{-1}) related weakly to the concentration. Similar results(flickering) were also obtained with higher concentrations of Mg^{2+}(2-10mM) in the pipette solution. The blocking action of Co^{2+} was little affected by the membrane potential while that of Mg^{2+} was slightly enhanced at negative potentials.

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Ba-INDUCED PACEMAKER ACTIVITIES AND RESPONSIBLE MEMBRANE CURRENTS. GOTO, M., GONDO, H. AND IKEDA, K. Dept. of Physiol., Faculty of Medicine, Kyushu University, Fukuoka 812

The pacemaker activities induced by Ba (0.1-0.3 mM) were studied on the bullfrog atrial muscle under voltage clamped and unclamped conditions with double sucrose-gap technique. The activity was elicited after membrane depolarization (~-60 mV) due to depression of anomalous K channels (I_{K1}), and continued for a while with an interaction of the inward (mainly I_{si}) and outward current (I_{xs}). Funny inward current (i_f) and extremely slow delayed outward current ($I_{x\,acc}$) did not participate in the activity. In the muscle became quiescent, isoproterenol (10^{-7} M) induced an oscillatory potential (OS) which developed to pacemaker activity, and a transient afterdepolarization (TD) when the activity was triggered. Co (1 mM) diminished TD first and then OS, thereby suggesting a presence of spontaneous and triggered Ca-indued Ca-release from SR. It was proposed that the current responsible for pacemaker potential is a mixture of the deactivation of I_{xs} and the activation of I_{Tl} (TD), and consequent depolarization generates the inward current (I_{si}).

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THE MECHANISMS UNDERLYING NIFEDIPINE BLOCKADE OF SLOW INWARD CURRENT (I_{si}) ON FERRET PAPILLARY MUSCLES. INAZAWA, M. AND KATZUNG, B.G.* Dept. of Physiol., Fac. of Med., Kyushu Univ., Fukuoka 812 and Dept. of Pharmacol., Univ. of California, San Francisco, San Francisco, C^ 94143, U.S.A.*

The blocking action of nifedipine on I_{si} was investigated by current or voltage clamp mode with single sucrose gap method. In 20 action potentials (APs) train, 0.2μM nifedipine depressed the overshoot (OS) of 1st AP but showed no more suppression of OS in the following APs at 0.5Hz stimulation. However, rate dependent suppression of OS developed at 2-3Hz. Under voltage clamp I_{si} was already depressed at 1st pulse in 20 clamp pulses train by nifedipine (tonic block). When the potential (V_i) between the pulses was held at -40mV, a gradual suppression of I_{si} following initial tonic block was observed at both 0.5 and 2Hz (use-dependent block). The degree of use-dependent block was larger at 2Hz than that at 0.5Hz. When V_i was -80mV, the use-dependent block disappered at 0.5Hz, but was still observed at 2Hz though the degree of block was smaller compared to that at 2Hz (V_i=-40mV). These results indicate that nifedipine inhibits I_{si} partly in a frequency- and voltage-dependent fashion and suggest that tonic block is caused in rested state channel and use-dependent block develops in activated channel.

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CHARGE MOVEMENT REGULATING THE INWARD RECTIFYING POTASSIUM CURRENT IN GUINEA-PIG PURKINJE FIBERS. MITSUIYE, T., IKEMOTO, Y. AND GOTO, M. Dept. of Physiol., Faculty of Medicine, Kyushu University, Fukuoka 812

Cs-sensitive inwardly rectifying potassium current (I_{K1}) and the background current (I_C), which was not blocked by 20mM Cs, were studied in 25 mM Na and Ca-free solution using ramp voltage clamp (400mV/sec). I_C was not affected by Na_o, Ca_o, Mg_o, Cl_o or Cs_o, but was markedly influenced by K_o and completely supressed in K-free solution. This finding excludes implications of these ions except K to I_C. I_{K1} reversed at potentials predicted by Nernst equation for K, showed strong inward rectification at more positive potentails than E_K with a negative slope conductance region, and did not flow at more positive potentails than the half of E_K. The slope conductance of I_{K1} at E_K was dependent on the square root of K_o. I_C reversed at the potential of half value of E_K. Its slope conductance was positive at the reversal potential (E_C) and dependent on K_o around E_C. These findings suggest that I_C is related to charge movement concerning the regulation of I_{K1} channel. A dipole model was proposed to explain I_C, I_{K1} and their dependency on K_o.

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VOLTAGE- AND TIME-DEPENDENT BLOCK OF g_{k1} BY Ba UNDERLIES THE Ba-INDUCED AUTOMATICITY IN SINGLE VENTRICULAR MYOCYTE OF GUINEA PIG. IMOTO, Y., EHARA, T. AND GOTO, M. Dept. of Physiol., Faculty of Medicine, Kyushu University, Fukuoka 812

The mechanism of Ba-induced automaticity in single ventricular myocyte of guinea pig was studied using whole cell clamp method. In the presence of barium (0.3mM), a small and continuous depolarizing current induced regular and repetitive firings. In voltage clamp experiments, Ba (0.1-5mM) strongly suppressed the membrane conductance, but this suppression had a time-dependent component. Thus the instantaneous I-V relation ($I\text{-}V_{in}$) obtained with the holding potential (HP) of highly depolarized level (e.g. +5mV) was N-shaped, as if there were little influence of Ba. However, the current during hyperpolarizing pulses showed a time-dependent change, which resulted in a steady state I-V relation ($I\text{-}V_{ss}$) of more linear shape. On the contrary, if HP was deep (e.g. -80mV), $I\text{-}V_{in}$ obtained with depolarizing pulses was more linear than $I\text{-}V_{ss}$. These time-dependent changes in current were not observed at voltages near E_k. Thus the blocking action of Ba on g_{k1} is voltage- and time-dependent, with stronger block at more negative potentials. These characteristics of Ba action can produce "block" and "unblock" phenomena even during cardiac cycle, which may contribute to the action potential repolarization and to the pacemaker potential, in Ba-treated myocytes.

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SIGNIFICANCE OF THE AFTER-HYPERPOLARIZATION ON Ba-INDUCED PACEMAKER ACTIVITY IN MYOCARDIUM. IKEDA, K., UMENO, T. AND GOTO, M. Dept. of Physiol., Faculty of Medicine, Kyushu University. Fukuoka 812

The roles of the after-hyperpolarization on Ba-induced pacemaker activity were studied in the bullfrog atrial muscle using double sucrose-gap techniques under voltage clamped and unclamped conditions. Ba (0.1-0.3mM) induced the pacemaker activity in the presence of isoproterenol (10^{-7}M), and in these conditions the following results were obtained. When the after-hyperpolarization was more intense, 1) the deactivation of delayed outward current (I_x) was facilitated and thus steepened the slope of the diastolic slow depolarization, 2) the rate of recovery from inactivation of the fast and slow inward currents (I_{Naf}, I_{Si}) increased to be ready for inducing the next firing, 3) the fast decay of I_x accompanied the corresponding increase in the membrane resistance and electrotonic potential, and produced a supernormal period in the excitability recovery curve, and 4) a transient after-depolarization superimposed on this phase which led to the next firing.

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ISOLATION OF THE Na-K PUMP CURRENT IN SINGLE VENTRICULAR CELLS OF GUINEA PIG. KIMURA, J. and Noma,A. Nat. Inst. Physiol. Sci., Okazaki

The Na-K pump current was isolated by blocking most other membrane currents using combined techniques of intracellular dialysis and the whole cell voltage clamp. Thus when the cell was voltage-clamped with a patch pipette containing Cs-rich solution while superfusing Ca-free Tyrode solution containing 0.9 mM Ba and 1 mM Ni, the Ca and K currents were blocked. Under those conditions, changing the Na concentration in the pipette from 1 to 33 mM in the presence of 5.4 mM external K induced an outward current of about 100-200 pA. Since this current was abolished by ouabain, it was identified as the Na-K pump current. The current-voltage relation at the steady state showed a significant voltage dependence, i.e. the current progressively decreased as the potential became more negative and it reached a steady level at positive potentials. Decreasing internal Na or external K decreased the magnitude of the current and shifted the I-V curve in the positive direction.

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ATP-REGULATED K CHANNEL IN GUINEA-PIG VENTRICULAR CELLS.
SHIBASAKI, T., NOMA, A., °KAKEI, M. AND IRISAWA, H. Natl. Inst. Physiol. Sci., Okazaki 444 and °1st. Dept. Intern. Med. Kagoshima Univ. Sch. Med., Kagoshima 890.

When intracellular ATP concentration of heart cells was decreased by internal dialysis under the whole cell voltage clamp, a time-independent outward current with marked fluctuations was activated. This macroscopic outward current showed following characteristics. 1) The reversal potential was near the K equilibrium potential. 2) It appeared when the ATP concentration in the pipette solution was lowered below 2 mM. 3) Inward rectification was observed positive to +25 mV. 4) The Lorentz components were observed in the power density spectrum of the current fluctuations with corner frequencies of 1.2 $\pm$ 0.5 and 9.3 $\pm$ 2.6 Hz. 5) The amplitude of the single channel current, estimated from the fluctuations, was 0.88 $\pm$ 0.10 pA at -35 mV. These characteristics are almost equal to those of the single channel current and indicated that the ATP-regulated K channels are responsible for the increase in the macroscopic outward current under various ATP-deficient conditions.

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OPTICAL MONITORING OF ACTION POTENTIAL AND CONTRACTION FROM EARLY EMBRYONIC RAT HEART.
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Spontaneous action potential and contraction in the early embryonic heart of the rat have been monitored optically using a voltage-sensitive merocyanine-rhodanine dye together with a multiple-element photodiode matrix array, and the onset of rhythmical action potential activity in the early phases of rat cardiogenesis was conclusively determined for the first time. Spontaneous rhythmical action potentials were first generated in the central part of the embryonic heart at the middle period of the 3-somite stage of development, at 9 1/2 days after copulation. Subsequently, contractions coupled with the action potential also appeared at the end of the 3-somite stage. Usually, at the 3-somite stage, spontaneous action signals were synchronized among the different areas in the heart. From this result, it is evident that the paired right and left cardiac primordia are fused completely at the time of initiation of spontaneous electrical activity. In the 3-somite embryonic heart, excitatory waves were conducted radially over the heart, at a uniform rate (0.4-0.8mm/sec), from the pacemaking area. However, the regional priority of pacemaking activity is not rigid but is flexible. Our present findings stongly suggest that conventional ideas concerning the onset of function of the embryonic rat heart need to be corrected.

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COMPARATIVE STUDY OF THE LEFT VENTRICULAR ACTION POTENTIAL IN EMBRYONIC HEARTS OF WKY AND SHR.
MIYAZAWA, T., SATO, S., SEKIYA, S.*※, TSUTSUMI, K.*※ AND OSADA, H.*※ Dept. of Clinical Physiol. Kanagawa Prefectural College, Yokohama 241. *Div. of Cardiology, Showa Univ. Fujigaoka Hospital, Yokohama 227.

Each fifteen prenatal rats of 15, 18 and 20 days of gestation age, whose left ventricules can be distinguished, were used. The heart of embryonic rat was excised and isolated, then it was fixed the free wall of left ventricle upside in cardiac muscle chamber. The action potentials were recorded from 30 to 70 sites on the specimen with conventional microelectrode methode, and stimulated by a bipolar electrodes with rectangular pulse of 4 msec duration and 1 Hz in frequency. The resulted were as follow: There can not find any significant difference between WKY and SHR with amplitude, depolarization rate of action potentials and the resting potentials on embryonic hearts. The duration of the repolarization phases(APD 50) of 18, 20 days of gestation age showed tendency of more prolongation in SHRs than WKYs, average value of prolongation SHRs being 90.5 ± 11.0 msec, whereas that WKYs 76.2 ± 12.6 msec in 20 days of gestation age.

It indicates that there is difference between SHR and WKY already in the prenatal period on their left ventricular action potentials.

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ELECTROPHYSIOLOGICAL STUDY OF THE AFFERENT FIBRES IN THE NASAL MEMBRANE. HOSHINO,T., YOSHIZAKI,K. and KOYANO,H. Dept. of Physiol. Akita Univ. Sch. of Med. Akita 010

Extracellular recordings of the nasal afferent fibres in the rabbit were made in vitro and in situ. Mechanoreceptors in the nasal membrane were investigated by more slightly touching to confirm the conduction velocity (more than 1.75 m/sec). These mechanoreceptors were not activated in response to constant air blow (7 l/min). The afferent impulses of thermosensitive receptors in the nasal membrane were recorded by application of a cooling physiological solution to the nasal membrane. Mechano and thermosensitive receptors were located all over the nasal membrane except for ventral nasal choncha and densely localized just behind ventral nasal choncha. The fast e.p.s.p.s were also recorded intracellularly from some pterygopalatine ganglion cells in response to the same cooling stimulation of the nasal membrane. In the tracheostomized rabbit, the afferent impulses were recorded from the maxillary nerve and remarkably increased by the inflow of cool and warm air (7 l/min, 7°C-24°C, 24°C-40°C to the nasal cavity at the room temperature 24°C. From these results, it was concluded that the sensation of air flowing in the nasal cavity could be elicited mainly by thermoreceptors in the nasal membrane which might be cold and warm receptors, and that the pterygopalatine ganglion cells and the afferent thermoreceptive fibres constitute the peripheral reflex arc to control the air conditioning.

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ELECTROTONIC STRUCTURE OF OTIC AND PTERYGOPALATINE GANGLION CELLS. YOSHIZAKI,K., HOSHINO,T. AND KOYANO,H. Dept. of Physiol., Akita Univ. Sch. of Med. Akita 010

Two compartmental models of the electrotonic structure of otic and pterygopalatine ganglion cell (OGC, PGC) were constructed, based on the response of the cell to a step of current injected. These electrical structures are too simple to describe the electrotonic structure of ganglion cell which has many complicated processes. However, the models are able to predict the passive response of the cell to any wave form of injected current. If the product of the resistance and capacitance of each compartment in the model is assumed to be the same, the membrane time constant of soma can be obtained by "peeling" technique as a longest time constant, thus all elements of the compartmental model can be calculated. In the more than 150 ganglion cells tested, the exprimental curves could be fitted by a two-compartment model consisting of an isopotential soma compartment attached to a single process compartment. The dendrite-to-soma conductance ratio (ρ) in these cells was also estimated from the parameters of model, 1.74 ± 1.04 (n=90) in OGC and 1.90 ± 1.31 (n=44) in PGC. In the only two cells of OGC, the experimental curve could be fitted by a three-compartment model consisting of a soma compartment attached to two serial process-compartments. The ratio (ρ) of one of these cells was 9.54. We concluded that the majority of rabbit otic and pterygopalatine ganglion cells is electrically "compact".

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CENTRAL CONVERGENCES OF UNMYELINATED SOMATIC AFFERENT INPUTS AND BARORECEPTOR AFFERENT INPUTS ON CARDIAC SYMPATHETIC EFFERENT NEURONES. NAKAO, M., SASAKI, M., SATO, A. Dept. of Physiol., Tokyo Metropol. Inst. of Gerontol., Tokyo 173

Arterial baroreceptor afferent stimulation depresses tonic activity of a sympathetic efferent nerve (Bronk et al., 1930s) as well as sympathetic reflex discharges evoked by electrical stimulation of myelinated somatic afferent nerves (Koizumi et al., 1971). Somatically produced sympathetic reflexes can be divided into two identified discharges; one (A-reflex) with early latency evoked by myelinated afferent excitation, and the other (C-reflex) with a longer latency evoked by unmyelinated afferent excitation (Schmidt and Weller,1970). The present experiment was undertaken to determine whether arterial baroreceptor excitation can inhibit the sympathetic C-reflex as it did the A-reflex. The sympathetic reflexes were evoked in a cardiac sympathetic nerve by electrical stimulation of the superficial peroneal nerve in anesthetized, vagotomized cats. Both A and C-reflexes were inhibited by baroreceptor afferent stimulation which was produced by increases in arterial blood pressure after intravenous injection of phenylephrine. These inhibitory effect of baroreceptor stimulation on the sympathetic reflexes was abolished after bilaterally cutting the sinus nerves. These evidences indicate that excitatory somatic afferent inputs and inhibitory baroreceptor afferent inputs interact within the central nervous system.

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CHANGES IN ADRENAL SYMPATHETIC NERVE AND CATECHOLAMINE SECRETION BY MOVEMENTS OF NORMAL AND INFLAMED KNEE JOINTS. SATO, A., SATO, Y. and SCHMIDT, R.F.* Dept. of Physiol., Tokyo Metropolitan Inst. of Gerontology, Tokyo 173

In our laboratory it has been studied for the last 3 years that the sympatho-adrenal medullary function in anesthetized rats was facilitated by noxious cutaneous pinching stimulation, while it was depressed by innocuous cutaneous brushing stimulation (1). It is interesting to know how joint afferent stimulation is responsible for the sympatho-adrenal medullary function. However, there has been few report concerning an effect of joint afferent nerve stimulation on autonomic functions. Thus, the present experiment was performed to investigate influences of passive knee joint movement in both noxious and innocuous ranges of both normal and inflamed knee joints, on sympatho-adrenal medullary functions using cats anesthetized with halothane. It was found that passive various knee joint movements, especially in noxious ranges of a normal joint, and also both in noxious and innocuous ranges of an inflamed joint, produced significant reflex increases both in adrenal sympathetic efferent nerve activity and in catecholamine secretion.

Ref. 1) Araki, T. et al., Neuroscience 12(1): 289-299, 1984.

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EFFECT OF INTRACEREBROVENTRICULAR INJECTION OF CORTICOTROPIN RELEASING FACTOR ON SYMPATHO-ADRENAL MEDULLARY FUNCTION IN ANESTHETIZED RATS. KUROSAWA, M., SATO, A., SWENSON, R.S.* and TAKAHASHI, Y. Dept. of Physiol., Tokyo Metropolitan Inst. of Gerontology, Tokyo 173

Corticotropin Releasing Factor (CRF) is suggested to be involved in the regulation of sympatho-adrenal medullary system in addition to controlling ACTH-adrenal cortex system. In the present experiment, effects of intracerebroventricular (i.c.v.) administration of CRF on adrenal sympathetic efferent nerve activity and adrenal catecholamine secretion rates were investigated in halothane anesthetized rats. I.c.v. administration of CRF (0.64 and 6.4 nmoles) resulted in a dose dependent increase in adrenal sympathetic nerve activity which began several minutes after injection and reached maximum values approximately 30-60 min later. Acute hypophysectomy did not influence the response of adrenal nerve activity evoked by i.c.v. administration of CRF. Following i.c.v. administration of CRF (0.64 nmoles) epinephrine and norepinephrine secretion, as measured from adrenal venous blood samples, were increased taking a similar time course of the adrenal nerve activity.

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CHANGES OF MUSCLE SYMPATHETIC ACTIVITY IN MAN DURING HEAD-UP/DOWN TILTINGS. IWASE, S., SAITO, M., ABE, H. and MANO, T. Dept. VI, Res. Inst. Environ. Med., Nagoya Univ., Nagoya 464.

Post-ganglionic sympathetic muscle activity (MSA) to the calf was recorded from the tibial nerve using a tungsten microelectrode in 5 healthy men of 19 to 27 years old during head-up and head-down tiltings. The subject was laid on a tilting bed, being tilted from -18° (head-down) to 70° (head-up). The data were analyzed by a data processing computer (Nihon-Koden ATAC-450). Post-stimulus time histograms of MSA discharges in each bursts triggered by ECG R-wave were analyzed to determine the discharge number and the latency of the R-wave locked MSA. The discharge number increased as the tilting gradient became large, establishing a significant positive correlation between the body axis component of gravity. The latency from R-wave to the peak of R-wave locked histogram ranged between 1.0 to 1.3 sec. These latencies did not change significantly during head-up/down tiltings. The maximum discharge frequency, as well as the full-wave rectified and digitally integrated value of each MSA bursts increased with the rise of head-up tilting gradient. It is concluded that head-up tilting causes not only increase of burst frequency as reported before, but also increase of frequency and amplitude of discharges in each bursts, while not modifying the reflex latency of the MSA.

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SUPPRESSION OF MUSCLE SYMPATHETIC ACTIVITY IN MAN UNDER WEIGHTLESSNESS SIMULATED BY WATER IMMERSION. MANO, T., IWASE, S., SAITO, M., YAMAZAKI, Y., KOHNO, M. and ABE, H. Dept. VI, Res. Inst. Environ. Med., Nagoya Univ., Nagoya 464.

Muscle sympathetic activity (MSA) to the calf was recorded using a tungsten microelectrode inserted into the tibial nerve in 4 healthy males under weightlessness simulated by water immersion, simultaneously with leg volume (LV), heart rate (HR), blood pressure (BP), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR). In the course of water immersion up to the neck of the standing subject, the MSA was gradually reduced with the rise of immersion level, accompanying the reduction of LV, HR and TPR, as well as the increase of SV and CO, while not accompanying the change of systolic and diastolic BP. Under these conditions, significant positive correlations were observed between MSA bursts per minute (burst rate) and LV ($p<0.001$) /HR ($p<0.001$)/TPR ($p<0.01$), while significant negative correlations were observed between the burst rate and SV/CO ($p<0.001$). No significant correlation was observed between the burst rate and systolic/diastolic BP. Based on these findings, it is concluded that the suppression of MSA in man under weightlessness simulated by water immersion would be caused by activation of low pressure volume receptors due to upward fluid shift in water, rather than high pressure baroreceptors activation.

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FIBERS IN THE ANASTOMOTIC BRANCHES CONNECTING THE BILATERAL PELVIC PLEXUS OF THE DOG. FUKAI, K. AND FUKUDA, H. Dept. of Physiol., Kawasaki Med. Sch., Kurashiki 701-01

The bilateral pelvic rectal plexus are connected by a few anastomotic branches. We aimed in this study to elucidate the types of fibers in the branches. Compound action potentials of the anastomotic branch in responses to the sacral root stimulation consisted of potentials of Aδ and C afferent fibers, and parasympathetic postganglionic C fibers and a small number of the preganglionic B fibers. Only C fiber peaks were obvious in responses of rectal strands originating from the ganglia when the contralateral sacral ventral roots were stimulated. The C fiber peaks disappeared after C6 was applied to the stimulating side of the ganglia, but were not affected by C6 applied to the recording side. These results show that most preganglionic B fibers synapse with the postganglionic cells in the homolateral pelvic plexus although some of them travel through the anastomotic branches and terminate on the contralateral ganglia. The postganglionic fibers which travel through the anastomotic branches do not synapse with any cells in the contralateral ganglia. All the postganglionic C fibers synapsed again with cells in the myenteric plexus.

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THE INFLUENCE OF HYPOTHALAMIC NEURONS ON THE CERVICAL VAGAL AND SYMPATHETIC ACTIVITY ON THE THERMOREGULATORY RESPONSE. MIYAMOTO,J.K., FUJITA,Y., MANO,H., SANADA,M., TANEMURA, S. and HIRAMA,Y. Dept. of Physiol., Saitama Kenritsu Eiseitandai, Urawa, 338

Changes in efferent discharges of cervical vagal and sympathetic nerves produced by increasing the body temperature and by injection of prostaglandin D_2 (PGD_2), adrenaline and phenoxybenzamine(αblocker) into the hypothalamus were studied in N_2O 65%, O_2 35% anesthetized and vagotomized rabbits. The increase of the body temperature from 35°C to 40°C produced a sustained increase of the cervical vagal and sympathetic efferent nerve discharges. In decerabrated animals a substantial decrease of these responses was seen. This indicates that the bulbo-pontine cardiorespiratory neurons are influenced by a supracollicular acceleratory mechanism probably mediated by the hypothalamus. PGD_2 and adrenaline injected into the hypothalamus produced invariably a sustained increase of the cervical vagal efferent discharges while the responses of the cervical sympathetic nerves were inconsistent. Phenoxybenzamine injected into the hypothalamus decreased or suppressed the vagal background discharges while the sympathetic efferent discharges increased with concomitant rise of arterial blood pressure. After the injection of αblocker the responses induced by PGD_2 and adrenaline were no longer seen. Thus, the acceleratory neuronal pathway of the vagi nerves into the diencephalon is mediated by adrenergic neurons and the action of PGD_2 seems to be exerted through such adrenergic neurons.

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UPTAKE OF [3H]DEOXYGLUCOSE, [3H]LEUCINE AND [3H]DOPAMINE IN ADRENAL CHROMAFFIN CELLS OF MICE. HIRANO, T., FUKUDA, A. AND NIIJIMA, A. DEPT. PHYSIOL., NIIGATA UNIV. SCH. MED., NIIGATA 951

The uptake and distribution of [3H]deoxyglucose, [3H]leucine and [3H]dopamine in adrenal medullary chromaffin cells was investigated by autoradiography.

Radioactive substances were injected intraperitoneally and the animals were fixed at 30 min after [3H]leucine and [3H]dopamine injection and at 45 min after [3H]deoxyglucose injection.

[3H]dopamine was taken up in chromaffin cells especially those situated in subcortical zones. Uptake in cortical cells was almost background level. There was no such regional difference in the uptake of [3H]leucine in the medulla. Cortical uptake was remarkably higher than medullary uptake. No regional difference in the uptake of [3H]deoxyglucose in the medulla was observed either. Neuronal cells in the medulla took up remarkably higher radioactivity than chromaffin cells. Cortical uptake of deoxyglucose was slightly lower than the uptake in chromaffin cells.

It is supposed that regional difference in the uptake of [3H]dopamine was not brought about by regional difference in glucose consumption in the medulla. It looks likely that medullary chromaffin cells ~~are~~ not generat~~ing~~ action potentials in vivo as neuronal cells in the medulla do.

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THE EFFECT OF HYPERTONIC NaCl AND SUCROSE SOLUTION ON THE EFFERENT RENAL NERVE ACTIVITY, IN THE NORMAL AND DECEREBRATED RAT. NIIJIMA, A., FUKUDA, A., YAMAGUCHI, K., Department of physiology, Niigata University School of Medicine, Niigata City, 951 Japan.

Intravenous administration of 2M NaCl or sucrose solution (0.2 ml) caused a decrease in discharge rate of the efferent nerve activity of the renal nerve filament in the normal rat as well as decerebrated and subdiaphragmatically vagotomized rat. However, the spinal transected rat showed no response following intravenous administration of 2M NaCl or sucrose solution.

After the lesion of the area postrema by suction method in the decerebrated and vagotomized rat, no change in efferent activity in the renal nerve activity was observed after administration of 2M NaCl or sucrose solution in the same situation.

These observations suggest that the nucleus tractus solitarius in the medulla oblongata, which has close connection with the area postrema, plays a role for monitoring concentration of NaCl and non-electrolyte substances in the plasma in the regulation of renal nerve activity.

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RELATION BETWEEN THE CHANGES IN ION CONCENTRATIONS OF PAROTID SALIVA AND THE FLUCTUATION OF PAROTID SECRETORY POTENTIAL INDUCED BY TONGUE STIMULATION. INOMATA, K., TAKAKUWA, M. AND KURAHASHI, M*. Dept. of Physiol. and Oral Physiol*., Sch. of Dent., Higashi-Nippon-Gakuen Univ., Ishikari-Tobetsu, Hokkaido 061-02

It is well known that tongue stimulation induces the fluctuation of parotid secretory potential. In previous meetings, we reported the facts as follows; When the same experiments were repeated several times in one subject, the time course of secretory potential did not always show the same patterns in spite of the similar salivary volumes (2.5-3.0 ml) in each experiment. We summed up the concentrations of each ion obtained at same time, and named this summation as the total charges. The time course of total charges was closely related to that of secretory potential. In this study, we used new equipments for measuring pH, Pco_2 and temperature. Therefore, it was possible to measure the total charges and secretory potential in unit time smaller than in previous study. The results obtained suggested again that the time course of secretory potential is closely related to that of total charges.

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EFFECTS OF CAERULEIN ON THE GASTRIC MOTILITY OF RATS. NEYA, T., JIN, Z. and NAKAYAMA, S., Dept. of Physiol. Okayama Univ. Med. Sch., Okayama 700

The effects of caerulein on the gastric motility of urethane-anesthetized rats were studied. Caerulein administered into lateral cerebral ventricle(ICV) or jugular vein(IV) caused predominantly an inhibition of the gastric motility but sometimes an excitatory or a biphasic response. An inhibitory response reduced after vagotomy and/or splanchnicotomy, or after guanethidine. The remaining response was abolished by tetrodotoxin but resistant to atropine and guanethidine. An excitatory response was abolished by atropine. Discharges of the gastric branch of vagus nerve were decreased by IV injection of caerulein but increased by ICV, whereas those of splanchnic nerve were increased by IV and ICV injection. These results suggest that caerulein caused an inhibition of gastric motility by centrally stimulating vagal non-adrenergic inhibitory nerves and splanchnic adrenergic nerves and inhibiting vagal cholinergic nerves, and by peripherally stimulating non-adrenergic inhibitory neurons of the myenteric plexus, while this peptide caused an excitation by stimulating cholinergic neurons of the myenteric plexus.

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PARTICIPATION OF ENKEPHALIN IN THE CONTROL OF PERISTALSIS.

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It was shown that the prominent processes of enkephalin-neuron provide fibers to the circular muscle. Experiments were made to clarify the physiological role of enkephalin using the modified Trendelenburg method in the quinea-pig small intestine. Effects of methionine-enkephalin (M-ENK), naloxone and substance P (SP) to the peristaltic movement produced by raising intraluminal pressure (1.0-5.0cm H_2O) were observed. The duration of peristalsis under the sustained pressure (5cm H_2O) was elongated by aplication of naloxone (10^{-7}Mol) in all preparations. The ratio of elongation varied 1.5+0.1 times (n=8, duodenum) to 26.0+7.7 times (n=19, ileum). M-ENK (10^{-7}Mol) inhibited the occurence or duration of peristalis. SP (5×10^{-8}Mol) lowered the threshold level of peristalsis and recovered the inhibitory action of M-ENK. It was suggested that the peristalsis in vivo may be inhibited by enkephalin due to suppression of SP release.

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EFFECT OF GASTRIN ON EXCITATORY RESPONSE OF GASTRIC MOVEMENT INDUCED BY STIMULATION OF CHOLINERGIC NERVE IN CONSCIOUS DOGS. FUJII, K. AND MUKAI, M., Dept. of Physiol., Hiroshima Univ. sch. Med., Hiroshima 734

Effect of gastrin on gastric motor excitatory response induced by stimulation of cholinergic nerve was studied in conscious dogs. (1) The excitatory response of gastric movement and increase of serum gastrin level induced by intravenous injection of 2-DG (100 mg/kg) and insulin (0.5 u/kg)consisted of a neuro-humoral response which was abolishable by d-chlorpheniramine maleate (d-CPM, 2.5 mg/kg, s.c.) and neural response which was abolishable by atropine (0.05 mg/kg, i.v.). The 2-DG- and insulin-induced increase of serum gastrin level was unaffected by d-CPM. (2) The gastric excitatory response induced by pentagastrin (2.0 μg/kg, s.c.) consisted of a humoral response which was abolishable by d-CPM and neural response which was not. The motor response which was not affected by d-CPM was abolished by atropine.

From these results, we speculated that gastrin released by 2-DG and insulin stimulates the cholinergic nerve elements in the intraluminal plexus and releases acetylcholine (Vizi et al., 1973), and stimulates the histamine secreting cells in the gastric wall and releases histamine, and consequentry, activity of the gastric smooth muscle was increased by released acetylcholin and histamine.

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RESPONSES TO NICOTINIC DRUGS OF THE HAMSTER SUBMANDIBULAR GANGLION CELL AFTER CHRONIC PREGANGLIONIC DENERVATION. SUZUKI, T. Dept. of Physiol., Tokyo Dent. Coll., Chiba 260

The effect of chronic preganglionic denervation on the nicotinic response of the hamster submandibular ganglion cell was investigated with intracellular microelectrode techniques in vitro. The nicotinic depolarizations of the carbachol (CCh) and dimethylphenylpiperazinium (DMPP) did not change after denervation. Denervation also did not change their threshold concentration and the concentration producing the maximal response. Furthermore, the dissociation constant of the drug-receptor complex, extrapolated as a agonist concentration producing 50% response in the experimental dose-relative effect curve was not altered after denervation. The numbers of CCh and DMPP molecules attached to a single nicotinic receptor were found out to be 1 and 2, respectively, and remained unchanged after denervation. Therefore, there seems to be a cooperative effect in the physico-chemical interaction between the receptor and DMPP. Thus, the effect of denervation on the nicotinic response of this parasympathetic ganglion cell was not demonstrated.

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EFFECTS OF N-HEPTYL ALCOHOL ON INTRACELLULAR CALCIUM ION ACTIONS IN CULTURED AUERBACH'S PLEXUS CELLS. MARUYAMA, T. Dept. of Applied Physiol., Tohoku Univ. Sch. of Med., Sendai.

The cultured Auerbach's plexus cells which were derived from the small intestine were studied in this experiment. The cells prolifereated well the cultured system. During the culture, the Auerbach's plexus cells connected with each other, some by their processes and the other by their cell bodies. The cells are coupled by low resistance channels that permit the cell-to-cell passage of the electrical current and florescein dye. N-heptyl alcohol not only blocked the cell-to-cell communication properties but also induced the membrane hyperpolarization in the cultured Auerbach's plexus cells. The hyperpolarizing responses induced by n-heptyl alcohol were blocked by the application of K^+ channel blockers TEA, Ca^{2+}-activated K^+ channel inhibitor of quinine and Ca^{2+} channels blocker of Co^{2+} and verapamil, but were not suppressed by caffeine.

These results suggests that the elevated intracellular calcium ions induced by n-heptyl alcohol regulates the cell-to-cell channels or activates Ca^{2+}-dependent K^+ channels in the cultured Auerbach's plexus cells.

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SEROTONIN RECEPTOR IN AUERBACH'S PLEXUS AND ITS ANTAGONIST. TAKAKI,M.,BRANCHEK,T.* & GERSHON,M.D.* Dept.Physiol.,Okayama Univ.,Med.Sch.,Okayama 700. *Dept.Anat.& Cell Biol.,College of P & S.,Columbia Univ.,New York,N.Y.10032,U.S.A.

3H-5-HT was used as a ligand and binding was assessed by filtration and radioautographic assays. One specific binding site of 3H-5-HT($Kd \cong 2nM$) is found in the ganglia of the Auerbach's plexus of the small intestine. The binding of 3H-5-HT to this enteric site is not antagonized by antagonists of $5\text{-}HT_{1a}$- or $5\text{-}HT_2$-CNS receptors. In contrast, a dipeptide of 5-hydroxytryptophan, N-acetyl-5-hydroxytryptophyl-5-hydroxytryptophan amide(5-HTP-DP) blocks binding of 3H-5-HT to the enteric site($ID_{50} \cong$ 1.2μM). We also evaluated effects of 5-HTP-DP on 5-HT responses and on slow and fast excitatory post synaptic potentials(EPSPs) evoked by stimulating interganglionic fiber tracts of myenteric neurons. 5-HT induced a long-lasting depolarization(29.0±2.6sec) of type II/AH neurons, similar to the slow EPSPs in these cells. 5-HTP-DP(1-20μM) blocked slow EPSPs in, and the response of, type 11/AH cells to 5-HT. In contrast, 5-HTP-DP did not block the effects of acetylcholine or substance P on type 11/AH neurons or fast EPSPs in type 1/S cells.

It is concluded that enteric 3H-5-HT binding sites are enteric neural 5-HT receptors, that 5-HTP-DP is a specific antagonist at these receptors, and that 5-HT is one of the mediators of slow EPSPs in the Auerbach's plexus.

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THE ACTION OF 5-HT ON SINGLE NEURONS OF THE RABBIT CILIARY GANGLION. TATSUMI,H., KATAYAMA,Y. & MORITA,K. Department of Autonomic Physiology, Medical Research Institute, Tokyo Medical and Dental University, Chiyodaku, Tokyo 101

Intracellular recordings were made *in vitro* from ciliary ganglion neurons. 5-HT (30μM) usually produced a transient depolarization (10-20mV) associated with a marked fall in input resistance. The amplitude of the 5-HT induced response was increased with the consentration of 5-HT (ranging from 1 to 100μM). In some cells, 5-HT induced response was followed by a small hyperpolarization. A depolarization was also evoked by ionophoresis of 5-HT in cells which produced 5-HT induced responses. The 5-HT induced responce progressively reduced in amplitude at intervals less than 10s. This tachyphylaxis was not apparent at interval more than 30s. The 5-HT induced depolarization persisted in calcium free / magnesium or TTX containing solutions. The peak amplitude of the 5-HT induced response was decreased by menbrane depolarization, and it reversed in polarity at about -10mV. The 5-HT induced response reversibly abolished in sodium free solution. (+)-tubocurarine and picrotoxin bloked the 5-HT response, but methysergide did not block it. These results indicate that 5-HT receptors may be endowed with the soma of the ciliary ganglion neuron. Futhermore, these suggest that the 5-HT induced response may be due to increase in membrane sodium and presumably potassium conductances.

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CAROTID SINUS NERVE REFLEX: REEXAMINATION OF NUCLEUS TRACTUS SOLITARII AS THE CENTER OF CIRCULATION AND RESPIRATION. MIURA, M., TAKAYAMA, K. Dept. of Physiol., 1st Div., Gunma Univ. Sch. Med., Maebashi 371

Using double-barrel electrode, we surveyed field potentials evoked by electrical stimulation of the afferent A delta fibers of the ipsilateral carotid sinus nerve (CSN) throughout the nucleus tractus solitarii of the cat (NTS response), and examined the ESB effects of the site of the NTS responses on circulation and respiration, and the effect of naloxone and substance P (SP) antagonist [D-Pro^2, D-Trp^{7,9}, Leu^{11}-SP] on the NTS responses. Since the NTS responses evoked in the rostral and commissure regions were inhibited by naloxone but not by SP antagonist and the ESB effect of the site of the NTS responses are consistent with the CSN chemoreceptor reflex response, opioid peptides may act as a neurotransmitter on terminals of the CSN chemoreceptor A delta fibers. Since the NTS responses evoked in the medial portions of the intermediate regions were inhibited by SP antagonist but not by naloxone and the ESB effect of the site of the NTS resoonses are consistent with the CSN baroreceptor reflex response, SP may act as a neurotransmitter on the terminals of the CSN baroreceptor A delta fibers.

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SITES OF ACTION FOR BRAIN-GUT PEPTIDES IN CULTURED NEURONS OF RAT BRAIN STEM. OKADA, J. AND MIURA, M. Dept. Physiol. 1st Div., Gunma Univ. Sch. Med., Maebashi 371

Six types of cultured neurons (small-sized oval, large-sized oval, fusiform, pyramidal, polygonal and multipolar) from the lower brain stem of 14- to 15-day old rat were perfused alternately with the normal medium and synaptic free medium. The neuronal activities of cultured neurons were facilitated, inhibited or unchanged following administration of representative 8 brain-gut peptides, as bombesin, CCK-4, CCK-8, motilin, neurotensin, secretin, somatostatin and VIP. Considering changes in these responses following replacement of the normal medium with the synaptic free medium, brain-gut peptides may be classified into 4 groups. Active responses to VIP, secretin and CCK-4 were unchanged in the synaptic free medium, indicating this group of peptides acts postsynaptically. Active responses to motilin and neurotensin were abolished in the synaptic free medium, indicating this group of peptides acts presynaptically. Active responses to somatostatin and bombesin were partly abolished and partly unchanged in the synaptic free medium, indicating this group of peptides acts either presynaptically or postsynaptically. The fourth group was CCK-8 to which all of the test neurons were insensitive.

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PROJECTION TO PARABRACHIAL NUCLEUS OF NEURONS WITHIN SOLITARY TRACT NUCLEUS ACTIVATED BY HEPATIC VAGAL AFFERENTS IN RATS. KOBASHI, M. AND ADACHI, A. Dept. of Physiol., Okayama Univ. Dent. Sch., Okayama 700

One hundred and ten neurons which were activated antidromically by electrical stimulation of the ipsilateral parabrachial nucleus (PB) were identified in the nucleus of the solitary tract (NTS) of the rats. Antidromic latencies varied from 17 msec to 50 msec. Since the distance from the stimulating site and recording site was roughly estimated at 7 mm, conduction velosity of mode value was 0.24 m/s. In these antidromically activated neurons, 25 neurons were excited and 17 neurons were inhibited in response to electrical stimulation of the hepatic branch of the vagus nerve. As result of the portal infusion of hypertonic saline about these hepatically driven neurons, 3 neurons increased their firing rates and 5 neurons diminished.

It was confirmed that the information related to the osmotic pressure from the hepatic vagal afferents projects to the PB through the NTS.

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DISCHARGE PATTERN CHANGES OF SOLITARIUS NUCLEUS NEURONS INDUCED BY TRIGEMINAL STIMULATION. TAKAGI, T., KAJIWARA, K., MATSUO, T. and SUGAYA, E. Dept. of Physiol., Kanagawa Dental College, Inaoka-cho, Yokosuka

The Reilly syndrome is considered to be experimentally or clinically induced disturbances in different organs mainly consisting of microcirculatory disorders due to the irritation of the autonomic nervous system, either in the peripheral or central portions. In cases of irritation of the somatic nerves in a specific area such as the oral cavity and pharynx where the sensory fibers are abundant, the same type of pathological changes as the Reilly syndrome occur. To elucidate the mechanism of the Reilly syndrome in oral medicine, the functional relationship between trigeminal input and the function of the solitarius nucleus neurons was examined. Cats of both sexes were used. The discharge pattern changes of nucleus solitarius neurons were examined when the infraorbital and mandibular nerves were stimulated. The trigeminal nerve input had three types of effects on the solitarius nucleus neurons, i.e., excitatory, inhibitory and indifferent. The distribution of neurons of these three types was random.

The above findings suggest that the trigeminal stimulation has an influence on the vagus nucleus neurons, and this may be one of the causes of the Reilly syndrome in oral medicine.

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APOMORPHINE SENSITIVE NEURONS IN THE NUCLEUS SOLITARIUS. IIZUKA, S., ISHIGE, A., SEKIGUCHI, K., ABURADA, M., HOSOYA, E. and SUGAYA, E.* Tsumura Research Institute for Pharmacology, Ami, Ibaraki and *Dept. of Physiol., Kanagawa Dental College, Inaoka-cho, Yokosuka

The solitary nucleus of the vagus nerve has been considered as a main vomiting center. However, studies concerning the vomiting center have not always obtained the same conclusion. In particular, stimulation of the solitary nucleus has not always been found to provoke vomiting. To clarify the effect of apomorphine on the nucleus solitarius neurons, the changes in discharge patterns of solitarus nucleus neurons by administration of apomorphine were examined. Cats were used. The microlelectrode position was identified by the fast green FCF. Unit discharge patterns were analysed with a ATAC-450 computer. Three types of neurons were found in the nucleus solitarius from the viewpoint of apomorphine sensitivity, i.e., excitatory neurons, inhibitory neurons and indifferent neurons. The distribution of these three types of neurons was random in the nucleus solitarius.

The above findings indicate that stimulation of the solitarius nucleus, a vomiting center, does not constantly evoke vomiting.

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DECREASES IN BLOOD PRESSURE IN RESPONSE TO L-GLUTAMATE MICROINJECTIONS INTO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS IN RATS. KANNAN, H., OSAKA, T., KASAI, M. and YAMASHITA, H. Dept. of Physiol., Univ. of Occup. Environ. Health Med., Kitakyushu 807

Using electrical stimulation it has been reported that the paraventricular nucleus (PVN) region of the hypothalamus has pressor effects on blood pressure. However, electrical stimulation excites axons of passage as well as cell bodies. To activate PVN cells more selectively, we used L-glutamate for chemical stimulation. In rats anesthetized with urethane-chloralose, focal electrical stimulation (10-25 μA, 50 Hz, 0.5 ms) within the PVN, where large antidromic field potentials by pituitary stalk stimulation could be recorded with the same electrode, produced a decrease in blood pressure. However stimulation with high frequency (100 Hz) and high intensity (50-100 μA) produced a transient increase followed by a decrease in blood pressure. Microinjection of L-glutamate (0.5 M, 40-80 nl) consistently led to a decrease in blood pressure, while the same amount of saline injection had no effect. The depressor response was not affected by cervical vagotomy but markedly reduced after intravenously administration of hexamethonium (4 mg/Kg). The present results suggest that activation of PVN neurons leads to a decrease in blood pressure brought about mainly through the sympathetic nervous system.

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ELECTROPHYSIOLOGICAL PROPERTIES OF GUINEA-PIG VENTROMEDIAL HYPOTHALAMIC NEURONS AND THEIR CHEMOSENSITIVITIES: AN IN VITRO STUDY. MINAMI, T., SUGIMORI, M., OOMURA, Y., MIZUNO, Y. AND NABEKURA, J. Dept. Physiol., Fac. Med., Kyushu Univ. 60, Fukuoka 812

Intracellular recordings were made from 103 ventromedial hypothalamic (VMH) neurons in slice preparations. Three types of neurons were identified, based on their electrophysiological and morphological characteristics. Type A cells were charactirized by a small after-hyperpolarization (AHP), a short time constant, high firing rate on intracellular current injection and an EPSP on local stimulation at the lateral edge of the nucleus. Type B showed a large AHP, a long time constant, high frequency firing on current injection and an EPSP accompanied with a hump. Type C exhibited a large AHP, low firing on current injection and an EPSP-IPSP response on local stimulation. Particularly, Type B showed the low-threshold spike which was abolished by Ca^{2+} free solution. Type C exhibited I_A current which was blocked by 4-AP. The electrophysiological charactiristics of these 3 types can be attributed to their specific ionic currents. In morphological studies, Type A were charactirized by two main dendrites, Type B with three dendrites, while Type C showed more than four dendrites and a large soma. With regard to the chemosensitivity, among 14 cells examined only 3 cells of Type C neurons responded by depolarization (10.0 ± 2.6 mV) with high glucose solutions (30 mM) associated with an increase in the input membrane resistance (25.8 ± 11.2 %). The mean reversal potential was -89 ± 2 mV in I-V plots. This responses can be attributed to the decrease in K^+ conductance. Also, bath application of mazindol, an anorexic agent, caused depolarization by 8.0 ± 3.4 mV with an increase in the input membrane resistance (23.0 ± 5.8 %) among 20 % of cells examined.

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EFFECT OF HYPOTHALAMIC STIMULATION ON THE NEURONAL ACTIVITY OF DORSAL VAGAL COMPLEX. NISHIMURA, H., INOKUCHI, A., MIZUNO, Y., MINAMI, T., SHIMIZU, N. AND OOMURA, Y. Dept. Physiol., Fac. Med., Kyushu Univ. 60, Fukuoka 812

The effects of hypothalamic stimulation on the neurons of the dorsal vagal complex (DVC), such as nucleus tractus solitarius (NTS) and dorsal motor nucleus of vagus (DMV), was investigated in urethane anesthetized rats. DVC neurons were identified by their characteristic response to subdiaphragmatic vagus stimulation with the mean latency of 88.3 msec, indicating that the axons of the DVC neurons are unmyelinated. Intracellular recording from DMV neurons revealed EPSP and IPSP that preceeded the antidromic response. One hundred and sixty identified DVC neurons were examined extracellularly for their response to ventromedial hypothalamic nucleus (VMH) and lateral hypothalamic area (LHA) stimulation. The number of spontaneously firing DVC neurons that responded with LHA stimulation was higher (76%) than that of VMH (63%). The spontaneously silent neurons responded much better for LHA stimulation as compared to VMH (VMH: 12%, LHA: 26%). The mean latency of response for VMH stimulation was longer (39.3 msec) than for LHA (33.4 msec). Majority of the spontaneously firing (87%) and some of the silent (38%) DVC neurons also responded to paraventricular nucleus (PVN) stimulation with mean latency of 34.6 msec. The pattern of response of DVC neurons to hypothalamic stimulation was mainly excitation and excitation - inhibition while in few cases inhibition was observed. There was no significant correlation between the pattern of responses and the site of hypothalamic stimulation or the type of DVC neurons. Intracellular recording revealed monosynaptic EPSP and EPSP-IPSP in DMV neurons on stimulation of VMH, LHA and PVN respectively. However, our study has not revealed any significant inhibitory pathway from VMH to DMV neurons, which was postulated from the behavioral and hormonal studies. Our electrophysiological studies indicated dense innervation of DVC neurons by PVN and LHA as compared to VMH.

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CALCIUM COMPONENT IN THE ACTIVE RESPONSE OF RABBIT OTIC GANGLION CELLS.
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Action potential recorded intracellularly from otic ganglion cells consisted mainly in spike, afterhyperpolarization (AH, about 400 msec duration) and long lasting hyperpolarization (LLH, 2-10 sec duration). Ionic basis of these active response was examined by extracellular application of TTX (50 nM), 4-aminopyridine (4-AP, 3 mM), low Ca^{++} (<0.1 mM and 2.0 mM Mg^{++}), TEA (10 mM) and Co^{++} (10 mM) and iontophoretic injections of Ca^{++} and EGTA. The spike was blocked by TTX and was prolonged by TEA. Both AH and LLH did not change significantly by 4-AP and TEA while both were blocked by Co^{++} and TEA + Co^{++}. Low Ca^{++} reduced duration and amplitude of the AH and blocked the LLH. Intracellular injection of Ca^{++} brought about hyperpolarization with a same duration of the LLH in a cell which has LLH primarily. Intracellular EGTA reduced duration and amplitude of the AH and blocked the LLH. These results suggest that (1) the spike is mostly caused by increase of g_{Na}, (2) the AH by extremely slight increase of voltage-dependent g_K and mainly by increase of a fast Ca^{++}-induced g_K and (3) the LLH by increase of a slow Ca^{++}-induced g_K presumably resulting from Ca^{++}-induced Ca^{++}-release in a cell.

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ALTERATIONS IN GASTRIC ACID SECRETION FOLLOWING HEPATIC VAGOTOMY AT A STAGE OF DEVELOPMENT IN RATS.
SAKAGUCHI, T., OHTAKE, M. and NIIJIMA, A. Dept. Physiol., Niigata Univ. Sch. Med., Niigata 951

Previous studies have revealed that the hepatic mechanism receptive to glucose in the portal blood can modulate gastric acid secretion in a rat. In the present study, the relation of acid response by hepatic vagus to developmental stages of a rat was examined. Animals were classified into five categories according to the body weights at the experiments (50, 100, 200, 300 and 400g). In all of the categories, it was observed that glucose injected into the portal vein reduced the acid output associated with insulin-hypoglycemia. However, hepatic vagotomy produced different effects for the five categories; the vagotomy failed to cause acid response when the rats were weighed about 300 and 400g, while in the rats weighing about 50, 100 and 200g acid outputs were elevated following the vagotomy. Results suggest that functioning of the hepatic vagus varied according to the developmental stages of animals.

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Ca^{++}- ACTIVATED K^+-CURRENT IN THE MYENTERIC PLEXUS OF THE GUINEA-PIG ILEUM.
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Intracellular recordings were made in vitro from voltage-clamped myenteric neurones (type 2) of the guinea-pig ileum. Slow outward current (Iout), which followed a depolarizing command pulse, was observed associated with an increase in membrane concuctance. Iout was decreased in amplitude by changing the holding potential to more negative and it reversed the polarity near potassium equilibrium potential (- 90 mV). The reversal potential of Iout was dependent on the potassium concentration of the external solution. Iout was reversibly abolished in calcium-free/high magnesium solution. Calcium blockers as manganese, cobalt and nickele also markedly depressed or eliminated Iout. Iout was often augmented by TEA (1 - 10 mM); but it was blocked by barium (1 - 3 mM). Substance P and serotonin caused a slowly developing inward current responses accompanied by a reduction of membrane conductance, and an inhibition of Iout. On the other hand, ATP enhanced Iout and produced a slowly developing outward current response accompanied by an increased membrane conductance. These actions of biogenic substances were markedly reduced or disappeared in calcium-free/high magnesium solution. It is concluded that the slow outward current (Iout) is due to an activation of calcium-activated potassium conductance and regulated by biogenic substances.

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ACTIONS OF LUTEINIZING HORMONE-RELEASING HORMONE IN THE MYENTERIC PLEXUS OF THE GUINEA-PIG JEJUNUM. KATAYAMA,Y., MORITA,K. & TATSUMI,H. Department of Autonomic Physiology, Medical Research Institute, Tokyo Medical and Dental University, Chiyodaku, Tokyo 101

Intracellular recordings were made *in vitro* from myenteric neurons of the guinea-pig jejunum. Luteinizing hormone-releasing hormone (LHRH) applied with superfusion (3 nM - 1 μM) induced concentration-dependent membrane hyperpolarizations in about 30 % of the neurons examined. The LHRH-induced hyperpolarization was associated with a decrease in membrane resistance, reversed in polarity at about -90 mV and reduced in amplitude in high K^+ solution, suggesting that the hyperpolarization may be due to an increase in K^+ conductance. The LHRH-induced potential was maintained in TTX containing solution, whereas it was reversibly depressed in Ca^{++} free/high Mg^{++} solution or Co^{++} containing solution. Therefore, Ca^{++} dependent processes might be involved in the LHRH-induced hyperpolarization of the myenteric neurons. Furthermore, slow EPSPs which were evoked by repetitive focal stimulation were reversibly inhibited by LHRH. After 5 to 10 min from the withdrawal of the peptide from superfusing solution, however, the slow EPSP was often augmented and afterwards returned to control size in 30 min. It should be added that the substance P-induced depolarization was not significantly altered by LHRH within the concentration range tested.

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COMPARISON OF THE EFFECT OF OPIOID PEPTIDES ON MICTURITION REFLEXES AND ANALGESIA. HISAMITSU, T. and OKADA, M. Dept. of Physiol., Sch. of Med., Showa Univ., Tokyo 142

The effects of intrathecal administration (i.t.) of μ-agonist morphine (MOR), δ-agonist DADL and κ-agonist dynorphin-B (DYN) on micturition reflexes and analgesia were investigated. Effects on nociceptive thresholds were observed using the vocalization test. Intrathecal MOR (4 to 8μg) and DADL (1 to 2μg) inhibited the micturition reflexes for 20 to 60min., whereas DYN (i.t.) did not depress the micturition reflexes up to the doses of 48μg (30nM). Inhibitory effects of MOR and DADL were reversed by naloxone (NAL, 100μg i.t. and 1mg/Kg i.v. respectively). In contrast, all three drugs produced analgesic effects. Intrathecal DYN (24μg) induced the elevation of pain thresholds which lasted for 45min. This analgesic effect was blocked by NAL (20mg/Kg i.p.). MOR (4μg i.t.) produced 60min. lasted analgesia but it was completely antagonized by NAL (100μg/Kg i.p.). DADL (2 to 10μg i.t.) produced analgesia but the pretreatment of NAL (1mg/Kg i.p.) blocked it. These results suggest that μ- and δ-receptors mediate the depressive roles both on micturition reflexes and nociceptive responses at the spinal site, in contrast, κ-receptor has a role on analgesia but not on micturition inhibitory system. Paraplegia was seen in 30% of DYN treated animals and it remained after the termination of the analgesic effect. NAL (20mg/Kg i.p.) did not change the paraplegia. This suggests that the paraplegia induced by DYN was not mediated by opiate receptors.

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EFFECTS OF AGE ON ULTRASONIC PLETHYSMOGRAM.
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Ultrasonic plethysmograms (UPG), carotid artery pulses (CAP), digital plethysmograms (DPG), and phonocardiograms (PCG) were recorded simultaneously on the men from 20 and 60 years of age. The UPG is a wave of the pulse of the cerebral arteries. In order to examine the arterial elasticity, the pulse conducting time of the peripheral arteries (IIa -C) was determined from the interval between the IIa of PCG and the incisura of DPG, and that of the cerebral arteries (C'-C) from the interval between the two incisura of CAP and UPG. The IIa -C did not alter from 20 to 40 years of age but shorten rapidly from 40 to 60 years of age. The C'-C shortened in proportion to the age. These results show that the peripheral arteries stiffen little from 20 to 40 years of age but rapidly from 40 to 60 years of ages, whereas that the cerebral arteries stiffen with advancing years. Blood pressure correlated roughly with the IIa -C but little with the C'-C. Thus there were several men who have the short C'-C, namely, the stiff cerebral-arteries, inspite of the low blood pressure. This result showes that the C'-C is a better indication than the blood pressure to examine the arteriosclerosis in the cerebrum.

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INTRACRANIAL PRESSURE AND BRAINSTEM ACTIVITY.
Matsuura,S., Kuno,M., Yasunami,T. Dept. Physiol., Osaka City Univ. Med. Sch., Abeno-ku, Osaka 545

Activation of the brainstem was observed to produce not only a change in blood pressure but also an increase or a decrease in intracranial pressure (ICP). We applied electric stimulations to bipolar electodes inserted into the brainstem areas for pressor and depressor responses of BP at different base line levels of ICP and BP to examine the effects of the activation in the cats anesthetized with chloralose. When stimulation of the sites for pressor response of BP produced an increase in ICP at control levels of ICP (within about 20 mmHg), the rate of increased amplidue of ICP to that of BP was diffefent for the activated sites but remained approximately same to different intensities of stimulation of each site. When a conditioning decrease in base line levels of BP and/or the increase in that of ICP were induced before stimulation of the site and the site was activated at the baseline levels of BP and/or ICP, a marked increase was observed in ICP response. In such case, the ratio of incresed response amplitude of ICP to that of BP frequently became more than 2 - 3 times larger than the ratio at control levels of ICP and BP.

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CONTINUOUS MEASUREMENT OF HUMAN DURAL SINUS PRESSURE AND SATURATION O_2 OF BLOOD IN THE SIGMOID SINUS USING AN OPTIC FIBER CATHETER.
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Intracranial pressure (ICP), brain circulation and metabolism were investigated in twelve patients under barbiturate therapy by using an optic fiber catheter to continuously monitor sigmoid sinus pressure (sig. P) and saturation O_2 of the blood in sigmoid sinus (S sig. vO_2). These patients all had elevated ICP caused by various cerebrovascular disorders. Administration of pentobarbital (2.1 + 0.7mg/kg/hr) reduced sig. P from 22.2 + 2.3mmHg to 9.8 + 1.4mmHg. S sig. vO_2 varied little during administration while in conscious patients, S sig. vO_2 fluctuated as much as 50-75%. A strong positive correlation (r=0.84) was seen between $PaCO_2$ and S sig. vO_2 during barbiturate administration. Following administration of nifedipine (10mg), sig. P remained remarkably constant at 10-15mmHg in spite of a drop in arterial pressure. S sig. vO_2 decreased and sig. P increased during tracheal suction or convulsive seizures. This suggests that sig. P might reflect ICP and that S sig. vO_2 can be used as an indicator to evaluate brain circulation and metabolism.

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(1- σ) · ω RELATION IN PULMONARY CAPILLARIES. UEDA, G., SAKAI, A., TAKEOKA, M., KOBAYASHI, T.', KUBO, K.', FUKUSHIMA, M.', YOSHIMURA, K.' AND SHIBAMOTO, T.' Dept. Environmental Physiol. and Internal Med., Shinshu Univ. Sch. Med., Matsumoto, 390

After Staub's chronic lung lymph collection technique applied to sheep, the permeability of capillary area was calculated by Kedem-Katchalsky method. Under three conditions such as altitude exposure,, left atrial pressure elevation and endotoxin injection, physiological data were obtained at control, test and recovery periods. Among them, the protein reflection coefficient σ and the permeability coefficient ω were plotted in X-Y plane. Here, 1-σ was Y and ω was X. On these, linear regression lines (Y = AX + B) were obtained. On the basis of these lines, first, the mean protein molar volume Vs was estimated by Vs = A * LPOS. Here, LPOSo is the control value of the hydraulic filtration coefficient. Next, the ratio of protein to water passage pore areas As/Aw was obtained by B = As/Aw. The values ranged from 0.6 to 0.8. For all the experiments, Y = 0.22X + 0.70. For $\omega > 0$, the condition is $L_D > L_P \sigma^2$ and for $\omega < 0$, the opposite. Thus, in dynamic conditions, when σ values are greater than c.a.0.5, ω can take negative values.

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ESTIMATION OF CARDIAC FUNCTION AND VASCULAR PARAMETERS BY RIGHT HEART BYPASS EXPERIMENTS IN THE DOG. TAKEUCHI, A., SATO, T., SHIRATAKA, M., and NARA Y. Departments of Medicine and Physiology, Kitasato University School of Medicine, Sagamihara, Kanagawa 228

With an attempt to analyze the hemodynamic characteristics of the left heart failure, right heart bypass experiments were performed in the mongrel dogs under Nembutal anesthesia. Relationships of cardiac output and pressures in the systemic and pulmonary arteries and veins were used to estimate afterload sensitivity (inverse of contractility), preload sensitivity (diastolic compliance of left ventricle) and systemic and pulmonary vascular resistances and compliances. Although the limited number of experiments in the preliminary studies does not yield the statistic of the parameters, changes of the calculated parameters by ligation of coronary artery or administration of dobutamine (positive inotropic agent) were in the direction as predicted by the physiological and pharmacological actions. It was concluded that both cardiac and vascular components were reflected in " venous return curve " which was defined by Guyton and widely used in the graphical analysis.

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TIME COURSE OF CHANGE IN BLOOD VOLUME AFTER MANIPULATION OF HYDROMINERAL BALANCE IN THE QUAIL, COTURNIX COTURNIX JAPONICA, AND THE EEL, ANGUILLA JAPONICA. TAKEI, Y. AND HATAKEYAMA, I. Dept. Physiol., Kitasato Univ. Sch. Med., Sagamihara 228

Change in blood volume was continuously measured following hemorrhage or intravascular injection of hypertonic saline in a terrestrial and an aquatic vertebrate. For this purpose, 2.5% Evans blue in saline was injected into the circulation and 50 μl of blood samples were taken at 0.25, 0.5, 0.75, 1, 3, 6, 9, 10, 11, 12, 13, 14 and 24 h after injection. Hematocrit and plasma dye concentration were measured. Data of dye concentration by 10 h after injection were then applied to a single or dual exponential function and goodness of the fit was evaluated in terms of the least residual square sum and greatest correlation. Blood volume was calculated from hematocrit and 0-time dye concentration extraporated from the function fitted best to 0.25-1 h data. The best function and combination of data by 10 h were selected in order to estimate the later (11-24 h) dye concentration. Finally, hemorrhage or 7% saline injection was made at 10 h after dye injection and the change in blood volume was calculated from the difference between the value actually measured and that estimated by the best-fit model. It was found that injection of 7% saline increased blood volume by 12.1 and 11.8% in 15 min and the decrease of blood volume caused by hemorrhage was restored in 4 and 10 h in the quail and the eel, respectively.

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COMPARATIVE RESPONSIVENESS OF THE RAPIDLY ACTING ARTERIAL PRESSURE CONTROL SYSTEM TO QUICK MILD HEMORRHAGE AND INFUSION IN CONSCIOUS RABBITS. HOSOMI, H., KATSUDA, S., MORITA, H., NISHIDA, Y., and KOYAMA, Y. Dept. Physiol., Kagawa Med. Sch. Kagawa 761-07, Japan.

The responsiveness of the rapidly acting arterial pressure control system to hemorrhage and infusion was investigated. Two catheters, one for pressure measurement and the other for inducing hemorrhage and infusion, were chronically implanted in 19 rabbits. A few days after the operation, each animal was quickly bled and infused (2 ml/kg body weight) at intervals of 5 min while it was conscious. The experiment was repeated 16 times in each animal. The 16 strings of data were pooled for each animal. The overall open-loop gain (G) of the rapidly acting arterial pressure control system was estimated from the individually pooled responses to hemorrhage and infusion. There was no significant difference between the G-values, i.e., 7.2±1.2 by hemorrhage and 7.1±1.2 by infusion (mean±SD), as evaluated by the paired t-test ($p>0.5$). Time to peak after hemorrhage and infusion were 3.0 ± 0.7 and 2.3 ± 0.3 sec ($p>0.05$), and settling time were 52.7 ± 9.6 and 53.4 ± 11.0 sec ($p>0.5$), respectively. Thus, the responsiveness of the rapidly acting arterial pressure control system to quick mild hemorrhage and to infusion appears to be similar.

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HYDRODYNAMIC STRUCTURE OF SECONDARY FLOW PRODUCED AROUND ARTERIAL BIFURCATIONS FUKUSHIMA, T., HARAKAWA, K., and AZUMA, T. Institute of Cardiovascular Diseases, Shinshu University School of Medicine, Matsumoto 390

The present study was carried out to determine the hydrodynamic structure of flow around arterial bifurcations and to correlate the findings wtih the possible involvement of fluid dynamics in atherogenesis and thrombogenesis. Steady and pulsatile flow disturbances were examined in a Y-shaped glass model simulating an arterial bifurcation and in transparent dog aortic arches by means of flow visualization. Each aorta was removed after fixation by 0.5% glutaraldehyde solution under a transmural pressure of 100 mmHg. It was then dehydrated by immersion in ethanol and was made transparent by treatment with methyl salicilate. The transparent vessel thus obtained was installed firmly in a glass chamber for hydrodynamic experiments. Flow disturbances observed at the bifurcations in steady flow have characteristics similar to those of secondary flow, called the horseshoe vortex, produced in front of a wall-based protuberance in a circular tube. The same flow pattern was clearly observed during the decelerating phase of a pulsatile flow. The secondary flow induced stagnant regions over the frontal and posterior wall just upstream from the apex of the flow divider. Comparison between the present results and topographical patterns of atherosclerosis reported in the literature suggests that it is in such low shear regions that lipid deposition tends to occur most.

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A PHYSICAL STUDY OF A LUMPED PARAMETER MODEL FOR THE SYSTEMIC ARTERIAL TREE. HARAKAWA, K., FUKUSHIMA, T., *USHIYAMA, Y., and AZUMA, T. Institute of Cardiovascular Diseases, *Central Research Laboratory, University Hospital, Shinshu University School of Medicine, Matsumoto, 390 Japan.

We have previously shown that a simple network model of the arterial system consisting of numerous symmetrical Y-junctions is equivalent to a modified Windkessel model, which is composed of four elements, i.e. inductance L, compliance C, characteristic resistance Rc, and peripheral resistance Rp. To consider the practical applicability of the four elements model, the present experiments were carried out. Pressure, flow, and pulse wave velocity were measured in the ascending aorta of six anaethetised rabbits, weighing 3.1-3.6 Kg. The values of L, C, Rc, and Rp were determined in order that the measured pressure pulse came into good agreement with a pressure wave of the model, reconstructed from the measured aortic flow wave using the estimated values of the four elements. By the aid of Weber's equation for pulse wave velocity, we can derive a magnitude of the Windkessel volume Vw from the compliance C and pulse wave velocity. The value of parameters were obtained as follows: $L=8.15\pm2.91$ gcm^{4}, $C=3.91\pm0.45$ ($\times10^{-5}g^{-1}\ cm^{4}s^{2}$), $R_c=1083\pm135$ $gcm^{-4}s^{-1}$, $R_p=2.78\pm0.46$ ($\times10^{4}gcm^{-4}s^{-1}$), and $Vw=9.64\pm1.71$ml. Referring to the previous theoretical study, these results give reasonable estimates of the geometrical and physical features of the systemic arterial tree.

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Measurement and Analysis of Turbulence in the Aorta -Nonstationary Spectrum Analysis-.
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Turbulent velocity fluctuations measured in the center of canine ascending aorta with a hot-film anemometer underwent a simple nonstationary spctral analysis, in which short segments of measured data are assumed being quasi-steady. As is well known, the fast Fourier transform (FFT) method of spectral calculation is extremely unstable when short data are used, while the autoregressive method(AR) or the maximum entropy method (MEM) is said to be suitable for such a purpose. In the present study, a new AR/ME spectral calculation method specially devised for the data ensemble was successfully used in order to characterize the nonstationary nature of the turbulence in the aorta. Though the pattern of spectra thus obtained itself shows the structure of turbulence in the aorta, it is sometimes clumsy to interpret especially when a number of spctra are calculated. For the sake of characterization of spectra calculated at each time point of the data, two different integral parameters are used in the present study. They are the (first order) characteristic frequency, which is well established in the research field of turbulence, and the second order characteristic frequency, which is a newly introduced parameter. The nonstationary nature of these two parameters of spectra are discussed with respect to some conditions of blood flow.

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MECHANISM OF SOMATOSENSORY AND FOREBRAIN INHIBITION OF VAGAL BRADYCARDIA IN RATS.
NOSAKA, S., NAKASE, N.* AND MURATA, K.* Dept. Physiol., Mie Univ. Sch. Med., Tsu 514.

In chloralose-urethane anesthetized rats, electrical stimulation of sciatic nerve (ScN) was found to suppress vagal bradycardia elicited by electrical stimulation of aortic depressor nerve (ADN). Afferent fibers responsible for the suppression belonged to A-delta and partly, C fiber groups. Exposure of hindlimb to heat (over 58°C) suppressed the vagal bradycardia, whereas other natural stimuli such as vibration, pinching and cold exposure had no effect. Electrical stimulation of hypothalamic pressor area (HP) similarly suppressed vagal bradycardia. Likewise, vagal bradycardia induced by electrical stimulation of hypothalamic depressor area (HD) was suppressed by ScN or HP stimulation. A study using Wall's method revealed that excitability of ADN terminals was not altered by either ScN or HP stimulation, precluding the possibility that the inhibition was presynaptic. Field potentials evoked in nucleus tractus solitarius (NTS) by ADN stimulation was not reduced by either ScN or HP stimulation. Destruction of NTS preserved HD-induced vagal bradycardia; inhibitory actions of ScN and HP on it were also preserved. Intraventricular administration of 6-OHDA abolished ScN inhibition but not HP inhibition of ADN-induced vagal bradycardia. These findings suggest that somatosensory and forebrain inhibitions of vagal bradycardia do not occur in NTS but probably at preganglionic cell level; adrenergic neurons are probably involved in ScN inhibition.

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ROLE OF THE VENTROLATERAL MEDULLA IN THE ARTERIAL BARORECEPTOR REFLEX.
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The ventrolateral medulla (VLM), a circumscribed region close to the ventral surface of the rostral medulla, is known to play a critical role in the maintenance of tonic sympathetic activity and arterial pressure. To examine whether the VLM participated in the arterial baroreceptor reflex, we made a unilateral electrical or chemical lesion (by a toxic dose of kainic acid) of the VLM in the split medulla preparation of the anesthetized rabbit in which neuronal connections across the midsagittal plane of the medulla was disrupted. The lesion markedly reduced the sympatho-inhibitory and depressor responses produced by stimulation of the ipsilateral aortic nerve(AN), while tonic sympathetic nerve activity as well as the reflex response from the contralateral AN was maintained. Neurons in the VLM were then examined for their response to electrical stimulation of A-fibers of the AN which consisted exclusively of barosensory afferents. Spontaneous activities of these neurons, if responsive, were always inhibited by stimulation of the AN. Activities of some of these neurons exhibited the cardiac-locked rhythm indicating convergence of the afferent input from carotid sinus baroreceptors. These results demonstrate that the VLM constitute part of the central mechanism subserving the arterial baroreceptor reflex.

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TIME-DEPENDENT EFFECT OF SECTIONING CAROTID SINUS NERVES ON THE VAGALLY MEDIATED BAROREFLEX. KAIZUKA, T., YOSHIDA, H., and HOSOMI, H. Dept. Physiol., Kobe Univ. Sch. Med., Kobe 650, Japan. Dept. Physiol., Kagawa Med. Sch., Kagawa 761-07, Japan.

The purpose of the reported experiments was to examine whether the vagally mediated baroreflex system (V-system) increases its feedback gain with time after sectioning of the carotid sinus nerves (CNS). In 10 dogs anesthetized with Nembutal, we determined the overall open-loop gain (G) of the rapidly acting arterial pressure control system. G was assessed as ($\Delta AP_I/\Delta AP_S$)-1, where ΔAP_I and ΔAP_S represent the immediate and steady-state decreases in arterial pressure at the aortic arch following a fast step-wise reduction in blood volume. ΔAP_I, ΔAP_S and G(INTACT) in the intact condition (30th min before sectioning of the CSN) were -1.8±0.1 kPa, -0.20± 0.01 kPa and 8.4±0.3 (mean±SE), respectively. The mean values of ΔAP_I, ΔAP_S and G after sectioning of the CSN (G(V)), first averaged within individual dogs and then averaged for ten dogs, were -2.53±0.07 kPa, -1.1±0.05 kPa, and 1.5±0.1, respectively. G(V) did not change with time over about 4 h after sectioning of the CSN. It is concluded that the V-system cannot augment its ability to restore arterial pressure in compensation for the lost function of the carotid sinus baroreflex system over 4 h after sectioning of the CSN in the anesthetized dog.

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HEMODYNAMICAL CHANGES OF SMALL VASCULAR BED IN THE RABBIT MESENTERY DURING DEPRESSOR REFLEX. MINAMIYAMA,M., HANAI,S., Dept. of Vascular Physiol., National Cardiovascular Center Research Institute, Suita, Osaka 565.

The blood pressure (IABP) in the intestinal artery (0.5 mm diameter) and the pressure (IVBP) in the intestinal vein (1 mm diameter) were recorded simultaneously using fine catheters. A small segment of the vascular bed, which is a parallel circuit consisting of microcirculatory networks in the mesenteric adipose tissue and those in the small intestinal wall, was assumed to be driven by the pressure difference (IABP-IVBP). The blood flow rate in the segment (ISF) was determined from the flow rate of the intestinal artery. In randomly selected arterioles (33 μm mean diameter) of the mesenteric transparent regions, continuous red cell velocity measurement was made and then the volume flow rate (IAF) was interpreted. In animals that showed medium reduction (30 - 40%) of aortic blood pressure during depressor reflex, IABP, IVBP, ISF and IAF consistently fell down but the ratios of this change differed among these parameters. The vascular resistance of the mesenteric route including a selected arteriole remained unchanged or even slightly increased, while that of the whole vascular segment decreased. It is suggested that the mesenteric arterioles are scarcely regulated but small arteries and/or arterioles in the small intestinal wall are dilatated owing to neural mechanisms during the reflex.

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INTERACTION OF CANINE CAROTID SINUS AND VAGALLY MEDIATED BAROREFLEXES IN THE CONTROL OF THE HINDLIMB VASCULAR RESISTANCE. KAWADA, M., NISHIDA, Y., KAMIKADO, C.*, ANABUKI, D.*, FUJITA, T.*, MORITA, H., KOYAMA, S., and HOSOMI, H. Pharmaceutical Developement & Resarch Laboratories, Teikoku Seiyaku, Kagawa 769-26, Japan. Dept. Physiol., Kagawa Med. Sch., Kagawa 761-07, Japan.

Interaction of carotid sinus and vagally mediated baroreflex control of the hindlimb vascular resistance was studied in sixteen dogs anesthetized with sodium pentobarbital and placed on constant flow hindlimb bypass. Two polyethylene catheters, one for systemic pressure measurement and the other for inducing hemorrhage, were placed at the aortic arch. Blood (2 ml/kg body weight) was withdrawn quickly and mean systemic arterial pressure and mean hindlimb perfusion pressure were monitored. We determined the overall open-loop gain (G) of the rapidly acting arterial pressure control system and change in hindlimb vascular resistance in response to hemorrhage (ΔHVR) under nerve intact condition (INTACT) (n=16), after sectioning the carotid sinus nerves ($\overline{CS}$) (n=8) or the vagi ($\overline{V}$) (n=8). G(INTACT), G($\overline{CS}$) and G($\overline{V}$) were 6.6±1.6, 1.7±0.7 and 2.0±0.8, respectively. ΔHVR($\overline{CS}$) and ΔHVR($\overline{V}$) were 46.0 ±8.8% and 54.7 ± 8.2% of ΔHVR(INTACT). Thus, G showed a facilitatory interaction, but ΔHVR a simple summation.

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ON THE LOCATION OF INTRACRANIAL BARORECEPTOR. HATAKEYAMA, I., KATAKURA, T. and NISIDA, S. Dept. of Physiol., Kitasato Univ. Sch. of Med., Sagamihara 228

Previously we indicated that the systemic blood pressure (SBP) markedly drops by elevation of pressure in carotid artery. It suggests the exstence of intracranial blood pressure receptor although we could not make any comments on its location. Internal carotid artery of rabbit mostly has two or more branches at the vicinity of carotid sinus. We have explored the effect of elevation of pressure in each branch on cardiovascular function and respiration. All branches except objective artery were ligated and a small quantity of blood was rapidly injected into common carotid artery. Then, the pressure in objective artery was considerably elevated. In general, the elevation of pressure in the largest branch did not produce any considerable drops of SBP, while the elevation of pressure in the smaller branch consistently produced remarkable drop of SBP. These branches run together through canalis caroticus and the latter distributed infundibular region of brain where is the most probable location of baroreceptor. It must be notified that this branch has no significant interconnection to the circle of Willis.

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COMPENSATORY REGULATION OF BLOOD SUPPLY TO THE BRAIN IN ANESTHETIZED RABBITS WHEN CEREBRAL ARTERIES ARE EXTRACRANIALLY OCCLUDED. SHIMIZU, T., 1st Dept. of Physiol. Fukushima Med. Coll., Fukushima 960

Acute changes of carotid flow(CF), systemic arterial pressure(SAP), heart rate(HR) and respiratory movements(RM) were studied when large cerebral arteries were surgically exposed near their origin and alternately or cumulatively clamped in various order and conbinations. The SAP, HR and RM were little changed, except typical carotid sinus reflexes, by vertebral(VA) and carotid(CA) occlusion, as far as one of external CAs, internal CAs and VAs was kept intact. A probe of electromagnetic flowmeter was mounted around a common CA and the external CA was clamped for the measurement of the internal CF. Following the separate occlusion of right VA, left VA and left common CA and cumulative occlusion of these arteries, the right common CF increased by 2.3, 8.1, 28.7 and 68.8% respectively. The right internal CF also increased by 5.6, 25, 25.8 and 85.5% respectively. The flow showed a tendency to increase after the ipsilateral cervical sympathetectomy and decreased by an efferent stimulation of the nerve. The results suggest that the decrease of blood flow through the cerebral arteries extracranially occluded is compensated by increasing the flow of other routes to the brain. An inhibition of the cervical sympathetic activity contributes to the particular compensation mechanism.

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CONTRACTILE RESPONSE OF EXTRAPARENCHYMAL ARTERY OF THE BRAIN TO NOREPINEPHRINE (NE) IN SUPERFUSATE WITH NORMAL AND ACID pH IN CAT. NAKAI, M. Dept. of Cardiovasc. Dyn., Natl. Cardiovasc. Ctr. Res. Inst., Osaka 565.

Hypercapnea is known to enhance responsiveness of cerebral blood flow (CBF) to stimulation of the sympathetic nerve. The present study was undertaken to investigate a mechanism which causes this enhancing effect of hypercapnea with isolated cat's middle cerebral artery (MCA). The vessel was excised, transferred into the bath filled with Krebs superfusate, and mounted on prongs attached to the isometric tension transducer. Values of the initial tension were transformed by employing the Oka-Azuma equation into equivalent transmural pressure (TP) which would give rise to the tension. This calculation process was verified by carrying out another series of experiment. The observed relationship between the TP and the contractile response to l-NE (5×10^{-7} M) indicated that the responsiveness was at most 1.1x higher with acid superfusate (n=13) than normal (n=12) when the TP was within a range between 30 to 60 mmHg. Additional in vivo experiment revealed that the TP of the MCA in hypercapnea was decreased to fall into this pressure range from a 1.4x higher level in normocapnea. We thus conclude that in hypercapnea the responsiveness of the brain artery to the transmitter NE may change to operate more efficiently, thereby backing up the unequivocal reduction of CBF upon sympathetic stimulation.

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DIFFERENCES IN RESPONSE TO VASOACTIVE SUBSTANCES BETWEEN INTERNAL AND EXTERNAL CAROTID ARTERY. KAWAI, Y. and OHHASHI, T. Dept. of Physiol., Shinshu Univ. Sch. of Med., Matsumoto 390

Effects of vasoactive substances on the isolated canine internal carotid artery (ICA) were examined to compare with those obtained in the external carotid artery (ECA). The results were as follows. (1) Norepinephrine (NE) produced a dose-dependent vasoconstriction in both arteries. The maximal tension developed by NE was significantly smaller in ICA than in ECA. (2) The response of ICA was mediated by the excitation of α_1- and α_2-adrenoceptors on plasma membrane of the smooth muscle cells, but the response of ECA mainly by the excitation of α_1-adrenoceptor. (3) Cocaine and hydrocortisone had a little and no potentiative effect on the dose-response curves to NE of ICA and ECA. (4) Norepinephrine caused no constriction in ICA and ECA which were preincubated in the Ca-free solution containing 1 mM EGTA. (5) Verapamil markedly suppressed the NE-induced constriction in ICA. The inhibitory effect of verapamil was significantly smaller in ECA than in ICA, suggesting there may be the difference of sources of activator calcium in NE-induced contraction between ICA and ECA.

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EFFECTS OF Ca^{2+}-ANTAGONIST (DILTIAZEM) ON THE NEURAL CONTROL MECHANISM OF CARDIOVASCULAR FUNCTION. ITO, H. Dept. Pharmacol., Sch. Med., Fac. Med., Univ. of the Ryukyus, Okinawa 903-01

In vivo experiments were carried out to analyze the effects of diltiazem on the ganglionic transmission in the rabbits. Under the anesthesia with pentobarbitone, arterial blood pressure and heart rate were recorded from the animal whose cervical sympathetic nerve trunks and vagi were dissected. An elevation in systemic blood pressure and an increase in heart rate upon electric stimulations on the right thoracic sympathetic trunk were depressed either by an intravenous application of diltiazem (300 μg/kg) or by a topical application by means of placing cotton pledgets soaked in diltiazem (2×10^{-3} M) solution onto the right stellate ganglion. This inhibiting effect of diltiazem was markedly observed when the stimulation of higher frequencies (10-20 Hz) were given, and at lower frequencies (1-5 Hz) no significant inhibition was observed. Hexamethonium, contrary, depressed cardiovascular responses by the sympathetic stimulation over a wide range through 1-20 Hz in the same extent. The present experiment reveals the frequency-dependent inhibition of ganglionic transmission by diltiazem in vivo, similarly to the result we had reported in in vitro studies previously.

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CENTRAL NEURAL CONTROL OF HEART RATE DURING CORONAY OCCLUSION. NINOMIYA, I.,MATSUKAWA, K.,HONDA, T. AND NISHIURA, N. Department of Cardiac Physiology, National Cardiovascular Center Research Institute, 5-125 Fujishiro-dai, Suita Osaka 565 Japan

Changes in efferent cardiac sympathetic nerve activity (CSNA) and heart rate (HR) due to 100-s anterior descending coronary artery occlusion from the control were measured in conscious, atropinized and anesthetized cats. In conscious state, at 20 and 90 s of occlusion, CSNA changed by 23 and -7%, respectively, whereas HR decreased by -5 and -17%, respectively. With atropinization, at 20 and 90 s of occlusion, CSNA changed by 6 and -32%, respectively, whereas HR changed by 0 and -8%, respectively. With anesthesia, at 20 and 90 s of occlusion, CSNA changed by 1 and -18%, respectively, whereas HR decreased by -2 and -14%, respectively. Correlation coefficients between changes in CSNA and HR to occlusion were +0.95, +0.90 and +0.96 for the three states, respectively. These findings suggested that bradycardia due to coronary occlusion was induced mainly by an increase in efferent cardiac vagal activity in conscious state, whereas it was induced by the decreased CSNA in atropinized and anesthetized states.

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THE UPTAKE OF THE CATECHOLAMINE IN THE ISOLATED BLOOD PERFUSED LEFT VENTRICLE.
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To analyse the influence of the loading state and the coronary blood flow on the balance of the coronary catecholamine input and output, we measured the coronary blood flow and the concentration of the norepinephrine and epinephrine of the coronary inflow and outflow by high-performance liquid chromatography under the control and hyperperfusion state of the coronary blood flow in the 7 isolated canine left ventricles, the volume and developed pressure of which were controlled. From the differences of the concentration of the catecholamine between the inflow and outflow, and the coronary blood flow we determined the uptake or release volume of the catecholamine (ng/min). Under the control and hyperperfusion state of the coronary blood flow the norepinephrine and epinephrine were taken up with the left ventricles. The uptake of the norepinephrine and epinephrine were not influenced by the loading state but the uptake of those catecholamine in the isolated left ventricles increased according to the extent of the increase of the coronary blood flow. We observed also that the inclination of the slope of the end-systolic pressure-volume relationship in the left ventricles was augmented with the extent of the increase of the coronary blood flow.

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AUTONOMIC CONTROL OF SMALL PULMONARY ARTERIES IN THE CAT.
SADA, K., SHIRAI, M. AND NINOMIYA, I. Department of Cardiac Physiology, National Cardiovascular Center Research Institute, 5-125 Fujishiro-dai, Suita, Osaka 565, Japan

Using an X-ray TV system, we analysed the autonomic control of small pulmonary arteries in anesthetized cats. Angiograms were taken before and after electrical stimulations of the cervical vagus (VN stim) and the stellate ganglion (SN stim). Internal diameters (ID) before and after the stimulations were measured at 140 sites and classified into four groups according to the control ID sizes, i.e., A) 400-500μm, B) 300-400μm, C) 200-300μm and D) 100-200μm. The relative ID, i.e., ID after the stimulations devided by those before the stimulations, were calculated at the sites. With SN stim, the mean relative ID decreased by 10-15 % in the A-C groups, whereas those did not change in the D group. After injection of β-blocker, those with SN stim decreased by about 20-30 % in the every groups. With additional injection of α-blocker, however, SN stim did not decrease. VN stim did not change those in any group. We concluded that ID of small pulmonary arteries were predominantly controlled by sympathetic than vagal nerves.

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NERVOUS CONTROL OF THE PORTAL VEIN IN RABBITS.
TAKEUCHI,T. and TERADA,N. Dept. of Physiol., Yamanashi Med. Col., Tamaho, Yamanashi 409-38

This investigation was designed to measure quantitatively the magnitude of neurogenic constriction of the portal vein. Rabbits were anesthetized with urethane, immobilized with galamine triethiodide and artificially ventilated with a respirator. Graded cerebral ischemia, which was able to increase stepwisely the sympathetic nerve activity from normal to the maximum, was produced during plethysmographic record of the volume change of the portal vein segment. Spontaneous constriction of the portal vein segment having 7.7±1.6 (SD) sec in period and 5.3±1.6 mm^3 in amplitude was observed in all rabbits studied. The portal vein segment showed the largest constriction of 23.5±5.2% of the control volume (303mm^3) with the maximum pressor response caused by severe cerebral ischemia. This value was equivalent in its magnitude to the constriction response produced by transmural electrical field stimulation of approximately 10 Hz. There were no significant differences among the largest percent decreases in portal vein segment with the maximum pressor response obtained under normo-, hypo- and hyper- volemic states. This constriction of portal vein segment disappeared after denervation.

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EFFECTS OF ALPHA ADRENOCEPTORS IN HEPATIC CIRCULATION IN ANESTHETIZED DOGS. TERADA, N., TAKEUCHI, T. and KOYAMA, S. Dept. of Physiol., Yamanashi Medical College, Tamaho, Yamanashi 409-38

The present study was designed to determine a possible participation of adrenoceptors in the control mechanism that links portal vein flow to hepatic arterial flow. Occlusion of the hepatic artery for one minute produced not significant change in portal vein vascular resistance (PVR), while, the occlusion caused a significant decrease in PVR after pretreatment with prazosin, as alpha-1-antagonist or with yohimbine, as alpha-2-antagonist. Occlusion of portal vein caused a decrease in hepatic arterial resistance (HAR), however, this decrease in HAR in the control group did not differ from that in animals pretreated with prazosin or yohimbine. HAR and PVR increased by inflating a balloon which was placed in the hepatic vein. These increases in HAR and PVR, however, did not change significantly even after pretreatment with either prazosin or yohimbine. It is concluded that alpha adrenoceptor participates in a control mechanism maintaining portal vein pressure constant when hepatic arterial pressure decreased.

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RELATIONSHIP BETWEEN VESICAL AND ARTERIAL PRESSURE. KUJIME, K., NAMIKATA*, Y., SAWATARI, H., UEBA*, Y., Kobe Univ. Sch. Allied Med. Sci., Kobe 654

It is well-known that pressure in the urinary bladder has effects on the cardiovascular system. However, either this mechanism or effects of arterial pressure(AP) on vesical pressure(VP) are still unclear.

In anesthetized male rats, firstly, we examined effects of VP on AP, heart rate(HR), and three different types of sympathetic nerve activities (SNA) i.e., hypogastric(HgNA), renal(RNA), and cervical(CvSNA). Rise in VP to 90 cmH2O increased all parameters in specific mode. VP elevated AP by 4%, HR by 3%, CvSNA by 17%, RNA by 27% and HgNA by 146%, respectively. Secondly, we examined the effect of AP on vesical movement(VM) measured by VP. During increase in AP with phenylephrine or coarctation of descending aorta, VM was suppressed. In contrast, during decrease in AP with nitroglcerin or hemorrhage, VM was elicited. Electrical stimulation of the aortic nerve decreased AP, however, elicited VM. These results suggested that changes in AP had an influece on VM not through the peripheral baroceptor but through the central baroceptor.

Thus, while VP has effects on AP through specific increase in each SNA, AP has effects on VP (VM) probably through the central nervous system.

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NEURAL CONTROL ON SPONTANEOUS ACTIVITY IN BOVINE MESENTERIC LYMPHATICS. OHHASHI, T. and AZUMA, T. Dept. of Physiol., Shinshu Univ. Sch. of Med., Matsumoto 390, and Juntendo Univ., Tokyo 113.

To investigate the effects of autonomic nerves on the spontaneous activity, responses of valvular and intervalvular segments of isolated bovine mesenteric lymphatics to 60-s trains of pulses (50V for 0.5ms at 2Hz) have been studied under the influence of various drugs. There was a marked difference in response to transmural stimulation between the valvular and intervalvular segments. In the valvular segment, the stimulation arrested the spontaneous activity. However, the rhythm of spontaneous contractions was markedly accelerated during the stimulation in the intervalvular segment. Both inhibitory and excitatory effects were completely blocked by tetrodotoxin and guanethidine, and unaffected by hexamethonium and atropine. No change in rhythm was brought about by the stimulation in the presence of propranolol and phentolamine in either segment. These results suggest that transmural electrical stimulation causes the release of norepinephrine from postganglionic sympathetic nerve terminals in the vicinity of pacemeker cells and that beta-adrenoceptors are predominant in the valvular region of the lymph vessel.

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RELATION BETWEEN PULSE WAVE PROPAGATION TIMES AND CIRCULATORY PARAMETERS. OKAI, O., HIGUCHI, Y. AND MAKINO, K. Department of Physiology, Kyorin University School of Health Science. Miyashita 476, Hachioji, Tokyo 192

Mongrel dogs were anesthetized with pentobarbital sodium (30 mg/kg). Various circulatory parameters were taken from them during intravenous infusion of norepinephrine (2 g/kg/min), isoproterenol (2 g/kg/min) and acetylcholine (10 g/kg/min). Here, a pulse wave propagation time from the tip of Q wave of ECG to the upward deflection point of aortic pressure wave at the ascending aorta is expressed as QP; the time from the same point of the pressure wave to the same point of femoral flow wave as PF; the time from the same femoral flow wave to the same point of plethysmogram measured at the foot back as FP; the time from the same point of the pressure wave to the same point of the plethysmogram as PP; A relation between the pulse wave propagation times and circulatory parameters was investigated. The results of these experiments indicated that cardiac output was almost linearly related to 1/QP; ascending aortic pressure to $(1/PP)^2$; femoral flow volume to $(1/FP)^2$; pressure difference between the ascending aortic root and the femoral region to $(1/FP)^2$; the periphral circulatory resistance was closely related to $QP/(PP)^2$.

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SPONTANEOUS CHANGES IN VASCULAR PRESSURE OF ISOLATED ELASTIC ARTERY. HAYASHIDA, N., OKUI, K., FUKUDA, Y.# Surgery, #Physiol., Chiba Univ. Sch. Med., Chiba 280

We recorded intravascular pressure of perfused cylindrical preparation isolated from rat common carotid artery. Spontaneous rhythmic changes in vascular pressure (about 0.5mmHg) was observed and its frequency increased with increasing baseline intravascular pressure (20cycle/min at 100mmHg). Spontaneous pressure change was disappeared by Ca^{2+} free solution or by application of phentolamine indicating that the initiation of pressure change depends upon extracellular Ca^{2+} and endogenous catecholamines. In the separate experiment, we applied artificial alternative pressure (0-80mmHg, 5Hz) to the arterial preparation and measured intravascular pressure swing. The pressure swing in response to such pressure load was larger in the spontaneously 'active' artery than in the phentolamine treated 'inactive' preparation, i.e., smaller pressure dampening in the former. The result suggests that spontaneous pressure change presumably due to rhythmic contraction of smooth muscles inhibits the dampening role of elastic artery.

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ENDOTHELIAL-DEPENDENT VASCULAR RESPONSES OF THE ISOLATED CANINE RENAL ARTERIES TO VASOACTIVE SUBSTANCES. ITOH, S., OHHASHI, T. and AZUMA, T.# Dept. of Physiol., Shinshu Univ. Sch. of Med., Matsumoto 390 and #Juntendo Univ. Tokyo 113

The present study was attempted to evaluate the role of endothelial cells in the responses of the isolated cylindrical preparations of canine renal artery to vasoactive substances. Intimal and adventitial sides of the preparation were separately perfused with normal Krebs solution. The change of outer diameter in the preparation was recorded by means of a photoelectric diameter gauge. The infusion of norepinephrine (NE) from intimal side caused a more marked decrease of the outer diameter than that from adventitial side. The response curve to NE was markedly shifted to right by pretreatment with 10^{-7} M prazosin but unaffected by 10^{-7} M propranolol or 10^{-8} M yohimbine. In addition, the response to NE in the everted preparation, was not affected by removing the endothelial cells from the preparation. With the removing in the everted preparation, the vasodilatory response to acetylcholine (ACh), on the other hand, was abolished and then reversed to vasoconstriction. The response to ACh was dose-dependently reduced by treatment with atropine. These results suggest there may be different sensitivities of smooth muscle cells to NE between intimal and adventitial side of the preparation. The endothelial-dependent relaxation of the preparation is mainly related to the excitation of muscarinic receptor on the endothelial cells by acetylcholine.

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DISTRIBUTION OF SYMPATHETIC VASOCONSTRICTOR TONE IN CONSCIOUS RATS
Iriuchijima, J. Dept. Physiol., Sch. Med., Univ. of Hiroshima.

Distribution of tonic sympathetic vasoconstrictor fiber discharge in various vascular beds was studied in conscious rats. In each rat an electromagnetic flow probe was chronically implanted around the common carotid, superior mesenteric, or renal artery or the terminal aorta. Peripheral resistance was calculated by dividing arterial pressure with flow. The intensity of sympathetic vasoconstrictor tone was estimated by the decrease in peripheral resistance on ganglion blockade with hexamethonium bromide (25 mg/kg, i.v.). Substantial tonic discharge to resistance vessels was concluded in conscious rats only in the carotid and renal areas and not in the superior mesenteric and hindquarter (terminal aorta) areas. In the latter two areas capacitance vessels may be receiving some tonic discharge, since cardiac output decreases on ganglion blockade. After renal denervation, the decrease in peripheral resistance on ganglion blockade was greatly diminished. Pentobarbital anesthesia diminished the tone to the carotid and renal areas but newly generated a tone to the hindquarters.

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NON-UNIFORM DISTENSIBILITY CHARACTERISTICS IN THE VENOUS SYSTEM OF THE LOWER EXTREMITIES WITH A SPECIAL REFERENCE TO MUSCLE PUMP.
MURASE, K., FUKUSHIMA, S., OHHASHI, T. and AZUMA, T# Dept. of Physiol., Shinshu Univ. Sch. of Med., Matsumoto 390 and #Juntendo Univ., Tokyo 113

Pressure-volume relationships in the isolated canine veins of the lower extremities were investigated to examine regional differences in their circumferential distensibilities. The distensibilities of the extramuscular portions of the femoral and lateral saphenous vein were lower than those obtained in the intermuscular portions of the veins. Administration of exogenous norepinephrine caused a more marked decrease in the distensibilities of the former portions of the veins, and electrical stimulation of sympathetic nerve fibers also developed a more brisk contraction in the same preparations. Aminergic nerve fibers demonstrated by glyoxylic acid innervated more densely in the extramuscular portions of the veins. On the other hand, few aminergic fibers were found in the intermuscular portions of the veins. Morphological observations suggested regional differences in amount and alignment direction of elastic and smooth muscle fibers in the walls of venous segment isolated from various portions. Moreover, many venous valves were frequently observed in the distal portion of the intermuscular lateral saphenous vein. We discussed the physiological significance of the functional and morphological differences with special reference to the effect of muscle pump.

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MEASUREMENTS OF CAPILLARY PERMEABILITY IN THE RABBIT SKELETAL MUSCLE USING Cr-EDTA.
SHIBATA, M., YOSHIDA, M., OHTA, I., KAMIYA, A.,*FUKUOKA, M. Research Inst. of Applied Electricity, Hokkaido Univ., Sapporo 060, * Dept. of Physiol., Kyorin Univ., Mitaka 181

Concerning transcapillary exchange of water-soluble substances, we tried a new method to measure the capillary permeability surface area (PS) from the dye clearance curve at a local tissue under a vital microscopic field. The microcirculation in the rabbit tenuissimus muscle was observed with a microscope-TV camera system. As a blue dye indicator, Cr-EDTA (MW:341) was solved in the suffusate of muscle and stained the muscle tissue. After rinsing the muscle with a dye free suffusate for a short period, the tissue concentration change of Cr-EDTA with time was measured using the output of the video-densitometor. PS value obtained by this method was well consistent with the values reported for sucrose. It was also confirmed that there may be some functional interaction between capillary wall permeability and capillary red cell velocity. It was suspected the mechanism is concerned with clearance of diffusion barrier against the capillary wall by red cell passage.

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NONINVASIVE MEASUREMENT OF CAPILLARY PRESSURE AND PRE- AND POST-CAPILLARY RESISTANCE RATIO IN THE FINGER SEGMENTS. SHIMAZU H.*, ITO H.* and YAMAKOSHI K.**, * Department of Physiol. Kyorin University School of Medicine, Mitakashi, Tokyo 181, ** Institute of Applied Electricity, Hokkaido University, Sapporo, Hokkaido

A new method for the noninvasive measurement of capillary pressure (Pcap) and pre- and post-capillary resistance ratio (Rv/Ra) in human finger segments was developed. The blood volume change in vessels of a finger was detected by using a transmittance infrared photoelectric plethysmograph during the gradual increase in occluding cuff pressure applying on the finger segment. The volume curve thus obtained rapidly decreased with the cuff pressure due to the compression of veins and arteries in the finger. There was a difference in the slopes of the volume curve between low and high cuff pressure levels. The capillary pressure was indirectly determined with the cuff pressure at the turning point of the volume curve. Capillary resistance ratio was calculated from mean arterial pressure, venous pressure and Pcap. The values of Pcap and Rv/Ra were compared with those reported by previous investigators.

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EXPERIMENTAL RAT HYPERTENSION WITH INCREASED PERIPHERAL FLOWS. Teranishi, Y. and Iriuchijima, J. Dept. Physiol., School of Med., Univ. of Hiroshima, Hiroshima 734

An electromagnetic flow probe was chronically implanted around the superior mesenteric artery or the terminal aorta in one-clip, one-kidney renovascular hypertensive rats. After recovery from operation, in the resting conscious state, both superior mesenteric and hindquarter (measured at the terminal aorta) flows were larger than the corresponding values in normal control rats. Such flow increase has never been observed in spontaneously hypertensive rats (SHR) and one-clip, two-kidney renovascular hypertensive rats in any vascular area. While vascular resistance is markedly elevated in the hindquarters in SHR and in the superior mesenteric area in one-clip, two-kidney rats, elevation of resistance was not marked in either vascular area in one-clip, one-kidney rats. In this kind of experimental hypertension, the vascular area with marked resistance elevation was the renal area: One kidney was removed and the compensatory increase in flow in the other was restricted by clipping.

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PO_2 IN TENDON SYNOVIAL FLUID OF RABBIT HINDLIMBS. KOMIYA,H.,HAYASHI,I.,IFUKU,H.,SATOH,T and Takemiya,T. Div of Physiol.,Inst. of Health&Sports Sci.,Univ.of Tsukuba. Sakura,Niihari,Ibaraki 305

In the condition of the stable systemic blood pressure under urethane anesthesia, continuous measurements of PO_2 were performed by polarographic method using a pair of platinum electrodes covered with polymer membrane. The size of electrode was 80μm in diameter, which was introduced into the tissue of the tibialis anterior and gastrocnemius by the aid of a fine needle after the checking of the standard calibration. The resting PO_2 in a tendon,synovial fluid and muscle (mmHg,mean±SD,n) was 42.06±18.06 (43),38.83±9.97(113),26.04±11.39(95) respectively. After the cutting of sciatic nerve , PO_2 in all tissues increased gradually to a steady level in 5-10min. The decreasing rate of PO_2 after the cessation of pure oxygen inhalation was much larger in the synovial fluid than in other two tissus. No evidence of overshoot change in PO_2 was observed in the synovial fluid after the release of 30sec occlusion in femoral artery. In 30sec muscle exercise induced by electrical stimulation of the sciatic nerve end at 5Hz , the decrease in PO_2 was small in the synovial fluid, as compared with the muscle. These data suggest that PO_2 in the synovial fluid can easily be affected by microcirculation in the surrounding tissue.

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EFFECTS OF RESPIRATORY HYPOXIA ON RED BLOOD CELL DEFORMABILITY AND IN VIVO PLATELET AGGREGATION. KIKUCHI, Y. and OHSHIMA, N. Biomed. Eng., Inst. Basic Med. Sci., Tsukuba Univ., Ibaraki 305

Blood flow properties may play a critical role in the oxygen transport to tissues when it must be maximized under hypoxia or during exercise. We examined red blood cell deformability and platelet aggregation in rats inhaled 10 % or 5 % O_2 gas. Deformability of the red blood cells was tested in vitro by squeezing them through micorpores of 5 μm in diameter and 10 μm in length under a pressure difference of 10 cmH_2O. The mean pore passage time of single red blood cells was determined as an index of their deformability. Blood samples used for this measurement were obtained via arterial cannulae and heparinized. Platelet aggregation was induced in vivo in a venule (diameter 50-60 μm) of the mesentery by exciting with light fluorescein sodium molecules which had been administered intravenously prior to the irradiation of the vessel. The red blood cells taken under hypoxia showed a considerable swelling but passed through the micropores much quicker than those taken under normoxia. Also the time for the vessel to be totally occluded by platelet thrombus was markedly prolonged under hypoxia or following hypoxia. A marked increase in red blood cell deformability and decrease in platelet aggregation were thus suggested to be induced as a response to respiratory hypoxia. These changes are obviously favourable for the oxygen transport to tissues.

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ANOMALOUS RESPIRATORY ARRHYTHMIA. HAYASHI, H. and TAKAYAMA, K. Dept. of Physiology, Saitama Medical School, Iruma-gun, Saitama 350-04, Japan.

We examined the respiratory arrhythmia by analyzing the ECG tachogram (cardiac interval) and respiration curve (thorax movement) in a man. 1. One to one correspondence was observed between rhythmic respiration curve and ECG tachogram. Phase delay of ECG tachogram to sinusoidal respiration of 4 sec cycle was about 180° as many investigators reported, however, that of 20 sec or 2 sec was approximately null. 2. Cardiac interval in expiratory breath holding (e.b.h.) was shorter than in inspiratory one(i.b.h.). 3. The duration of one respiration during e.b.h. was several times shorter than that of ECG tachogram. 4. Rapid shift from e.b.h. to i.b.h. within 1 sec brought about a transient dip (shortened cardiac interval) in ECG tachogram, but gradual shift did not show the dip. 5. Exercise either diminished or enhanced the amplitude of the dip according to the tone of autonomic nerves. 6. There was a positive correlation between the level of ECG tachogram in e.b.h. and the amplitude of the dip. 7. Ventricular extrasystole was not infrequentely oberved during the dip.

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EFFECTS OF PLASMA CATECHOLAMINES ON INTERNAL DIAMETER OF SMALL PULMONARY ARTERIES IN THE CAT. SHIRAI, M., SADA, K. & NINOMIYA, I. Department of Cardiac Physiology, National Cardiovascular Center Research Institute, Fujishiro-dai, Suita, Osaka 565, Japan

Using an X-ray TV system, effects of plasma catecholamines on internal diameter(ID) of small pulmonary arteries(100-500 μm in ID) were studied in anesthetized cats. After sympathetic ganglion blockade(C_6, i.v.) and vagotomy, ID responses to norepinephrine(NE) and epinephrine(EP), 7-10 μg, i.v., were analyzed at 367 and 260 arterial sites, respectively. Both NE and EP induced three types of ID response, i.e., no change(No), constriction(Co) and dilatation(Di). With NE, relative frequencies of No , Co and Di were 42, 30 and 28%, respectively. On the other hand, with EP, those of No, Co and Di were 23, 3 and 74%, respectively. In almost all arterial sites, after administration of β-blocker both NE and EP induced Co, whereas after administration of α-blocker they caused Di.

These results suggest that the ID response of small pulmonary arteries to plasma catecholamines depends on the balance between α-receptor mediating vasoconstriction and β-receptor mediating vasodilatation.

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A STUDY OF THE ELECTRICAL IMPEDANCE PLETHYSMOGRAPHY IN THE CENTRAL CAUDAL ARTERY OF THE RAT. SASAOKA, K., Physiol., Tokyo Dent. Coll., Chiba City, Chiba 260

The present study verified the relationship between changes in mean flow and CR in the peripheral vascular system. The measurement of the impedance(Z) by electrical impedance plethysmography and the blood flow (F) by electromagnetic flowmetry was carried out simultaneously using the central caudal arteries of 35 male Wistar rats under sodium pentobarbital anesthesia. The artery was occluded distal to the measuring positions. The present study confirmed that in the formula, $f(t) = \alpha z'(t) + \beta z(t)$, which was derived from the formula $F = \alpha\, dZ/dt + \beta Z$, α and β expressed were coefficients. The former formula was equivalent to the latter formula without consideration of direct current. The equation was confirmed by comparison of the values for the peak to peak of left side and of right side. The relationship $CR=\alpha/\beta$ was noted when CR was the time constant in the peripheral vascular system. The average mean flow under maximum occlusion was 1/ 50, while CR of the maximum occlusion was 30 fold higher than the average before occlusion.

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MEASURMENTS OF P-P,R-R AND P-R INTERVALS IN UNANESTHETIZED ANIMALS. NISHIURA, N.,NINOMIYA, I. AND *YONEZAWA, Y. Department of cardiac Physiology, National Cardiovascular Center Research Institute, 5-125 Fujishiro-dai, Suita Osaka 565, and*Department of Electrical Engineering, Hiroshima Institute of Technology, Itukaich, Hiroshima, 738 Japan

For analysis P-P,R-R and P-R intervals of the ECG signals in freely moving unanesthetized animals, we have developed a 2 channel telemeter consist of gain control amplifiers and PWM-FM transmitter. This gain control transmitter system can adjust the magnitude of input signal and increased the signal to noise ratio.The ECG signals can be transmitted continuosly over 50 hours with two botton type lithium batteries. The transmitted FM signal is demodulated by a receiving system and the demodulated PWM signal is transformed into the analog signals.

In this study, P and R waves of ECG were recorded directly from the left atrium and ventricle. The onset of P and R waves are discriminated and transformed into the standered pulses. The intervals of P-P,R-R and P-R standard pulses are measured and analysed by a minicomputer(MINC). Using this system, we obtained 24 hour histograms of P-P,R-R and P-R intervals in freely moving cats.

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RESPONSES OF EXTRACELLULAR FLUID SYSTEM TO ISOTONIC INFUSION AND ASSESSMENT OF MICROCIRCULATION USING ^{51}Cr-EDTA. ITOH, T., *MIKI, K. AND MORIMOTO, T. Dept. Physiol., Kyoto Pref. Univ. Med., Kyoto 602, * Dept. Physiol., Univ. Occup. Environ. Hlth., Kitakyusyu 807

The parallel model of extracellular fluid (ECF) system reported by us (Jpn. J. Physiol. 34:641, 1984) was further studied regarding its responses to isotonic infusion. Parameters of K_1 and K_2 have been introduced as "mobility indices" to depict the distribution kinetics of ECF tracer, ^{51}Cr-EDTA. In this report, the constancy or variability of these parameters was also tested.

After the equilibrium of injected ^{51}Cr-EDTA in spleno-nephrectomized dog, 10ml/(kg·10min) of lactated Ringer was infused and, simultaneously, tracer concentration in blood, hematocrit and subcutaneous interstitial pressure were continuously monitored for 70 min. From these data, continuous time course of the infused water was resolved to distribute itself into three compartments of ECF. Using this precise time profile and modified time-dependent parallel model, mobility indices were concluded to increase transiently in accordance with infusion. They changed in parallel to the rate of transvascular fluid shift and, therefore, reflected the increment in simple diffusion and solvent drag of tracer across exchange vessels. Mobility indices, determined from whole-body experiments, are considered useful for the assessments of microcirculation.

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ARTERIAL BARORECEPTOR REFLEX IN GROWING RATS. SAITO, M., TERUI, N., NUMAO, Y. and KUMADA, M. Inst. of Basic Medical Sciences, The Univ. of Tsukuba, Ibaraki-ken 305

In order to characterize the neural control of the circulation in an organism undergoing a rapid somatic growth, we compared the arterial baroreceptor reflex (ABR) in two groups of male Wistar rats anesthetized with urethane (i.p.). The first group consisted of 36 rats of 6-7 weeks old, known to exhibit the greatest increase rate of the body weight during the life span, whereas the second group consisted of 30 full-grown rats of 20-22 weeks old. When arterial pressure (AP) was controlled by phenylephrine or nitroglycerin (i.v.), the reflex change in renal nerve activity was elicited over the AP range 15-30 mmHg lower than that of full-grown rats. The difference was attributable to the lower operating range of arterial baroreceptors in the former group. Although no difference was observed between the two groups with respect to the reflex fall in AP in response to graded electrical stimulation of the aortic nerve, the accompanying bradycardia was significantly smaller in 6-7 weeks old rats primarily due to the difference in the vagally mediated component of the reflex response. In conclusion, the ABR of growing rats, as compared to full-grown counterparts, is characterized by deviation of the operating range of arterial baroreceptors as well as the ABR itself to lower APs and poor development of the vagally mediated reflex bradycardia.

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RHEOLOGICAL STUDY ON PRESSURE-VOLUME RELATION OF BULLFROG AORTA

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Aorta sinistra from Rana catesbeiana of 1 cm length were tied both sides. The inner pressure, which was generated by a micrometer-syringe injection of the amphibian Ringer solution, was measured. The pressure P and the injected volume V are represented as the following differential equation; $dP/dV = aP+b_0+b_1V+b_2V^2+b_3V^3$. The mean values of each parameter at 20°C (20 examples) were obtained as follows; $a=-0.07(/\mu l)$, $b_0=2.4(mmHg/\mu l)$, $b_1=-0.0008(mmHg/\mu l^2)$, $b_2=-0.0091(mmHg/\mu l^3)$, $b_3=0.00089(mmHg/\mu l^4)$ and the integral constant $P_0=0.78(mmHg)$. As the temperature was increased from 0°C to 40°C, the initial pressure parameter b_0 became larger (2.1 at 0°C, 2.6 at 40°C) and the collagenous elasticity parameter b_3 smaller (0.0014 at 0°C, 0.00076 at 40°C). The P-V curves of each temperature cross each other approximately at the physical pressure (20-40 mmHg). A. ischiadica(20 examples) and V. abdominalis (10 examples) were also investigated and $a=-0.15$; -0.19, $b_0=7.0$; 0.83, $b_1=-0.74$; -0.064, $b_2=0.23$; 0.030, $b_3=0.022$; 0.0059 and $P_0=0.54$; 0.022 at 20°C were obtained, respectively.

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Response of renal nerve in endotoxic hypotension to stimulation of medullary pressor area. Kagawa Medical School. Dept.of Physiol. S. S. Koyama, H. Hosomi, Y. Nishida, and H. Morita

The present study was designed to determine if there is a central abnormality in the sympathetic efferent outflow through the brainstem in endotoxic hypotension. MBP after endotoxin (1 mg/kg,iv) fell significantly. Simultaneously with dereases in MBP, NA and HR also decreased significantly. Peak responses in MBP and NA to repetitive stimulation of medullary pressor area (MPA) were attenuated significantly after endotoxin. Onset and peak latencies of renal nerve potentials (RNP) evoked by the electrical stimulation of the MPA at 5 min after endotoxin were significantly shorter than that before endotoxin, followed by a recovery to the preendotoxin level. Peak and total activities of RNP were reduced significantly. However, responses in MBP, NA and RNP to the stimulation of the thoracic sympathetic chain did not differ significantly between before and after injection of endotoxin. These data confirm the presence of a central neural abnormality in central sympathetic outflow in the hypotension induced by E. coli endotoxin.

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TRANSIENT INCREASE IN SYMPATHETIC RENAL NERVE ACTIVITY AT THE BEGINNING OF EATING IN AWAKE CATS.
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To investigate underlying neural mechanisms inducing cardiovascular responses to eating, we attempted to record sympathetic renal nerve activity (RNA), heart rate (HR) and arterial pressure (AP) during eating in 6 conscious cats. The cats were trained to eat under a condition that their body trunk movements were restrained. Food was repeatedly presented for 10 sec at intervals of 100 sec. After the abrupt presentation of food, eating started with a latency of 1.6 sec. During eating, RNA, HR and AP increased by 100%, 22% and 11% respectively. The increase in RNA suggests that enhancement in sympathetic nerve activities evoked cardiovascular responses to eating. Moreover, the fact that the increase in RNA occurred almost concomitantly with the onset of eating, whereas the change in AP followed it by 3 - 4 sec, suggests that the increase in RNA at the beginning of eating was not evoked reflexly either by food intake or by the change in AP, but directly by higher central nervous system.

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CHARACTERISTICS OF SPINAL SYMPATHETIC TONE IN THE RAT.
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Spinal sympathetic tone in conscious rats one hour after spinal transection at C8 when arterial pressure recovered to about 90 mmHg was compared to the tone in intact rats. Cardiac output was measured with an electromagnetic flow probe chronically implanted around the ascending aorta. For measurement of pressure and sampling of blood, an indwelling catheter was inserted into the terminal aorta. Concentrations of adrenaline and noradrenaline in the plasma were measured by the radioenzymatic method of Peuler and Johnson. Arterial pressure and cardiac output were lower after spinal transection than before. On the contrary, total peripheral resistance was even higher after transetion. Concentration in the plasma of adrenaline and noradrenaline were 371±195 and 438±212, respectively, in intact conscious rats and 93.9±52.7 and 283±95.3, respectively, one hour after spinal cord transection. These findings suggest that spinal sympathetic tone in predominantly for resistance vessels and not for capacitance vessels and adrenal medulla and that these organs have separate innervations.

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MOUSE HEMOPOIETIC COLONIES IN CULTURE FORMED BY POKEWEED MITOGEN-STIMULATED-SPLEEN CONDITIONED MEDIUM. NAGASAWA, T., OKADA, T. KINOSHITA, Y. Physiol., Osaka City Univ. Med. Sch., Osaka 545

Several investigators have reported hemopoietic colonies expressing multilineage differentiation in culture. Using pokeweed mitogen-stimulated spleen cell-conditioned medium (PWM-SCM), we observed the development of multilineage colonies in culture of spleen cells obtained from 5-fluorouracil (5-FU)-treated mice. One ml of culture medium contained $2x10^6$ spleen cells from normal BDF^1 mice or $6x10^5$ spleen cells from 5-FU-treated mice, α-medium, 0.8% methylcellulose, 30% fetal bovine serum, 1% deionized bovine serum albumin, 10^{-4}M mercaptoethanol, 10% PWM-SCM and with or without 2 U of human urinary erythropoietin (EPO). Colonies were counted on day-12 to 16. Spleen cells of normal mice and 5-FU-treated mice were $9.9x10^7$ and $6.0x10^7$ cells/spleen, respectively. Mixed hemopoietic colonies in vitro by PWM-SCM contained erythroid cells, granulocytes, macrophages, megakaryocytes and mast cells. Mixed colonies in culture of normal mice with EPO, 5-FU-treated mice with EPO and 5-FU-treated mice without EPO were 26.1%, 63.4% and 68.3%, respectively. Erythroid colonies of 5-FU-treated mice with or without EPO were 90.2% and 75.6%, respectively. These results suggest that multipotent stem cells are not affected by 5-FU treatment and PWM-SCM contains multipotent colony-stimulating factors and erythropoietin-like factor.

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Membrane lipids of rabbit's reticulocytes induced by phenylhydrazine. Masao,Kitahara. and Susumu,O'hata. Dept. of Physiol, Tokyo Medical college, Shinjuku-ku, Tokyo.

Reticulocytosis due to hemolysis was induced by 4 deys subcutaneous injection of phenylhydrazine hydrochloride (pH 7.0, 0.6mg/kg) into a rabbit. Bloodsample was spun up 2,500 g for 15 min, and was excepted buffy coat, its final concentration was approximately 100%.

Reticulocytes and eryehrocytes were extracted with Forch's procedure, and the each Folch's upper phases were developed on thin-layer chromatography. The TLC was carried out on Silica Gel G (Merk) using chloroform:methanol: water (65:25:4) and n-propanol:water (70:30), and reagents were Anthron, Orcinol, α-naphthol and Resorcin.

Reticurocytes appeared to contain some bands of glycolipids, as yet unidentified.

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DEFORMABILITY OF HUMAN ERYTHROCYTES: EFFECT OF CALCIUM CONTENT AND ENERGY METABOLISM. MAEDA, N., KON, K. and SHIGA T. Dept. Physiol., Sch. Med., Ehime Univ., Ehime 791-02

The effect of calcium (Ca) on the erythrocyte deformability was examined by a high-shear rheoscope, with varying the cellular Ca content with ionophore (A23187) and $CaCl_2$, in relation to energy metabolism, especially to cell age.

(i) Loading Ca, intact erythrocytes flattened, and the cell volume and energy charge gradually decreased. By the rheoscope technique, a distinct transition of deformable to undeformable cells was observed under the shear stress of 140 dyn/cm^2, without alteration of deformability of deformable cells to ellipsoidal disc. Undeformable cells increased parallelly with the progress of cellular dehydration, and the membrane fluidity of lipid portion decreased. Further loading of Ca induced echinocytosis, in where all cells were undeformable. (ii) After Ca-loading, high density cells fractionated by the density gradient centrifugation of Percoll were undeformable and older in age (with lower G-6-PDH activity), while low density cells deformable and younger. (iii) Younger cells fractionated from intact cells restored their shape, cell volume and deformability upon Ca-loading, while older cells reduced their cell volume and became undeformable. In conclusion, the preferential accumulation of Ca is induced in aged cells with lower energy metabolism and leads the decreased deformability due to the cellular dehydration and the altered membrane organization.

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D-GLUCOSE TRANSPORT ACROSS ERYTHROCYTE MEMBRANE STUDIES BY OSMOTIC FRAGILITY. II. —— EFFECT OF INHIBITOR. FUKUSHIMA, M., KIKUCHI, H., UYESAKA, N. AND SHINAGAWA, Y. Dept. of Physiol., Nippon Medical School, Bunkyo-ku, Tokyo

Osmotic fragility of human erythrocyte was tested by means of the usual method. Remarkable increase in osmotic fragility was caused by the mixture of NaCl and D-glucose (1:1) solution. Cytochalasin B, phloretin and phlorizin inhibited the osmotic fragility induced by the mixture, and the degree of inhibition increased as concentration of glucose did. This suggests that the increase in osmotic fragility depends mainly upon sugar transport across erythrocyte membrane. The increase in osmotic fragility was inhibited also by Ca^{++}, however this phenomenon was not occured so remarkably in high or low concentration of glucose. This fact shows that the manner of inhibition of Ca^{++} is different from that of cytochalasin B. Addition of human blood plasma (0.06-0.006 mg/ml) inhibited the increase in osmotic fragility but lower quantity of plasma accelerates it, inversely. Blood plasma incubated at 56°C for 30 min represented more effective inhibition than native plasma, but incubated blood plasma (91°C, 30 min) did smaller one than native plasma. The effect of acceleration of native plasma was larger than that of incubated plasma (56°C, 91°C). These results show that there are several kinds of inhibitory substances besides Ca^{++}, and acceleratory substances in human blood plasma.

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SIMILARITY AND DISSIMILARITY OF PLASMINOGEN ACTIVATORS IN DIFFERENT ANIMAL TISSUES. HORIE, N., SHIMIZU, H., OHTA, N. AND OKAMOTO, U., Fac. Nutr., Kobe-Gakuin Univ., Kobe 673.

In comparative studies of plasminogen activator(PA) in some different tissues, the method to adopt "plasminogen and fibrinogen copolymerized SDS-PAGE" was successfully used. The electrophoresis of 2M $NaClO_4$ extracts of the spleen, lung, liver, kidney and heart in rabbit, guinea pig, hamster, rat and mouse was performed. High molecular weight PA(approximately 50K dalton) was detected in all tissues. Low molecular weight PA(approximately 30K dalton) was detected in the lung, kidney and spleen. The PA was partially purified from 2M $NaClO_4$ extract of porcine spleen by three stepts; the salting out with ammonium sulfate, affinity chromatography on Benzamidine Sepharose and finally gelfiltration on Sephacryl S-200. Sephacryl S-200 gelfiltration indicated a peak of PA at 49K dalton in molecular weight. Anti-human urokinase rabbit IgG inhibited this PA activity with fibrin plate and electrophoresis, but anti-porcine heart PA rabbit IgG did not. Even though, similarity of immunological and physiological properties between PA and urokinase attracted special attention of the authors.

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EFFECTS OF LOCAL VIBRATION EXPOSURE ON BLOOD AND VESSEL. HIGASHI, T., WATANABE, M., TAKASHIMA, N.*, SAGAWA, S.*, NISHIDA, K.*, ISOMOTO, A., TYUMA, I. AND # KURODA, M.* Dept. Physicochem. Physiol. and # Cent. Res. Lab., Med. Sch., Osaka Univ., Osaka 530

The effects of local vibration exposure on blood and vessel were studied concentrating on the factors of thrombosis and hemostasis. (Experiment I) The blood flow in forearms of 5 healthy males was cut off with an oppressive band of sphygmomanometer. The palm was exposed to vibration (number of vibrations: 35Hz, acceleration: 5G, amplitude: 2mm p-p) for 5 minutes, and blood was drawn from vein in forearm as a sample of "vib(+)." As a control of "vib(-)", blood was also drawn from same vein after cutting off the blood flow in forearms for 5 minutes without vibration exposure. The whole blood viscosities (cP) at the shear rate of 76/sec were vib(-)/vib(+) = 5.0/5.4, 4.3/5.1, 4.4/4.9, 5.0/6.2, 4.7/5.5 respectively. This result seems to be due to the hemoconcentration because the values of hematocrits, and the concentrations of fibrinogen and serum protein increased by 10-20%. (Experiment II) The palms of 10 healthy males were exposed to vibration for 5 minutes keeping the normal blood flow in the forearms. The fibrinolytic activity of euglobulin fraction increased after vibration in spite of no hemoconcentration. If the endotherial cell of vessel functionally takes part in it, a possibility of vibration load test is anticipated as a functional diagnosis of arteriosclerosis.

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EFFECTS OF FIBRIN ON THE ACTIVATION OF PLASMINOGEN BY UROKINASE. TAKADA, A., MAKINO, Y., TAKADA, Y. Dept. Physiol., Hamamatsu Univ., Sch. Med., Hamamatsu-shi, Shizuoka 431-31

When human plasma was added with urokinase (UK) or UK plus thrombin, plasminogen was activated better by UK in the presence of clot than in the plasma as measured by the hydrolysis of S-2251. Then fibrinolysis and fibrinogenolysis were measured by enzyme immunoassay (EIA). When the plasma was clotted, fibrinolysis by UK was enhanced more than fibrinogenolysis in the plasma. When Glu-plasminogen(plg) and fibrinogen were mixed with UK or UK plus thrombin, Glu-plg was activated better by UK in the presence of fibrin as measured by the hydrolysis of S-2251, or fibrino(geno)-lysis by EIA. The release of Bβ peptides from fibrin was also enhanced than that from fibrinogen, when plasma was activated by UK or UK plus thrombin. In a purified system Bβ peptides were released faster from the mixture of Glu-plg, fibrinogen and UK than from the mixture of Glu-plg, fibrin and UK, but the presence of α_2AP, or α_2AP and α_2M in the purified system resulted in faster release of Bβ peptides from fibrin than from fibrinogen.

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EFFECTS OF HYPER GRAVITY ON THE BLOOD COAGULABILITY AND FIBRINOLYTIC ACTIVITY. ITO,A.,SUGISHITA,E.,SAKAI,J.,NAKASHIMA,A.,SUGIE,I. (Dept. of 1st Physiol.,Aichi Med. Univ. Nagakute-cho 480-11),SATAKE,H.(Inst. of Equilibrium Res.,Gifu Univ. Sch. of Med., Gifu 500), WATANABE,S.(Dept. of Aerospace Physiol.,Res.Inst.Environ.Med., Nagoya Univ. Nagoya 464)

To investigate the effects of hyper gravity for a short-term exposure on the hemostatic mechanism of blood, clotting and fibrinolytic activities were calculated on animal. Male golden hamsters, 8-10 week-old and weighning 110-150g, were centrifuged at -2Gx, -3Gx and -10Gx for 30 min. and blood was obtained from heart immediately and after 60 min. In the case of -2Gx and -3Gx, the results indicate significantly increased Ht, shortening of r-time, prolongation of k-time and decreased ma on thrombo-elastgraph, decreased fibrinogen and prolongation of prothrombin time. Furthermore, anti-plasmin in plasma was decreased after results of activation of fibrinolytic system. On the other hand, in the case of -10Gx, the results indicate decreased Ht, prolongation of activated partial thromboplastin time, increased small-size of platelet and appearance of plasminogen activator in plasma. From the these activations of coagulation and fibrinolysis, it is suggested that to expose hyper gravitation would be the risk condition for thrombosis and bleeding.

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A STUDY ON THE HUMAN PLATELET FC RECEPTOR. ARIGA, T. AND *OSHIBA, S. Dept. of Nut. and Physiol., Nihon Univ. Coll. of Agri. and Vet. Med., Tokyo 154. *Dept. of Physiol., Nihon Univ. Sch. of Med., Tokyo 173

Immune complexes and aggregated IgG bind to human platelet through the Fc fragment of IgG. The Fc receptor on platelet is still under research. We, therefore, attempted to prove the existence of the receptor by using methyl 4-azidobenzoimidate (MABI), a photosensitive hetero-bifunctional cross-linking reagent. Molecules of MABI-(^{125}I)IgG and MABI-(^{125}I)Fc cross-linked to a platelet were estimated to be about 5,000 and 2,000, respectively. The presence of excess IgG reduced those molecules. By cross-linking the IgG or Fc to platelet, a glycoprotein of an apparent molecular weight 250,000 was removed from the platelet after forming giant molecules, although the typical glycoproteins which were detectable with radioiodination of platelet by lactoperoxidase method remained unchanged. The glycoprotein of Mr=250,000 was thus assumed to be a Fc receptor, as was the one isolated by J. Hawiger and C. M. Cheng with their affinity column.

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STUDIES ON THE FIBRINOLYTIC ENZYME SYSTEM IN THE HEPATOBILIARY SYSTEM (XIII)—PRODUCTION AND SECRETION OF THE PLASMINOGEN ACTIVATOR FROM CULTURED HEPATIC CELLS—

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Studies were made on the release of plasminogen activator from the primary cultured rat hepatic cells, and some of its enzymatic properties. The fibrinolytic activity of the cultured rat hepatic cells was observed by Todd's fibrinolysis autograph and the released fibrinolytic activity into the medium was measured by Astrup's fibrin plate method.

The results were obtained as follows; 1) Cultured rat hepatic cells produced plasminogen activator (PA) and secreted it into the culture medium. 2) The molecular weight of this PA was determined to be 57,000. 3) The isoelectric points were determined to be 7.3, 8.0 and 8.2. The affinity of this PA to fibrin was high in contrast to the low affinity to fibrinogen. 4) Since the enzymatic properties of the hepatic cell plasminogen activator resemble those of Bilokinase but differ from Urokinase, Bilokinase is considered to be of hepatic cell origin.

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THE EFFECT OF WATER SOLUBLE RADIOGRAPHIC CONTRAST MEDIA UPON ERYTHROCYTE MEMBRANE

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The effect of various radiographic contrast media(RCM) upon erythrocyte membrane was investigated by the measurement of osmotic fragility, volume change, shape change and viscosity of cell suspension taking osmolality, viscosity and iodine contents into account. Marked decrease in osmotic fragility induced by RCM was observed. The volume change of erythrocytes suggested that permeability of RCM across membrane is lower than that of NaCl. Metrizamide solution caused remarkable shape change (echinocyte III) even under physiological condition. Several recognizable stages of discocyte-echinocyte transformation were found out as the osmolality of Diatrizoate solution increased. Ioxaglate solution caused little shape change. Striking increase in viscosity and yield stress was induced by Metrizamide. Increase in viscosity induced by Diatrizoate was smaller than that done by Metrizamide. On the other hand there was little substantial difference between Ioxaglate and NaCl solution. The above result indicates clearly that Ioxaglate is better contrast medium than Diatrizoate and Metrizamide concerning on the physiological behavior of erythrocyte membrane.

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SHAPE CHANGE AND MECHANICAL FRAGILITY OF ERYTHROCYTES MEMBRANE II.

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In order to investigate membrane stability, quantitative and simple method for measuring mechanical fragility of erythrocytes making use of impact-force was developed. An advantage of this fragility test is that it can be carried out under physiological conditions. A personal computer was introduced to quantify this method further, and quantitative measurement of impact-force wave was carried out satisfactorily. Red cell shape changes induced by impact-force were several recognizable stages of echinocyte-spherocyte transformation. Most characteristic shape change induced by impact-force was fragmentation of erythrocytes. Effect of chloropromazine(CPZ), N-ethylmaleimide(NEM) and ethanol(EOH) upon membrane fragility of erythrocytes were investigated. Remarkable increase in osmotic fragility induced by CPZ, slight increase in it done by NEM and little increase in it by EOH were observed. However marked decrease in mechanical fragility induced by EOH, moderate decrease in it done by CPZ and a little decrease by NEM were found out. These results indicate clearly that mechanical fragility of erythrocytes gives us unique information concerning molecular environment of membrane, which is not detected by other methods such as osmotic test and spin labeling.

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ON THE METHODS FOR BLOOD SAMPLING AND INFUSION FROM SMALL ANIMAL AT PRESSURE.
MIZUSHIMA, Y., IWASAKI, M., SHIDARA, F.,[1]NAKAYAMA, H. and SEKI, K. Japan Marine Science and Technology Center, Yokosuka 237. [1])School of medicine, Tottori University, Yonago.

The methods for sampling blood and infusion to study effects of high ambient pressure on circulatory physiological and pharmacological funcion. In the presious report the profile of the apparatus which is consisted of small chamber and pressure equilibrium bulb for blood sampling was presented. In the present study, some modifications were made to clear problems arose from the previous report. In order to sample an appropriate deal of blood from catheterized animal without destruction of erythrocyte or coagulation, the difference of pressure between the inside of the animal chamber and the sampling bulb was maintained in 0.2kg/cm^2 at any experimental pressure, resulted in successfull sampling more than 5ml. The blood sampled as above proved to be the good condition even for biochemical analysis. Modification was also made to infuse vehcles into catheterized animal at constant flow velocity, In which another bulb with electrical pump was equipped.These methods proved to be appropriate enough in blood sampling and vehcle infusion across the difference of the ambient pressure. Using these methods, a cat was compressed up to 11bar($He-O_2$)and blood was sampled from the catherterized venus decompressed in the sampling bulb, and formal and biochemical analysis were made. It revealed blood sampled as above showed exactry the same conditionas in normal pressure.

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ON THE INHIBITION OF LEUKEMIC CELL PROLIFERATION BY ANTI-LEUKEMIC AGENTS. MEASUREMENT BY THE CELL CULTURE AND SIMULATION BY THE CELL KINETIC MODEL.
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Considering the kinetics of the cell cycle, a compartment model for describing the dynamic relationship between the administered drug and the number of leukemic and normal white blood cells in the bone marrow has been established. This model can describe also the fluctuations of those cell counts in the peripheral blood of acute leukemic patient. In this study, the simulation of the inhibition of leukemic cell proliferation under various drug concentration were compared with experiments of the cell culture with a cell line derived from human monocytic leukemia, J-111. The mode of both simulated and cultured cell proliferation was exponential. The mean doubling time of J-111 was estimated as 35.6 hours. As indicated by the simulation, the surviving fraction diminished exponentially as exposure time went when J-111 was exposed to a cell cycle nonspecific drug, daunorubicin. On the contrary, the dose response curve to daunorubicin failed to support the result of the simulation. In the case of a cell cycle specific drug, behenoyl ara-C, the simulation agreed approximately with the cell culture.

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EFFECTS OF ACETAZOLAMIDE(AZ) AND SITS ON Na^+ AND HCO_3^- IONS APPEARING IN THE PLASMA DURING A CO_2-RICH GAS PASSAGE THROUGH DOG'S WHOLE BLOOD. MINAGAWA, T., ASANO, K., OHIRA, T., OGAWA, A., SHIMABUKURO, S. AND HIRAKAWA, S. 2nd Dept Intern Med Gifu Univ Sch Med, Gifu 500

We have previously reported that there occurred "transport" of Na^+ and HCO_3^- ions in one-to-one relation from red blood cells into plasma, side by side with chloride shift, when dog's whole blood was exposed to a CO_2-rich gas mixture. In the present study, to examine the effects of AZ and SITS on the "transport" of Na^+ and HCO_3^- ions in question, a CO_2-rich gas mixture was passed through dog's whole blood after pretreatment with AZ or SITS. In addition, the approximate net "flux" was calculated for Na^+(FNa^+), Cl^-(FCl^-) and HCO_3^- ions($FHCO_3^-$) by the end of 5 min after starting a CO_2-rich gas passage, where pH, PCO_2 and PO_2 were 7.23, 50.4 mmHg and 195.0 mmHg, respectively, and comparisons were made between AZ or SITS group and untreated control group. As a result, AZ was found to decrease FNa^+, FCl^- and $FHCO_3^-$, while SITS caused FCl^- to be decreased as compared with the control group.

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ZYMOGEN PRECURSOR FORMS OF UROKINASE (PRO-UROKINASE) IN FRESH HUMAN URINE. SUMI, H., MARUYAMA, M., TSUSHIMA, H., YAMAMOTO, T. AND MIHARA, H. Dept. of Physiol., Miyazaki Med. College, Kiyotake, Miyazaki-gun 889-16

During isolation of fibrin-binding urokinase (UK) from fresh human urine using a fibrin monomer/Celite column, the presence of zymogen precursor forms was suggested. The main molecular form isolated revealed a molecular weight (mol. wt.) of approximately 100,000 and the minor one was estimated at 150,000-200,000. In contrast, commercially obtained UK preparations contained mostly active enzymes with mol. wts. of 53,000 and 32,000, respectively, and the remaining high molecular forms amounted to less than 2.0% of the total amount. Rabbit anti-UK serum completely inhibited the fibrinolytic activity of all molecular species.

The mol. wt. 100,000 preparation had a very low specific activity (ca. 300 IU/mg protein) and its fibrinolytic and pyro-Glu-Gly-Arg-pNA amidolytic activities were partially enhanced by the addition of trace amounts of human plasmin. In that process, the appearance of two additional protein bands with mol. wts. of 53,000 and 32,000 was also confirmed by zymography.

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ACID STABLE TRYPSIN INHIBITOR IN THE BILE. YAMAMOTO, T., MARUYAMA, M., TSUSHIMA, H., MIZUMOTO, H., IKEDA, R., HAMASUNA, S.*, SUMI, H. AND MIHARA, H. Dept. of Physiol., Miyazaki Med. College, Kiyotake, Miyazaki-gun, 889-16

In patients with malignant tumors (billiary tract carcinoma, pancreas head carcinoma) and gall stones, a trypsin inhibitor which is very stable in acid was identified in the bile as well as in the urine and plasma. Bile trypsin inhibitor (BTI) activities of malignant tumors and gall stones were 3.5±2.8, 2.5±2.3 U/ml, respectively. There was no significant difference between malignant tumors and gall stones. However, these values were almost equivalent to urinary trypsin inhibitor (UTI) and plasma acid stable trypsin inhibitor activities which we reported previously in the normal subjects. BTI was heat stable like UTI. In contrast with UTI which is a slow reactant to trypsin, BTI immediately reacted to tryspin.

BTI was partially purified by DEAE-cellulose, gel filtration and isoelectric focusing. By gel filtration and isoelectric focusing BTI of malignant tumors showed molecular weight of about 26,000 and pI of 4.3. Immunological identity of BTI to UTI was confirmed by double immunodiffusion.

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INTRACELLULAR K^+ ACTIVITY AND REABSORPTIVE FUNCTION OF RAT KIDNEY PROXIMAL TUBULAR CELLS IN URETERAL OBSTRUCTION. SUZUKI, S., *SAITO, Y. AND *NISHIYAMA, A. Dept. of Urology, *1st. Dept. of Physiol., Tohoku Univ. Sch. Med., Sendai 980.

The effect of complete unilateral ureteral obstruction (UUO) of 1 day (1D), 3 days (3D) and 1 week (1W) on peritubular membrane potential (E_m), transtubular potential (E_t), intracellular K^+ activity (A_K^i), the lissamine-green appearance time to the peritubular capillary (T_{LG}), the number of functioning nephrons (FN) and reabsorptive capacity of the single nephron were assessed. In normal rats E_m and A_K^i were -69mV and 81mEq/l respectively. In the UUO both E_m and A_K^i were progressively reduced with the duration of obstruction. However, among tubules a heterogeneous distribution of V_m and A_K^i values was noticed. In all tubules A_K^i's were still above electrochemical equilibrium, suggesting that Na/K pump activity was preserved in UUO. The effect of obstruction on E_t (-2.6mV in control rat) was marginal. The T_{LG} and FN in 1D rats were 3.7sec (142% control) and 5.5 x $10^3/cm^2$ (36.4% control) respectively. Further progressive decreases were seen in 3D and 1W rats. Water flux in 3D rat were 0.0065 nl/sec·mm (22.4% control) and the magnitude was proportional to the E_m values. Results indicated that in UUO where the Na/K pump activity of the peritubular membrane was preserved proximal tubules maintain their reabsorptive capacity so do the GFR relatively intact.

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AGE-RELATED CHANGES OF URINATION IN MICE. NAGASAKI,H. Physiol. Yamanashi Med. Univ. Yamanashi 409-38

In order to know the reason why aged people wake up more frequently by urination during sleep than young people, the time course of urination was measured after intraperitoneal loading of water and saline in young (1.5 month old) and old (21 month old) mice under three different temperatures, 23 ± 1, 36 ± 3 and 6 ± 0.5 °C. The volume of loaded water was 1 ml in young animal (body weight 16 ± 0.3 g, 36 animals) and 2 ml in old animal (body weight 31 ± 0.6 g, 26 animals).

In young animal urination began 60 min after loading under room and hot temperature while it was 30 min under cold temperature, resulting in the average recovery of the loaded water by 89 ± 3 % in young animal during 120 min. Conversely in old animal urination began120 min under room temperature and 180 min under hot and cold temperature. The recovery rate was 26 ± 7 % during 240 min.

The present result showed the delay of urination in old animal after loading of water both in the onset of urination and in the recovery rate of loaded water until 5 hrs after loading. The fact suggests that urination continue ater 5 hrs after loading of water to make an awake during sleep in old animal.

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EFFECTS OF GLIBENCLAMIDE ON THE URINARY CONCENTRATING SYSTEM. TAKEDA, S.*, KUSANO, E., HOMMA, S.* and ASANO, Y. Dept. of Cardiology, Jichi Medical School, Tochigi 329-04

Among sulfonylurea antidiabetic drugs, glibenclamide (GB) is known to decrease the vasopressin action in the kidney, while chlorpropamide(CPMD) enhance the vasopressin action by increasing cortico-papillary gradient of solutes (Kusano et al. J. Clin. Invest. 72:1298-1313, 1983). To clarify the mechanism of action of GB, we examined in vivo treatment of this agent on water excretion rate by water load of 5% body weight, cortico-papillary gradient of solutes and cAMP metabolism in microdissect-ed medullary collecting tubule (MCT) and ascending limb of Henle's loop (MAL) from rats with diuresis induced by 5% dextrose drinking. Treatment of SD rats with GB results in increased water excretion during water load of 5% body weight. It also results in increase of urine flow without change in creatinine, total solute excretion and cortico-papillary gradient of solutes. Furthermore, it has no detectable effect on vasopressin-stimulated cAMP content in MCT and MAL. These results suggest that GB may increase urine flow by the mechanism other than depression of ADH action in the kidney.

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CHARACTERISTICS OF DIURESIS AND NOCTURIA DURING A 7-DAY DRY SATURATION DIVE AT 31 ATA. SHIRAKI, K., SAGAWA, S., KONDA, N., TAKEUCHI, H., and HONG, S. K. Dept. Physiol., Sch. Med., Univ. Occup. Environ. Hlth, Kitakyushu 807; JAMSTEC, Yokosuka 237; and Dept. Physiol., SUNY at Buffalo, USA.

Four male divers were exposed to a dry 31 ATA He-02 environment (31.5°C, 60% rh) for 7 days. Urine was collected diurnally (0700-2200 h) and nocturnally (2200-0700 h) before (predive 1 ATA air), during and after (decompression and postdive 1 ATA air) exposure to 31 ATA, in order to assess diurnal changes in renal functions. A typical hyperbaric diuresis associated with a reduction in urine osmolality was observed in the face of a constant creatinine clearance. Osmolal clearance (Cosm) increased ($p<0.05$) while negative free water clearance decreased ($p<0.05$) at 31 ATA, indicating that the above diuresis has both osmotic (~40%) and water diuresis (~60%) components. Plasma level of ADH decreased nearly 50% ($p<0.05$) at 31 ATA, which recovered only partially during the postdive period. Although urine flow increased at pressure during both day and night, its magnitude was 2-fold greater at night as compared to daytime. Moreover, the diurnal diuresis was entirely due to an increase in free water excretion while the nocturnal diuresis was largely due to an increased Cosm (~80%). These results indicate that the hyperbaric diuresis is induced by inhibition of tubular reabsorption of water (diurnal) and certain solutes (nocturnal).

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THE EFFECT OF INTERACTIONS AMONG RED CELLS ON CELLULAR DEFORMATION. KON,K., MAEDA,N. AND SHIGA,T. Dept. Physiol.II, Ehime Univ., Sch. Med., Ehime 791-02

The red cell deformation has been studied only in dilute suspension, but not in high hematocrit (Ht). This report deals with the cellular deformation at high Ht suspension.

Because of difficulty in observing the deformation in high Ht, partially hemolyzed cells were prepared (MCH= 16pg/cell). Cells were suspended in dextran solutions (dextran; 4-21%, Ht;7-38%) and the deformation in a shear flow was observed by a microscope combined with a viscometer (rheoscope). Partially hemolyzed cells showed the same degree of deformation as intact cells.

Results (i) Under the conditions in which cells were deformed to ellipsoidal disk, the degree of deformation was enhanced as increasing the suspension viscosity regardless of Ht. (ii) At low shear stress, no ellipsoidal deformation was observed, but the non-ellipsoidal deformation became remarkable as increasing Ht. (iii) Whe a tracer amount of intact cells was added to hardened cells (glutaraldehyde treatmer.), the deformation of intact cells was suppressed.

In conclusion, the cellular deformation is enhanced by increasing Ht of deformable cells but not for hardened cells.

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QUANTITATIVE MEASUREMENT OF PLATELET ADHESION ON FOREIGN SURFACE, STUDIED BY TV IMAGE ANALYZER. SHIGA, T. and MAEDA, N. Dept.Physiol.II, Sch.Med., Ehime Univ., Ehime 791-02.

The in vitro platelet adhesion on artificial membrane was studied by a rheoscope, consisted of inverted microscope, transparent cone-plate viscometer, VTR, TV image analyzer and computer. Human platelet rich plasma was flowed at a certain shear rate ($1.5<\dot{\gamma}<6.0$ /s) for 3 - 10 min at 25°C, then the aggregation in stream and the adhesion on surface (e.g., polymethylmethacrylate) developed. Next, the shear rate was increased to 23 /s (τ =0.28 dyn/cm^2) in order to fade out the TV images of flowing platelets, then (1) the number and the area of adhered platelets (spots) were encoded and (2) the size distribution of adhered spots was measured. The time course of adhesion and its dependence on shear rates, ADP concentration and platelet number could be reproducibly measured. The size distribution was nearly of Poisson type.

The deformation of adhered platelets occured after ca. 5 min; at high [ADP], the aggregation in stream was dominant rather than the growth of adhesion spots; at high $\dot{\gamma}$, the adhesion decreased. Therefore, for routine test, $\dot{\gamma}$=3 /s, [ADP]=0.3 μM, [platelet] =200,000 /μl are recommended. An empirical equation, [adhered area (μm^2)/spot] = a · log[platelet] + b , was obtained.

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THE N-B TRANSITION OF BOVINE PLASMA ALBUMIN IN NEUTRAL REGION —— SUBDOMAIN-BREATHING STATE. ERA, S., SHIGEMI, J., ASHIDA*, H., NAGAOKA, S., KUWATA, K., SOGAMI, M., SHIGA**, K. AND WATARI**, H. Dept. of Physiol., Sch. of Med., Gifu Univ., Gifu 500, *Defence Med. Col., Tokorozawa 359 and **Natl. Inst for Physiol. Sci., Okazaki 444

The N-B transition of bovine plasma albumin (BPA) was studied by measuring fluorescence polarization and lifetime of tryptophyl fluorophors, CD-resolved secondary structure (f_α, f_β), ^{1}H-NMR spectra and cross relaxation times. Though the rotational relaxation time of whole BPA was 110-120 ns, those of tryptophyl fluorophors were 70 ns in the N- and B-forms, suggesting tryptophyl fluorophors being mobile. Though changes were seen in $[\alpha]_{233}$ (Wallevik (1973) and Avruch et al (1969)), suggesting some loss of the organized secondary structure, no parallel changes were seen in $[\theta]_{208}$, $[\theta]_{222}$ and f_α . There was no significant difference in cross relaxation times between N- and B-form. The distances between Trp-214 and Tyr-411 of human serum albumin in the N- and B-forms obtained by fluorescence energy transfer, were 18 and 24 A°, respectively (Hagag et al (1982)). These evidence might suggest that changes in the conformation in the N-B transition involve only side chain conformation and/or disulfide bonds, resulting in the increase in the mutual movement of the subdomains without loss of subdomain rigidity and helical content (the subdomain-breathing state).

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TWO DIFFERENT COMPLEMENT SENSITIVITIES OF RED CELLS FROM PAROXYSMAL NOCTURNAL HEMOGLOBINURIA.

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Paroxysmal Nocturnal Hemoglobinuria (PNH) is known as one of the aquired hemolytic anemia associated with intravascular hemolysis fluctuating severity and the occurence of hemoglobinuria. The pathogenetic mechanisms leading to hemolysis remaines still obscure and an effective therapy to improve hemolytic conditions has not been presented. In the present study, we try to investigate the sensitivity to human complement mediated hemolysis of PNH red cells using the complement lysis sensivivity test as reported by Rosse and Dacie (J. Clin. Invest., 45, 736, 1966). Complement was obtained from normal volunteers and the anti-I, cold aggultinin, serum was taken from a patient with a chronic cold aggultinin disease with an aggultination titer of 1/16800 against adult erythrocytes. Complement lysis sensitive titler ($CLSH_{50}$) was 1.5 ± 0.7 (n=7) in normal, (I 2.8, III 23.8) in case I, (I 1.5, III 36.4) in case II, (II 5.6. III 31.3) in case III and (I 4.2, II 6.8, III 16.4) in case IV. The proportions of complement sensitive red cells were 67% in case I, 44% in case II, 38% in case III and 3% in case IV.

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DEGRANULATION OF RAT MAST CELLS INDUCED BY SENDAI VIRUS.

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Sendai virus (HVJ) has the activity of cell fusion and the virus is reported to cause depolarization of the membrane and increase membrane permeability. HVJ-RL strain grown in the eggs and tissue culture cells (BHK-21) was fractionated with sucrose density gradient centrifugation. Dynamic records of cytological changes of virus treated mast cells with a time-lapse cinematography showed cytoplasmic edema followed by the drastic granule extrusion, just like that observed in calcium ionophore treatment. TEM revealed cavities containing many altered granules, which indicates the fusion of the perigranular membrane. Up to 70 % of the histamine was released from purified mast cells by the exposure of cells to HVJ in the presence of Ca^{2+} at 37°C, depending on the hemagglutination titre of the virus. HVJ grown in BHK cells is lacking in cell-fusing activity, and the virus could not liberate histamine from mast cells. After the cleavage of F to F1 and F2 glycoproteins by tripsinization, virus from cultured cells restored the activity of histamine liberation. These results suggest that sequential fusion of the cytoplasmic and perigranular membrane, induced both by HVJ and calcium ions, corresponds to the granule secretion of mast cells.

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RENAL RESPONSE TO HEAD-OUT IMMERSION IN HUMAN AT NIGHT.

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Renal, hemodynamic and hormonal responses to thermoneutral head-out immersion (HOI) were examined in 6 normal fluid-restricted subjects during the day (0900-1400 h) and during the night (2300-0400 h). During the day, HOI was followed by increases in urine flow and sodium excretion. During the night, diuresis and natriuresis induced by HOI were attenuated. During HOI, cardiac output increased significantly during both the day and the night, and no significant difference was observed between the equivalent immersion period during the day and the night. The mean plasma levels of renin activity (PRA) and aldosterone (Paldo) in the time control were slightly lower in the night than those in the day. By HOI, PRA and Paldo decreased significantly during the day but not during the night. Neither PRA nor Paldo during HOI differed between day and night. Plasma levels of ADH decreased significantly during HOI, but no difference was observed in the levels during HOI between day and night. The attenuated HOI-induced diuresis at night cannot be explained by ineffective HOI-induced central translocation of fluid at night. Thus, we may conclude that there exists a circadian variation in responsibility of the renal tubules to the renin-angiotensin-aldosterone system and ADH system.

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THE HYDRATION AND H-NMR RELAXATION TIMES IN SOLUTIONS OF URINE COMPONENTS.

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The aqueous solutions of different human urine components were examined on the NMR relaxation times T_1 and T_2.

They were prepared in 5 mm and 20 mm NMR-tube each as an aliquot of 0.5 and 5 ml at different concentration (0.1 to 5 M for NaCl, KCl and urea, 0.1 to 0.8 M for creatinine) by using pure water with 10 % D_2O for NMR field lock.

With the increase of the concentration, T_1 values showed a tendency for creatinine, NaCl and urea to decrease but for KCl to increase, and T_2 values decreased exponentially from 3.01 sec of pure water to 0.26 for creatinine, to 1.44 for urea, to 2.35 for NaCl and to 2.91 for KCl. From the results, we tried to calculate the extent of each component in the hydration (h, mol/mol) using the formula

$1/T = hc(1/T_b) + (1 - hc)(1/T_f)$; c, the molar concentration of each solution, T_f, T of pure water, T_b, T of each components in the highest concentration.

Assuming the T_b in sec 0.2 for creatinine, 1.2 for urea, 2.0 for NaCl and 2.5 for KCl, the h values were 50, 8, 6 and 2 mol H_2O/mol, respectively.

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QUANTITATIVE ANALYSIS OF HIGH FREQUENCY OSCILLATION IN PHRENIC AND CRANIAL NERVE ACTIVITY.

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Paralyzed rabbits were maintained by artificial ventilation under monitoring end-tidal O_2 and CO_2 levels. After bilateral section of vagus, depressor and sinus nerves, spontaneous efferent discharges of the phrenic(Ph), hypoglossal(XII) and facial(VII) nerve were recorded. The power spectral densities and the coherence functions for these nerve activities were computed. In the power spectrum of Ph, XII or VII, a common peak of high frequency oscillation (HFO: Dittler 1910; Cohen 1973) was observed. As the spectral curve of this peak fitted well to the Lorentzian curve, the peak-frequency and the peak-area power were estimated using the least squares method. Ventilated normally ($F_{E_{CO_2}}$=3.7±0.1%; mean ±SE, n=15), the peak-frequency of HFO in Ph, XII and VII were 96.5±0.7, 95.6±0.3 and 95.4±0.4 Hz, respectively (n=13-15), and the maximum coherence between Ph and XII or VII at the peak of HFO was 0.82±0.03 (Ph-XII, n=15) or 0.82±0.04 (Ph-VII, n=15). The peak-area power of HFO in Ph, XII and VII decreased during hyperventilation ($F_{E_{CO_2}}$=2.4 ±0.1%, n=15) and increased during hypoventilation ($F_{E_{CO_2}}$=5.2±0.1%, n=14) significantly ($p<0.05$). The peak-frequency of HFO in Ph was significantly ($p<0.01$) lowered during hyperventilation and elevated during hypoventilation in parallel with HFO in XII and VII. A regulatory role of HFO in Ph, XII and VII in the maintenance of respiration was suggested.

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CCK MODULATION ON NALOXONE-REVERSIBLE RESPIRATORY SUPPRESSION INDUCED BY THIN-FIBER MUSCULAR AFFERENTS.

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In chloralose-urethane anesthetized, vagotomized, paralyzed and artificially ventilated cats, electrical stimulation of thin-fiber muscular afferents causes naloxone-reversible respiratory suppression a few min after cessation of the stimulation ("after suppression"). However, the magnitude of the suppression varied much among experiments. It has been reported that the activity of opiate systems is influenced by various neuropeptides, and that endogenous CCK may physiologically antagonize opiate analgesia and be involved in opiate tolerance. The effects of proglumide (CCK antagonist) on "after suppression" were investigated in cats. Systemic proglumide (0.02 and 0.2 mg/kg) did not cause substantial effects on resting phrenic nervous activity. At doses of 0.02 and 0.2 mg/kg, proglumide significantly potentiated "after suppression" produced by stimulation at 5 min and 40-60 min after dosage, respectively. There is a possibility that low dose and high dose of proglumide had a tendency to enhance and to attenuate the appearance of "after suppression", respectively. These results suggest that CCK systems may be involved the action of the opiate system on the respiratory regulation system.

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DEPRESSION OF THE INSPIRATORY ACTIVITY BY CEASING THE BLOOD FLOOD TO THE UPPER PART OF THE BRAIN STEM, AND THE LEVELS OF ALVEOLAR P_{CO_2} AND P_{O_2}.

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The effects of occlusion of the basilar artery and the external artery on the inspiratory activity were studied in anesthetized, vagotomized and paralyzed cats. The basilar artery was previously ligated at about the same level as the origin of the roots of the sixth cranial nerve. Bilateral occlusions (two min) of the external carotid artery caused a significant depression in the integrated phrenic nerve activity (PNA), which disappeared within one min under hypocapnic condition. The depression effect on PNA reduced under hypercapnic or hypoxic condition. Systemic artery blood pressure began to fall markedly after one min of the occlusion and, then, to increase above a control, which returned to a control level usually within 2 - 3 min. Falling in systemic blood pressure produced transiently a gasping type in PNA. This effect of occlusion on the systemic blood pressure or PNA was also observed on carotid sinus nerve denervated cats. These results indicate that the upper part of the brain stem has an intrinsic role on the inspiratory activity.

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POSSIBLE FUNCTIONAL FLEXIBILITY (PACEMAKER SHIFT AND BILATERAL INTERACTION) IN THE BRAIN BRAINSTEM RESPIRATORY NEURONAL ORGANIZATION IN THE LAMPREY.
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'Natural' respiratory pacemaker site probably is 'ipsilaterally' located somewhere (such as trigeminal, facial or their intermediate zone) in the rhombenchalic basal plate in lampreys. The present study tested mainly the effect of microstimulation (2 msec, pulse duration, 20-50 μA, delivered to one side of the facial region) on respiratory potentials intracellularly recorded from the contralateral side of the same region. The distance from stimulating site to recording was 1-2 mm. Result 1) Respiratory prepotentials sometimes were not accompanied by spike potentials, indicating subthreshold oscillation of spontaneous depolarization. 2) Respiratory spiking preceded by prepotentials could be entrained driving pulses with frequencies just higher than its own. Onset latency of driven spikes was 10-20 msec. 3) With time after arrest of breathing by curarization, respiratory discharges gradually decreased in cycle frequency (from 2 to 0.15 Hz), increasing in spike number per cycle (from 1-2 to 3-5/cyc.), while these changes could be reversed by artificial ventilation. The results suggest that pacemaker shift and oxygen delivery by microflow of cerebral blood are important in the maintenance of breathing rhythm-generation especially in the case of brain lesions in the lamprey.

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EFFECT OF STRYCHNINE ON PERIPHERAL RESPIRATORY ACTIVITIES IN BRAIN STEM-TRANSECTED CATS.
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Respiratory activities were recorded from the phrenic (inspiratory) and pharyngeal constrictor (expiratory) nerves. Transections of the brain stem at the middle pons in anaesthetized, paralyzed, and bivagotomized cats caused apneustic respiration. After the pontile transections, the apneustic pattern of respiration was gradually normalized in many cats. The injection of strychnine to the normalized animals could facilitate and prolong the inspiratory activity, and finally it could induce apneusis again. Vagal inhibition on inspiratory activity was less effective in strychnine-induced apneusis than in normal respiration. Peripheral expiratory nerves showed tonic activity during strychnine-induced apneusis. These results suggest that strychnine-induced apneusis in brain stem-transected animals with normalized respiratory patterns may be produced by disinhibitory action of the drug on the pontomedullary structures without pneumotaxic centers.

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AN INTRACELLULAR STUDY ON EXPIRATORY NEURONS OF THE BOTZINGER COMPLEX.
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In Nembutal anesthetized cat, we have studied electrophysiological properties of expiratory neurons located in the Botzinger complex (BOT-E neuron) and those of superior laryngeal nerve (SLN) motoneurons. Extracellular study showed that the BOT-E neurons and SLN motoneurons were recorded in the same area around the retrofacial nucleus. However, the former was distributed in a more ventrolateral part of the nucleus of the latter. The membrane potential from some SLN motoneurons was shown to begin to depolarize by EPSP at the onset of phrenic nerve discharges and to hyperpolarize with the waning of phrenic nerve discharges. The membrane potential from the BOT-E neurons started to depolarize at the onset of expiration and was suddenly hyperpolarized by IPSPs just before the onset of the inspiratory phase. Stimulation of the SLN afferents induced polysynaptic EPSPs in SLN motoneurons (the mean latency : 3.8 ± 0.5 msec) and polysynaptic IPSPs in BOT E-neurons (5.9±0.65 msec).

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DIFFERENTIAL RESPONSES OF THE RAT RESPIRATORY CENTER IN VITRO TO H^+ AND CO_2.

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The primary stimulus to central chemoreceptors in the mammal has been considered to be H^+. In contrast, our recent studies have shown that CO_2 is also capable of stimulating the central chemoreceptor. In the present work, we examined whether H^+ and CO_2 activate the same population of central chemoreceptors. The brain stem, cervical cord and attached phrenic nerve were excised from neonatal rats under ether anesthesia and superfused with a physiological saline solution. The perfusion fluid was equilibrated with CO_2 (0-7%) in O_2, and the $NaHCO_3$ concentration was adjusted to maintain the pH at desired levels. Respiratory activity was recorded from the phrenic nerve. The phrenic nerve discharges were integrated for each 0.1 sec period. The peak integrated value of each respiration was used as a criterion of the respiratory magnitude. When the pH was reduced with constant P_{CO_2}, both the magnitude and frequency of respiration increased. When P_{CO_2} was increased at constant pH, the respiratory magnitude increased, but the frequency remained unchanged. The CO_2 effects were seen only transiently, whereas the H^+ effects were exerted as long as a given pH level was maintained. It is concluded that H^+ and CO_2 activate different subpopulations of the central chemoreceptor for respiratory control.

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TRANSIENT AND STEADY-STATE RESPONSES OF BULBOSPINAL EXPIRATORY (BS-E) NEURONS TO HYPERCAPNIA AND ISOCAPNIC HYPOXIA.

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Activity of BS-E neurons was recorded intracellularly and extracellularly in the nucleus retroambigualis of decerebrated, spontaneously breathing cats. Spinal projection of BS-E neurons was determined by antidromic stimulation at the contralateral C4 segment. Isocapnic hypoxia resulted in an excitatory effect on BS-E neuron activity, as evidenced by an increase in rhythmic slow depolarizing potentials with an elevation of spike frequency (augmenting pattern). Hypercapnia (5-9% CO_2) caused a biphasic response; an initial excitation followed by a late inhibition. The inhibitory effect was characterized by a shift toward hyperpolarization throughout the respiratory phase; the onset of the expiratory firing was delayed and the rate of firing was decreased. These findings indicate that the stimulation of peripheral chemoreceptors exerts an excitatory effect on BS-E neuron activity but hypercapnia causes an inhibitory effect either directly or via central chemosensitive areas.

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BRAINSTEM CONTROL OF SPINAL RESPIRATORY NEURONS.

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We previously reported that respiratory neuron activities could be recorded in the intermediolateral zone of the gray matter of the upper cervical (C1-C2) cord in lightly anesthetized cats.

In the present experiments, brainstem projections to the cervical respiratory neurons were examined. Using extracellular microelectrodes, medullary and cervical inspiratory neuron discharges were simultaneously recorded, and then a medullary recording electrode was switched to a stimulating electrode. Most of the cervical inspiratory neurons were antidromically activated by microstimulation (20-50 μA) with latencies of 2-8 msec. Contralateral stimulation of N.retroambigualis activated 93% of the cervical units examined, while ipsilateral stimulation activated 55% of them. Contralateral and ipsilateral stimulation of N.tractus solitarius activated 65% and 55% of them, respectively. Electrical stimulation (200 Hz, 10-50 μA) of N.raphe magnus (P4-P10) was found to inhibit both the cervical respiratory neuron activities and phrenic motor discharges. On the other hand, stimulation of the dorso-caudal part of the medulla(P8-P10) along the midline decreased the cervical neuron discharges, but prolonged the phrenic nerve discharges.

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RECOVERY OF FORELIMB VOLUNTARY MOVEMENT FOLLOWING REINNERVATION OF FORELIMB MUSCLES BY THE PHRENIC NERVE IN THE CAT. FUJITO, Y., KAWASAKI, H. and AOKI, M. Dept. of Physiol. Sapporo Medical College, S.1, W.17, Sapporo 060

We examined whether cats can compensate movement disorders when once-denervated arm muscles are reinnervated by a respiratory nerve. Right phrenic and musculocutaneous nerves were cut for cross union and the proximal stump of the phrenic nerve was united to the peripheral stump of the musculocutaneus nerve. In surgical operations, cats were anesthetized with pentobarbital sodium. Reinnervation of arm muscles by the phrenic nerve was confirmed by retrogradely labeled phrenic motoneurons following injection of HRP to the M.biceps brachii. EMG recordings from arm muscles and video recording of arm movements were simultaneously conducted to analyze behaviors of the cross-innervated forelimb. Teflon coated stainless steel wires were chronically implanted in the right M. biceps brachii or M.brachialis, M.triceps brachii and the left diaphragm to record EMGs from freely moving cats. EMGs of elbow flexors synchronized with those of the left diaphragm. During stereotyped movement such as walking, EMGs of right elbow flexors did not synchronize with walking cycles. In contrast, during voluntary goal directed movements, cats could reach their right forepaw to goals smoothly with elbow flexion which was associated with EMGs of right elbow flexors, indicating functional compensation of forelimb movement disorders by the respiratory nervous system.

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EFFECTS OF COLD AIR INTO THE ISOLATED INNERVATED UPPER TRACHEA ON THE PATTERN OF BREATHING IN RABBITS. SASAKI K.,UCHIDA H., NEMOTO H. AND KINOSHITA M. The 4th Dep. of Internal Med., School of Med.,Univ. of Toho,Meguro-ku,Tokyo. #153.

In chloralose-urethan anesthetized and tracheostomized rabbits(n;6), we introduced cold dry air(0°C, 100-200 ml per min.,30 seconds) into the isolated and innervated upper trachea with retrograde flow, and measured time of inspiration(Ti,sec), time of expiration(Te,sec), respiratory frequency(f; 60/(Ti+Te),rates/min) and electrical integration of the action potentials of the right phrenicus nerve($\bar{H}$, arbitrary units). These variables in baseline were .67 $\pm$ .02, .78$\pm$ .11, 43.8 $\pm$ 3.5, and 16.1 $\pm$ 1.7 for Ti, Te, f and $\bar{H}$ (mean $\pm$ SE),respectively, and,during cold air, were changed into .75 $\pm$ .02 (Ti, $p<0.001$(***)), .84 $\pm$ .10(Te, $p<0.01$(**)), 38.8 $\pm$ 2.6(f, **) and 18.2 $\pm$ 1.6($\bar{H}$, $p<0.05$(*)) with vagi intact. On the other hand, with vagi cut,cold air diminished frequency(*) only by the elongation of Te(1.06 to 1.11 *) and increased $\bar{H}$ (20.2 to 21.7 *). These changes of Te and $\bar{H}$ were still observed after bilateral sections of superior laryngeal nerves.

These results suggest that cold dry air increases neural outputs and suppresses respiratory frequency with prolongation of both Ti and Te, but that the elevation of neural outputs and the changes of Te are extravagally controlled in the cold air-induced changes of the pattern of breathing.

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CHARACTERISTICS OF INSPIRATORY FACILITATION AND INHIBITION CAUSED BY HIGH FREQUENCY AIRWAY INFLATION IN RABBITS. HOMMA, I., ONIMARU, H., OOUCHI, M. and ICHIKAWA, S. Dept. of Physiol., Showa Univ. School of Med., Shinagawa-ku, Tokyo 142

Experiments were performed on 12 rabbits anesthetized with urethane (Ure)-chloralose(Chl) (initial dose: 450mg Ure + 45mg Chl/kg i.v.). The left C_5 phrenic nerve was dissected free, cut distally, desheathed and prepared for recording. The right vagus nerve was also dissected free, cut orally, and dissected fine to record unitary afferent activity. Peak intratracheal pressure of high frequency inflation (HFI) was measured at 12cmH_2O. Respiratory rate was increased by inflating at frequencies below 37Hz and was decreased by higher frequencies. Slow adapting receptors (SAR) were activated by HFI and afferent pulses recorded from the vagus nerve increased more during relatively high frequency inflation. Rapid adapting receptors (RAR) were also activated by HFI, but the afferent pulses were almost equal at each frequency between 10 and 100Hz. Results indicate that HFI at frequencies below 37Hz dominantly facilitate RAR to reflexely increase respiratory rate and frequencies above 37Hz dominantly facilitate SAR to reflexely decrease respiratory rate.

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THE EFFECT OF CO_2 ON RESPIRATION IN HIBERNATING HAMSTER

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Hibernating hamster shows typical periodic breathing pattern which have long term apnea for several minutes following several breathings. Breathing number in grouped breathing is paralleled with the length of apneumatic phase. The period of apnea was considered to expand along with progressing stage to more deep hibernation. When body temperature was increased by rising of ambient temperature, the respiratory period shortened with an inverse pattern which was observed at progressing stage to deep hibernation. The breathing period, therefore, may be depend on core temperature. When ambient CO_2 content was rised gradually at very small step, respiratory rate and depth were increased, apnea shorteded and disappeared at about 5.9% CO_2 (45 mmHg). In case of acute exposure to certain environmental CO_2 level, respiratory rhythm was changed and required several minutes for stabilizing it. From these results, even in hibernating hamster, the respiration was considered to be regulated normary, and its regulation were related not only with arterial blood gases but also with intracelluler pCO_2 or pH level.

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CONVECTIVE AND DIFFUSIVE GAS MIXING IN TERMINAL AIR WAY.

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For the analysis of O_2 gas distribution of terminal bronchial tree (from 18 to 24th generation), Navier-Stokes equation and convection-diffusion equation of two dimension coupled and solved numerically by finite element method. The time dependent solution of flow, pressure and concentration distribution are obtained by method based on explicit numerical integration schema in time. If only diffusion equation is considered, the time dependent concentration reaches to its steady distribution within 0.2 sec. But if Navier-Stokes equation are coupled to convection-diffusion eauation and flow velocity is set to $V_0 SIN(2\pi t/T)$, V_0 = 0.03 cm/sec, T = 4.0 sec, for the boundary conditions at the alveolar wall, it takes 2.0 sec to reach steady distribution of concentration. Further our calculation has shown that there must be inhomogeneous concentration distribution of O_2 depended on the histry of asymmetric branching angle of air way.

As a conclusion our model of convective-diffusive O_2 transport in the terminal air way suggest existence of stratified inhomogenity and uneven distribution of O_2 depended on branching histry of air way.

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RECOVERY FROM POSTHYPERVENTILATION HYPERPNEA AND ITS CHANGE DURING THE MENSTRUAL CYCLE.

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The time course of the decrease in ventilation immediately after 1 min of voluntary isocapnic hyperventilation was studied in 10 healthy menstruating women. The hyperventilation test was done at different levels of P_{ACO_2}, ranging from resting to hypercapnic (60 mmHg) levels. The subjects underwent the test in each of the follicular and luteal phases of the menstrual cycle. The time constant (τ) of the recovery from hyperpnea occurring after hyperventilation was, on an average, 19.5 sec in the follicular phase with the resting P_{ACO_2} of 40.8 mmHg while it was 24.9 sec in the luteal phase with the resting P_{ACO_2} of 37.8 mmHg. The τ was inversely related to the P_{ACO_2} level during the hyperventilation test, with regression equations of $\tau=63.8-1.1P_{ACO_2}$ in the follicular phase and $\tau=71.8-1.3P_{ACO_2}$ in the luteal phase, the difference being not significant. The higher time constant seen in the luteal phase appears to be accounted for by the lower resting P_{ACO_2}, compared to the follicular phase.

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CARDIO-RESPIRATORY RESPONSES DURING EXERCISE IN HYPER-, HYPO-, AND NORMOXIC CONDITIONS.
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For the purpose to evaluate the validity of so-called "cardio-dynamic" hypothesis of Wasserman et al. in connection with the role of carotid body in the exercise hyperpnea, ventilation, cardiac output and gas exchange variables in response to a step work load of 70 W were studied in men using a bicycle ergometer in three different inspired oxygen conditions, i.e., 14, 21 and 41 %. The slope of CO_2 response curve obtained at rest was steeper in hypoxia and gentler in hyperoxia than in normoxia. Oxygen consumption did not differ with the inspired oxygen in both the phases of rest and exercise. At the steady-state of exercise (phase 3) minute ventilation ($\dot{V}E$) increased inversely proportionally with the reduction of inspired oxygen. The time constant of rising phase (phase 2) of $\dot{V}E$ became prolonged in hyperoxia while that of $\dot{Q}$ remained unchaged. There was a close correlation between both the time constants of $\dot{V}E$ and VCO_2. VE increased abruptly at the very early period after exercise onset in normoxia. On the other hand, no such an initial response was observed in $\dot{Q}$ and heart rate. As the results, end-tidal pCO_2 at the initial period of exercise was hypoxic in hypoxia and hypercapnic in hyperoxia. These results suggest that the cardio-dynamic hypothesis is valid as an explanation for phase 2 and 3 but invalid for phase 1. Some neurogenic stimuli, either peripheral or central origin would stimulate carotid body via sympathetic at the begining of exercise.

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Control of ventilation related to diminished CO_2 elimination in CO_2 breathing.
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Diminished CO_2 output during 3 and 4.5% CO_2 inhalation was consecutively followed for 60 min in 6 healthy subjects. Time profile of CO_2 output change was considered to represent CO_2 wash-in curve in the body. By eponential peeling technique CO_2 equilibrium in the slow compartment was excluded, and the process of CO_2 accumulation in the rapid compartment of the body fluid was estimated. Slow attainment of steady state CO_2 response was in large part explained from this CO_2 accumulation. Overall loop gain of the CO_2 - ventilation feedback control system was low until 20 min. But this was reasonably corrected when considered that this was due to the process of CO_2 accumulation. Depression of CO_2 elimination affected to partially compensate the level of loop gain which was greatly decreased by P_{ICO_2} increase. We concluded that diminished CO_2 elimination during CO_2 inhalation accounts well for the time profile of ventilation control, in terms of both ventilatory response and the overall loop gain of CO_2 - ventilation feedback control system.

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COMPARISON OF HYPOXIC AND HYPERCAPNIC VENTILATORY RESPNSES BETWEEN NON-ATHLETES AND JUDO ATHLETES WITH MODERATE OBESITY.
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Ventilatory responses to hypoxia and hypercapnia of 24 non-athletes(C)were compared with those of 21 judo athletes(J). The degree of obesity was comparable in each group. However, amount of body fat was significantly higher in the former than the latter group. Hypoxic ventilatory sensitivity as measured by parameter A in the hyperbola response equation was 756±501 $l \cdot min^{-1} \cdot mmHg$(mean±SD)in the C and 533±368 in the J group, respectively. The difference was insignificant. Hypercapnic ventilatory sensitivity as measured by paramater S in the linear response equation was significantly higher in the C(2.74±0.99 $l \cdot min^{-1} \cdot mmHg^{-1}$)than the J group(1.38±0.63)($P<0.01$). The relationships between S and body weight(BW)were significant in the J($r=0.55, P<0.01$)and in borderline significance in the C group($r=0.41, 0.55<P<0.1$). Also, A showed augmenting tendency with increasing BW, though the corelation coefficients between both parameters were insignificant. Such positive correlations of augmenting S and A with increasing BW were disappeared when S and A were eraluated in terms of lean body mass. On the contrary, S and A were significantly correlated with the degree of fat accumulation in both groups. We concluded that less hypoxic and hypercapnic ventilatory sensitivity in the judo athletes can be accounted for by less amount of body fat.

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COMPARISON OF VENTILATORY RESPONSE TO DEAD SPACE APPLICATION AND CO_2 INHALATION AT THREE O_2 LEVELS. MARUYAMA, R., MASUYAMA, H., NISHIBAYASHI, Y., HONDA, Y.
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In fifteen normal male subjects, ventilatory response to external dead space application (DS run) and CO_2 inhalation (CO_2 run) were compared at three O_2 levels, i.e. hyperoxic (P_{ETO_2}>200mmHg), normoxia (P_{ETO_2}≒100mmHg) and hypoxia (P_{ETO_2}≒60mmHg), respectively.

These studies indicate that at three O_2 levels ventilatory response ($\dot{V}_E$) was larger in the DS run than the CO_2 run. Although the respiratory rate was not significantly different between two runs. Therefore, higher $\dot{V}_E$ was accounted for by larger V_T. P_{ETCO_2}-$\dot{V}_E$ response slope was steeper in the DS run than in the CO_2 run. This difference between two runs increased with increasing degree of hypoxia. The peripheral chemosensitivity may contribute to enhance vnetilatory activity in the DS run.

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REPRODUCIBILITY OF VENTILATORY RESPONSE TO HYPERCAPNIA AT REST. HIRUTA,S., KANAO,Y., MIYAMURA,M. Lab. Work Physiol. Res. Cent. Hlth. Phys. Fit. Sports, Nagoya University, Chikusa-ku, Nagoya

To examine reproducibility of the ventilatory response to CO_2, resting hypercapnic ventilatory response for each subject was measured twice with 2-13 days interval in twenty-four healthy male. The slope (S) of ventilatory response curve to CO_2 was determined by rebreathing method. The slope of high responders significantly decreased at the second measurement as compared with the first. This decrease was caused by the fact that, increment rate of ventilation at higher P_{ACO_2} was smaller than that at lower P_{ACO_2}. However, the decrease became insignificant when the slope of a tangential line obtained by approximating the response curve to a curve of secondary degree was used instead of the slope of a linear line.

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VENTILATORY RESPONSES TO EXERCISE AND HYPERCAPNIA BEFORE AND AFTER PHYSICAL TRAINING. MIYAMURA,M., HIRUTA,S., KANAO,Y. Lab. Work Physiol. Res. Cent. Hlth. Phys. Fit. Sports, Nagoya University, Chikusa-ku, Nagoya

Ventilatory responses to exercise and hypercapnia were determined in the 5 male students before and after physical training. The subjects were belong to the Badominton club of our University and trained for about 4 years. Maximum oxygen uptake and maximum pulmonary ventilation were measured by the Douglas bag during maximal treadmill exercise. The slope of ventilatory response curve to CO_2 at rest was determined by rebreathing method. It was found that maximum oxygen uptake and ventilation after training was increased significantly, and the slope of ventilatory response to hypercapnia was decreased after training. These results indicate that physical training have an effect on not only aerobic power, but also the hypercapnia ventilatory drive at rest.

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DIFFUSION RATE FACTOR OF CO_2 OUT OF RED BLOOD CELLS IN THE PULMONARY CAPILLARY.
MOCHIZUKI, M., KAGAWA, T. Department of Physiology, Yamagata University, School of Medicine, Yamagata 990-23

The simultaneous partial differential equations for diffusions of O_2, CO_2 and HCO_3^- ions were solved by using a disc red cell (RBC) model. In addition to the changes in Po_2, Pco_2 and HCO_3^- content caused by the diffusion, the changes in So_2, the Bohr-effect, the Haldane-effect and the Pco_2 change due to the HCO_3^- shift were computed. Moreover, the change in extracellular HCO_3^- caused by the plasma buffer system was also taken into account, assuming that the time constant of CO_2 dehydration reaction in plasma in reduced to 1.5 sec by carbonic anhydrase in the capillary wall. For the parameters such as the buffer values and the Donnan ratio, the values so far measured by us and previous authors were applied. The diffusivity across the RBC boundary for O_2, CO_2 and HCO_3^- ions was expressed by the respective transfer coefficients, which were obtained in RBC suspension by a stopped flow method. The calculated data demonstrated that the CO_2 dehydration reaction out of the RBC was not completed during the contact time of 0.4 to 1.0 sec, suggesting possibility to estimate the contact time from the measured venous-arterial CO_2 content difference, the CO_2 diffusion rate factor, and the time average of the Pco_2 gradient across the RBC membrane. The calculated contact time coincided well with that estimated from the pulmonary diffusing capacity for CO and the cardiac output.

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EVALUATION OF VENOUS ADMIXTURE IN THE PULMONARY O_2 AND CO_2 EXCHANGE.
DOI, K., NIIZEKI, K., KAGAWA, T. AND TAKAHASHI, K*. Dept. of Physiol., Yamagata Univ. Sch. Med., Yamagata, 990-23 and * Dept. of Internal Medicine, Yamagata Univ. Sch. Med., Yamagata, 990-23.

For measuring the amount of venous admixture (shunt) in the pulmonary O_2 and CO_2 exchange in man a new approach based on a two compartment model with the reciprocal $\dot{V}A/\dot{Q}$ ratio has been developed and proved to be valid and practical. A total of thirty four studies were made on ten normal and healthy subjects (ages, 20 to 63), eight males and two females in a sitting position at rest. Each of $\dot{V}A/\dot{Q}$ line, the equi-RE lines and the arterial and alveolar Po_2-Pco_2 lines was computed by using the measured arterial and end-tidal Po_2 and Pco_2, gas exchange ratio during the steady state breathing, $avDo_2$ by a rebreathing method and the O_2 and CO_2 dissociation curves. Graphical analysis demonstrated that the equi-RE line drawn through the measured arterial Po_2-Pco_2 point coincided well with the arterial Po_2-Pco_2 line computed in the reciprocal $\dot{V}A/\dot{Q}$ model by selecting an appropriate volume ratio of the compartment I to II. Then, this method made it possible to find $\dot{V}A/\dot{Q}$ equality which is preresquisite for determining the shunt alone and also to estimate the extent of effect on $\dot{V}A/\dot{Q}$ inequality of alveolar dead space and venous admixture and detected virtually the degree of the shunt in five subjects (= 13 studies) out of ten subjects in the present study. Thus, the results provided a support evidence in validity and usefulness of this approach.

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RELATIONSHIP BETWEEN ERYTHROCYTE BUFFER VALUE AND HEMATOCRIT.
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To analyze the effect of hematocrit(Hct) on CO_2 transport capacity, whole blood and true plasma CO_2 dissociation curves were measured by varying Hct and estimated the intracellular buffering capacity(βc). Blood samples with various Hct were prepared from the venous blood of 5 healthy subjects after centrifuging at 37°C. These samples were equilibrated at a Pco_2 range from 20 - 80 Torr Pco_2 and 200 Torr Po_2. Blood gas analyses were made with the respective electrodes and Natelson's analyzer. The intracellular pH(pHc) was determined by freeze-thaw method. In a Hct range from 0.18 to 0.82, Donnan ratio of bicarbonate and pHc/pHp relationship were not significantly affected by Hct variation. With increasing Hct , true plasma bicarbonate content,$(HCO_3^-)p$, decreased and the slope of $(HCO_3^-)p$ to the Pco_2 increased, whereas the intracellular bicarbonate content showed a smaller variation. The calculated βc took a upward convex curve against Hct, showing a maximum βc of about 60 mmol/l,RBC pHc around 45 % Hct. The βc was inversely related to pHc: The slope of βc to pHc was linealy related to the Hct. Namely, the bicarbonate change per unit volume of RBC was augumented with decreasing Hct.

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OXYGEN DIFFUSION COEFFICIENTS OF CELL MEMBRANES STUDIED BY MEANS OF A NANOSECOND FLUORESCENCE DEPOLARIZATION. KOYAMA, T., ARAISO, T. Division of Physiology, Res. Inst. Appl. Electr., Hokkaido University, Sapporo 060

Microviscosity and wobbling diameter of cytoplasmic membranes can be separately measured by means of a nanosecond fluorometer and diphenyl hexatriene. Diffusion coefficient for oxygen in the cytoplasmic membranes can be estimated from Einstein-Stokes equation with the following two assumptions; (1) the measured microviscosity is valid for oxygen passage through the membranes, (2) the oxygen molecule is approximately a sphere having a mean diameter of 2 Å. The diffusion coefficient was different among species and among organs in one species. The diffusion coefficient of white ghosts of red blood cells was; 0.84 in sheep, 1.25 in human, 1.49 in rat and 1.89 in rabbits at 37 °C, each in the unit of x 10^{-7} cm^2/sec, while the wobbling diameter (the diameter of a hypothetical cylindrical space in which DPH molecules can wobble) was 10.7, 11.5, 12.1 and 10.7 Å in respective species. In frogs the diffusion coefficient of oxygen was 0.78, 3.15 and 3.83 x 10^{-7} cm^2/sec in red cell ghosts, lung cells and myocytes, respectively, and the wobbling diameter, 12.1, 12.4 and 13.4 Å, respectively, at 30 °C. It seems that both the oxygen diffusion coefficient and the wobbling diameter of cell membranes are large in organs having a high metabolic rate.

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THE MINIMUM DISTANCE BETWEEN HEMOGLOBIN AND ERYTHROCYTE MEMBRANE. A PULSED LASER FLUORESCENCE STUDY. ARAISO, T., NITTA, J. and KOYAMA, T. Division of Physiology, Res. Inst. Appl. Electr., Hokkaido University, Sapporo 060

The time resolved fluorescence measurement of diphenylhexatriene (DPH) which was embedded in the lipid bilayer of intact erythrocyte could be performed with aid of pico-second pulsed laser excitation. By energy transfer from DPH to hemoglobin, the fluorescence life time decreases according to distance between DPH and hemoglobin. Fluorescence decay of DPH in erythrocyte ghost membrane obeyed biexponential curve: as having τ_1 = 4.5nsec (9%), τ_2 = 11.4nsec (91%). The fast components may correspond to the fluorescence of DPH near membrane proteins. For the intact erythrocyte, the fluorescence decay of embedded DPH could be simulated by three components exponential curve with τ_1 = 0.24nsec (3%), τ_2 = 2.5nsec (33%) and τ_3 = 7.7nsec (64%). Thus, three different values were calculated for hemoglobin-DPH distance, 33Å, 43Å and 60Å. The shortest value may be caused by the interaction between band 3-binding-hemoglobin and DPH. The other two values indicated that the DPH molecules lie mainly at two different positions. Since the distance of the two positions was 16Å which is very close to the length of DPH (17.5Å), those position would be attributed to outer and inner phospholipid layers. From above data a value of 10Å was calculated for the minimum distance between the surface of hemoglobin and the surface of inner lipidlayer.

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INFLUENCE OF LOW OXYGEN-AFFINITY BLOOD ON OXYGEN UPTAKE BY EXERCISING GRACILIS MUSCLE. KOHZUKI, H., ENOKI, Y., TOMITA, S., AND SAKATA, S., Dept. of Physiol., Nara Med. Univ., Kasihara, Nara 634.

To clarify the effect of low oxygen-affinity blood (LAB) on oxygen transport to tissue, we perfused resting and isometrically contracting canine gracilis muscle (n=6) with LAB and normal oxygen-affinity blood (NAB). LAB was prepared by incubation of red cells with phosphoenolpyruvate in ACD. P_{50} (oxygen half-saturation pressure) for NAB and LAB were 36.6 and 45.5 Torr, respectively. Vo_2, as determined by the Fick principle, at rest andafter recovery forLAB were not significantly different from those for NAB. Also, Vo_2 and tension developed during exercise for LAB were not significantly different from those for NAB. In both the cases, Vo_2 increased with increaseing tension. This relationship was the same for NAB and LAB. But, oxygen pressures of the effluent venous blood (Pvo_2) at rest, during exercise and recovery for LAB were significantly higher than those for NAB. Thus, we concluded that the higher Pvo_2 induced by LAB does not influence on oxygen uptake by the resting and exercising skeletal muscle.

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PROJECTION OF UPPER STRUCTURE TO THE SPINAL PHRENIC MOTONEURONS IN CATS: AN HRP STUDY USING MICROINJECTION METHOD. ONAI, T. AND MIURA, M. Dept. Physiol. 1st Div., Gunma Univ. Sch. Med., Maebashi 371

We studied, with the horseradish peroxidase (HRP) method, supraspinal structure projections to the phrenic motoneuron pools in the anterior horn of C5 segment of the cat spinal cord. A double-barrel coaxial electrode was used, comprised of an inner electrode for recording evoked potentials and outer electrode for injecting HRP. We determined the HRP injection site in which the antidromic potential of the phrenic motoneurons was evoked by stimulation of the phrenic nerve. Out of 4 experiments, some 1222 HRP-labeled cells were identified. They were distributed in only 2 regions of the medulla oblongata, the ventrolateral part of the nucleus tractus solitarii (NTS) and the ventrolateral quadrant of the nucleus retrofacialis (Rf) and ambiguus (Amb). The distribution is as follows: (1) the contralateral NTS (16.7%); (2) the ipsilateral NTS (4.8%); (3) the contralateral Rf (6.0%); (4) the ipsilateral Rf (3.8%); (5) the contralateral Amb (30.2%); (6) the ipsilateral Amb (18.7%).

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MECHANISM OF THE RESPIRATORY CENTER ESTIMATED FROM CHANGES IN VAGAL EFFERENT RESPIRATORY DISCHARGES. HISAMITSU, T. and TAKESHIGE, C. Dept. of Physiol., Sch. of Med., Showa Univ., Shinagawa-ku, Tokyo 142

The vagal efferent unit discharges appearing in the finely dissected central end of the cervical vagus nerve and respiration were recorded simultaneously in urethan anesthetized rabbits. Some neurons on one side of the vagal nerve continuously discahrged. These discharges became periodic, increasing firing rates during inspiration and decreasing during expiration, and new discharges appeared concomitantly with inspiration or expiration after the other side of the vagus was severed. Such periodic discharges once again became continuous, and newly-appeared discharges were extinguished by low frequency stimulation of the other side of the vagus. The continuous discahrges were inhibited by high frequency afferent stimulation of the dissected vagus nerve. Central respiratory mechanisms were proposed as following: Two kinds of inspiratory (IC_1 and IC_2) and expiratory (EC_1 and EC_2) centers exist in the respiratory center. 1) IC_1 is inhibited by high frequency vagal afferent stimulation and the inhibitory effect of IC_1 on the expiratory center subsides, and hence expiration was induced by high frequency vagal stimulation. 2) EC_2 is inhibited by low frequency vagal afferent stimulation and the reciprocal inhibitory effect of EC_2 on IC_1 disappeared. As a result, inspiration was induced by low frequency vagal stimulation.

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THE STIMULATING EFFECT OF INJECTION OF HIGH CO_2 BLOOD INTO THE VERTEBRAL ARTERY ON INSPIRATORY ACTIVITY IN CATS. KUWANA, S. and NATSUI, T. Dept. of Physiol., Teikyo Univ. School of Med., Itabashi-ku, Tokyo 173.

Integrated phrenic nerve activities (PNA) were recorded as an indication of output of the respiratory center in twenty-nine anesthetized cats. After vagotomy and carotid sinus neurectomy, cats were paralyzed and artificially ventilated. Injection of high CO_2 blood ($pCO_2 \geq 160$ mmHg) into the vertebral artery (1.7-3.1 ml/min, 10-30 sec) produced a transient increase in the PNA. This increase seems to be due to the stimulating effect of CO_2 on the central chemoreceptors. In eight cats, this stimulating effect disappeared by the bilateral occulusion of the anterior inferior cerebellar arteries (AICA). In nine cats, the stimulating effect disappeared by the occulusion of both the AICA and the posterior inferior cerebellar arteries (PICA). These results indicate that the central chemoreceptors in respiratory control system mainly located in perfused area of the AICA. Whereas, in eleven cats, despite of simultaneous occulusion of several branches (containing the AICA and PICA) from the basilar artery in the ventral surface of medulla, the PNA increased slightly by injection. Therefore, it is assumed that, if circulation of the AICA stopped, this chemosensitive area was perfused by the blood of the PICA and/or the basilar artery.

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ROLE OF CO_2 OSCILLATION IN THE CONTROL OF RESPIRATION

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Role of tidal fluctuation of $Paco_2$ in the control of respiration was evaluated in urethane-chloralose anesthetized dogs. As ventilatory stimuli, intravenous CO_2 infusion using an artificial lung (endogeneous CO_2 loading, ENDO-CO_2) and CO_2 inhalation (exogeneous CO_2 loading, EXO-CO_2) were employed. In the former, isocapnic hyperpnea could be observed, whereas, in the latter, response was hypercapnic hyperpnea. As a possible parameter which conveys information of increased CO_2 output, we adopted the maximum rate of increase of $Paco_2$ (Max ($dPaco_2/dt$)) calculated from continuous measurement of arterial pH with an ISFET pH sensor. In ENDO-CO_2, increase in $\dot{V}co_2$ resulted in proportional increase of Max($dPaco_2/dt$), whereas they remained in the control values in EXO-CO_2. A newly proposed model of respiratory control in ENDO-CO_2, involving the feedforward drive of respiration originating from the CO_2 oscillation as $\dot{V}_E = b0 \cdot Paco_2 + b1 \cdot Max(dPaco_2/dt) + b2$ where b0, b1 and b2 were calculated from the results of above experiments, readily explained the isocapnic hyperpnea or augmented CO_2 sensitivity in ENDO-CO_2.

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CHANGE IN Pco_2 DUE TO HYDRATION REACTION IN RED BLOOD CELLS

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The Pco_2 change in the red blood cell (RBC) caused by CO_2 diffusion is greatly influenced by intracellular CO_2 hydration reaction. In order to describe the above influence quantitatively, a new equation was derived, modifying the Henderson-Hasselbalch equation. The equation was validated experimentally by measuring the Pco_2, HCO_3^- and pH changes after mixing a RBC suspension with saline solutions having different Pco_2. The measured Pco_2 changes agreed well with the values calulated from the initial experimental conditions before mixing. The buffer value, β_c, used for the calculation was also measured in the similar procedure. The β_c value obtained in human subjects (n=18) was 73.8 ± 9.7 mmol/l,RBC/pH_c (mean ± SD), which was compatible with the data of previous authors.

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EVALUATION OF CARDIAC OUTPUT AND PULMONARY CAPILLARY BLOOD VOLUME DURING EXERCISE.

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The gas exchange ratio (RE) during rebreathing linearly decreases with increasing alveolar P_{CO2} (P_A). When a contact time (tc) is about 0.8 sec, the slope of the RE–P_A relation (Φ) is equal to that of CO_2 dissociation curve at the middle between true- and oxygenated-venous P_{CO2} (α'). However, the shorter the tc, the lower the Φ becomes. Thus, we derived a correcting factor of Φ for obtaining an accurate $avDo_2$. By using the corrected $avDo_2$ (= factor·α'/Φ), the cardiac output ($\dot{Q}$) during exercise was calculated. The tc value being requisite for evaluating the correction factor was obtained by comparing the experimentally determined $vaDco_2(P)$ with that caculated from the diffusion equations. Here, $vaDco_2(P)$ is the veno-arterial CO_2 content difference resulting from P_{CO2} difference. The pulmonary capillary blood volume (Vc) was calculated as the product of $\dot{Q}$ and tc. The tc, $\dot{Q}$ and Vc values were 0.70 ± 0.01 sec, 6.50 ± 0.16 l/min and 76.0 ± 2.6 ml (mean ± S.E.) at rest for five healthy male subjects. The corresponding values at the heaviest exercise were 0.42 ± 0.01 sec, 16.2 ± 0.37 l/min and 114 ± 3.2 ml, respectively. The relation between $\dot{Q}$ (l/min) and O_2 consumption $\dot{V}o_2$ (l/min) was expressed as $\dot{Q} = 3.51 + 7.7 \cdot \dot{V}o_2$ ($r = 0.95$, $n = 151$) and that between Vc (ml) and $\dot{Q}$ (l/min) as $Vc = 41.8 + 4.8 \cdot \dot{Q}$ ($r = 0.86$, $n = 151$).

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ESTIMATION OF THE CONTACT TIME WITH CO AND CO_2 REBREATHING METHOD.

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We developed a rebreathing method for evaluating the contact time through the lung capillary. According to Mochizuki et al. (1984) the cardiac output ($\dot{Q}$) is obtained from O_2 and CO_2 concentrations in rebreathing air. By dividing the CO_2 output by $\dot{Q}$, the CO_2 quantity diffusing out of the red cell (RBC) is calculated during the contact time (t_c). Thus, by dividing the above diffusion quantity by the CO_2 diffusion rate factor and the time avrage of Pco_2 gradient across the RBC membrane, the t_c is obtained. In order to validate the above method, the O_2 and CO_2 concentrations were measured along the rebreathing time together with pulmonary diffusing capacity for CO (D_Lco). When alveolar Po_2 (P_Ao_2) was about 120, 250, and 400 Torr, the D_Lco measured in 6 normal subjects were 28.4 ± 2.6, 20.4 ± 1.7, and 16.1 ± 1.0 $ml \cdot min^{-1} \cdot Torr^{-1}$, respectively. From the D_Lco/P_Ao_2 relation the transfer coefficient of CO across the RBC membrane was estimated to be 1.5×10^{-6} $cm \cdot sec^{-1} \cdot Torr^{-1}$, yielding Fc(CO) = 0.013 $sec^{-1} \cdot Torr^{-1}$ at P_Ao_2 = 120 Torr. Since $D_Lco = \dot{Q} \cdot Fc(CO) \cdot Hct \cdot t_c$, the t_c was evaluated from D_Lco and P_Ao_2 by using the $\dot{Q}$ value obtained from the CO_2 method. The t_c estimated from D_Lco was 0.67 ± 0.15 sec, being consistent with that obtained from the CO_2 method, 0.66 ± 0.10 sec, suggesting the validity of the calculated CO_2 diffusion rate.

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AN ASSESSMENT OF LOOP 'GAIN' OF CO_2-VENTILATION FEEDBACK CONTROL SYSTEM IN HYPEROXIA.

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The differences in loop gain of the CO_2-ventilation feedback control system obtained by external dead space ventilation (Gco_2 DS) and by CO_2 inhalation (Gco_2 INH) were elucidated in eight healthy male subjects. They breathed into a closed circuit and were subjected to the CO_2 steady-state test. This procedure was conducted with 250, 500 and 750 ml added dead space (DS), consecutively. About 1h after these DS runs, a further steady-state CO_2 inhalation test (INH) was carried out, maintaining $P_{ET}co_2$ levels equivalent to those in the 250, 500 and 750 ml DS runs. Gco_2 was calculated by dividing the slope of the CO_2 response curve (S) by that of the metabolic hyperbola (S_L). The absolute value of Gco_2 DS was larger than that of Gco_2 INH. This was accounted for by both a higher slope of the CO_2 response curve and a lower slope of the metabolic hyperbola in DS run than those in INH run.

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NO DIURNAL CHANGES IN OXYGEN CONSUMPTION IN THE WINTER BULLFROG

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Bullfrogs commercially supplied on November were maintained in the water tank (15 to 18 C), with feeding cat foods once a week until measurement started. A metabolism(130x100x100mm) and a blank (70x100x100) chamber were made in series, and located in water bath thermally regulated. They were shaded and lighted 12 hrs daily. Carbon dioxide produced by the frog was absorbed by soda lime and a diminition of O_2 contents was replenished through an electromagnetic valve controled by an output of a pressure amplifier measuring pressure difference(ΔP) between a blank and a metabolism chamber. Thus at the end of 6 days of experiment, only remnant CO_2(0 to 0.3%) and O_2 contents similar to air were detected. And then the frog appeared as vivid as before experiment. $\dot{V}_{O_2}$ was calculated from the slope of ΔP changes for every hour. $\dot{V}_{O_2}$ became steady state in the third day and mean values(ml/100g/h) were 0.48±0.13(SD,n=5, mean body weight±SD=270.4±60.3g), 1.41±0.144(n=5, 292.4±45.4g), and 4.38±1.03 (n=10, 263.8±62.7g) at 5, 15, and 25 C, respectively. No diurnal changes in $\dot{V}_{O_2}$ were observed in any temperature examined.

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Suppression of respiration induced by opioid peptide into the region of the nucleus parabrachialis medialis. Eguchi,K., Simbulan,D., Tadaki,E. and Kumazawa,T. Dept. of Physiol., Sch. of Dent., Aichi-Gakuin Univ., Chikusa-ku, Nagoya. and Dept. of Nervous and Sensory Function, Res. Inst. of Environ. Med., Nagoya Univ., Chikusa-ku, Nagoya.

In chloralose-urethane anesthetized, bilaterally vagotomized, immobilized and artificially ventilated cats, the respiratory suppression mediated by endogenous opiate in the nucleus parabrachialis medialis(PBM) and it's adjacent area was studied. Single electrical stimulation of the PBM region evoked inhibition and excitation of phrenic nerve activity when stimulation was applied in an inspiratory phase and in an expiratory phase, respectively. By repetition of electrical stimulation applied only in the expiration a long-lasting suppression of respiration indicated by integrated phrenic nerve activity could be observed. This suppression was reversed by systemic administration of naloxone (0.5 mg/kg). In 8 out of 20 cats tested microinjection of opioid peptide (DAGO:(D-Ala^2, MePhe^4,Gly-(ol)^5)enkephalin, less than 1μl of 0.5μM) into the PBM region showed naloxone- reversible respiratory suppression occurring within 1 min after injection which was simillar to that induced by the electrical stimulation. Most of all effective sites were histologically identified in the PBM and it's adjacent area including the nucleus Kölliker Fuse. In the same cat, however, application of DAGO in the site where electrical stimulation was effective on respiratory suppression could not show suppression of respiration although in limited cases.

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THE PYLOROMUCOSAL-PANCREATIC EXOCRINE SECRETION RESPONSE OF DOGS. OKADA, H. AND FURUKAWA, N. Dept. of Physiology, Kawasaki Medical School, Kurashiki 701-01

The effect of mucosal stimulation of the gastric pylorus was studied in chloralose anesthetized dogs and decerebrate dogs in which the gastric pylorus was separated from the gastric body with ligature and the pyloric sphincter was occluded by submucosal ligature. In experiments on 7 dogs an average rate of pancreatic secretion was 18.9 µl/5 minutes. After infusion of 5 ml $CuSO_4$ solution into the pylorus, the secretion increased in every dogs to an average of 80.3 µl/5 minutes. This was accompanied by increased protein and bicarbinate secretion of 382% and 736% respectively. Gastrin value in the serum was 133 pg/ml in control, and it was increased to an average of 397.6 pg/ml (N=6) 20 minutes after the infusion. In 5 dogs applied intravenously proglumide (400 mg/kg), an antagonist of gastrin, a similar increased pancreatic secretion was produced by the pyloric infusion of $CuSO_4$ solution. Such pancreatic responses markedly reduced after bilateral cervical vagotomies (N=6). Pancreatic responses in vagotomized dogs following the infusion did not abolish by each pretreatment of atropine sulfate (0.5 mg/kg) or proglumide (400 mg/kg), but abolished by both treatments.

These results provide evidences for the existence of the pyloromucosal pancreatic secretion reflex that is developed via the vago-vagal pathway and another cholinergic pathway. Furthermore, a part of the increased secretion following the pyloric mucosal stimulation results from increased gastrin.

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THE EXOCRINE PANCREATIC REFLEX FOLLOWING DISTENSION OF THE CANINE GASTRIC PYLORUS. FURUKAWA, N. AND OKADA, H. Dept. of Physiology, Kawasaki Medical School, Kurashiki 701-01

In dogs provided with the pyloric pouch in which the pyloric sphincter was occluded by submucosal ligature and acute cannulation of the main pancreatic duct, the effect of graded pyloric distensions (10-40 ml) with Tyrode's or 1/10 N HCl solutions on the pancreatic secretion was studied. In both 28 chloralose anesthetized dogs and 10 decerebrate dogs, the graded distensions by either solutions caused usually graded rise of pancreatic secretion rate which was accompanied by increased protein and bicarbonate outputs. Such increased responses were greatly reduced by bilateral cervical vagotomies, but did not abolish. This remained response was still produced after additional splanchnicotomy, and it rather increased than that before splanchnicotomy. The increased response following pyloric distension in vagotomized dogs was observed even after caeliac and superior mesenteric ganglionectomy, but abolished by external ligature around the pyloric sphincter or i.v. injection of C_6 or atropine sulfate (0.5 mg/kg). Marked pyloric rhythmic contractions following splanchnicotomy in vagotomized dogs were accompanied with increased pancreatic secretion. Such a secretion response was abolished by external ligature of the sphincter or i.v. injection of atropine or C_6. This study shows the existence of the pyloro-pancreatic reflex via a long route vago-vagal pathway and a short route one.

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THE EFFECT OF OLEATE IN THE INTESTINE ON PANCREAS AND BILE SECRETION IN THE RAT. MIYASAKA, K., KITANI, K., The First Laboratory of Clinical Physiology, Tokyo Metropolitan Institute of Gerontology. 35-2 Sakaecho, Itabashi-ku, Tokyo-173.

Fat digestive products in the proximal intestine have been reported to increase pancreatic secretion, while the same materials in the colon inhibited pancreatic secretion in the dog. In the present study, we investigated th effect of sodium oleate infused into either the duodenum or the terminal ileum on bile and pancreatic secretions in the conscious rat. Rats were prepared with cannulae draining pure bile and pancreatic juice separtely and three cannulae (two duodenal, the other ileal). During the experiments, 40 mM taurocholate containing 7 mg/ml bovine trypsin were infused into the duodenum replacing bile pancreatic juice throughout. The intraduodenal infusion of oleate increased pancreatic secretion as well as plasma secretin concentration, whereas it did not change bile secretion. In contrast, the intraileal infusion of oleate exerted no effect on pancreas, while it decreased bile secretion. It was concluded that oleate in the duodenum stimulated pancreas and probably released secretin, while the administration of the same material in the terminal ileum significantly suppressed bile secretion.

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THE GALL-BLADDER EMPTYING BY THE VAGUS AND CCK IN DOGS. NARUSE S, YASUI A, OZAKI T & YANAIHARA N. National Institute for Physiological Sciences, Okazaki 444

The effect of vagotomy on gall-bladder emptying by cholecystokinin (CCK) was studied in dogs. After 18 h fast the animals were anesthetized by chloralose. The gall-bladder was canulated with a polyethylene tubing and was connected to a pressure transducer. A duodenal loop, prepared by ligating the pyloric ring and the distal duodenum, was perfused with saline at 2 ml/min. Bilirubin and amylase concentration were measured. An intravenous infusion of CCK (100 pmol/kg-h) increased the gall-bladder pressure and amylase secretion. The bile was evacuated into the duodenum 6 min after the start of the infusion. Bilateral cervical vagotomy reduced the gall-bladder and pancreatic responses to CCK significantly. The electrical stimulation (8V, 5msec, 5p/sec) of vagi at the neck increased the gall-bladder pressure immediately. The onset time of the appearance of bile, however, was 5 min. A simultaneous infusion of CCK with vagal stimulation shortened the appearance time to 4 min and increased the bilirubin output. The effect of bilateral cervical vagal block with 1% lidocaine on gall-bladder emptying to CCK (50 pmol/kg-h) was tested in concious dogs with chronic bile fistulae. Both gall-bladder pressure and bile responses to CCK were significantly reduced by this treatment. It is concluded that vagotomy reduces the sensitivity of the gall-bladder to CCK in the dog.

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STRUCTURE-FUNCTION RELATIONSHIP OF CCK TO CANINE GASTROINTESTINAL MOTILITY. KUWAHARA, A., SEGAWA, J., AND *YANAIHARA, N.
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Structure-function relationship of cholecystokinin(CCK) to caine gastrointestinal motility were studied in anesthetized dogs using strain gauge force transducers. Intravenous injections of CCK-8, Glt-CCK-8, pGlu-CCK-8, Suc-MePhe CCK-7 increased gastric and duodenal motility in a dose dependent manner (100-800 ng/kg/h). D_{50} of CCK-8 in the gastric antrum and the proximal duodenum were 640 and 348 ng/kg/h, respectively. Substituting of the Gly residue with D-Trp or Sar in CCK-7 (Pht-[D-Trp_3]-CCK-7, Suc-[D-Trp_3]-CCK-7, and Suc-[Sar_3]-CCK-7) did not affect gastric and duodenal motility. CCK-8 non sulfated form (CCK-8NS) slightly increased gastric and duodenal motility in a high dose (1.6-2.0 μg/kg/h). These results suggest that Gly residue in position 3 as well as the sulfation of tyrosin in position 2 is essential for combining receptors in the gastrointestinal system.

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EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) ON THE ACETYLCHOLINE (AcCho) STIMULATION OF THE RAT SUBMANDIBULAR GLAND. ENDO, M.*, FUJITA, K.*, PRAPUTPITTAYA, C., KIMURA, F. *Dept. Oral Surg. and Dept. Physiol., Yokohama City Univ. Sch. Med., Yokohama 232.

Possible cooperation of AcCho and VIP was studied in the rat submandibular gland. Salivary secretion through cannula was recorded via a photo-cell drop counter and blood flow was determined by blood flow meter using H_2-clearance technique. Single injections of AcCho (10-100 pmol/kg BW) and VIP (4-100 pmol/kg BW) produced a dose-dependent increase in blood flow. A near maximum increase in blood flow was produced by 60 pmol of AcCho or 100 pmol of VIP. AcCho was effective in eliciting salivary secretion at a dose higher than 60 pmol, but VIP was ineffective. Combined injections of VIP and AcCho produced a pronounced increase in blood flow in a dose dependent manner, and AcCho at a dose lower than 60 pmol became effective to elicite salivary secretion when VIP was added at a dose higher than 40 pmol. Injection of atropine (1 mg/kg BW) blocked the effects on blood flow and salivary secretion produced by injection of AcCho, but not of VIP. The results suggest that in the rat, as reported in other animals, VIP potentiates salivary secretion and blood flow induced by AcCho, and AcCho and VIP act synergistically at the level of effector cells to regulate salivary secretion.

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GLUCOSE ABSORPTION IN VENTROMEDIAL HYPOTHARAMIC(VMH) OBESE RATS. INOUE,S., TSUCHIYA, M.,* TAKAMURA,Y.* 3rd Dept. Int. Med., Yokohama City Univ. Sch. Med.

We have developed a new method which enabled us to observe the continuous time course of glucose absorption by an everted intestine. In this method the upper end of a sac was tied over a small polyethylene sampling tip and from this openning an aliquot(10ul) was collected for samples of the serosal side every 12 minutes. The same amount of fluid was also collected from the openning window at the wall of medium instrument for samples of the mucosal side at the same time interval. The collected fluids were analyzed for glucose concentration by using a Beckman Autoanalyzer. Using the method, we found that VMH lesioned rats had significantly decreased glucose concentrations in serosal and mucosal sides at middle portion of small intestine(dynamic phase), and had decreased glucose concentrations in serosal side at all three portions and decreased glucose concentrations in mucosal side at upper portion(static phase).

The results indicated that glucose absorption does not increase in VMH lesioned rats and metabolic abnormalities in postabsorption play an important role on the development of VMH obesity.

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STUDIES ON PENETRATION PHENOMENA THROUGH INTESTINAL MEMBRANE — new analytical method by computer program and intestinal penetration of L-phenylalanine, L-tryptophane and insulin. NAKANO,S., TERAO,T., NAGAMI,K., MIYOSHI,M., & YOSHIOKA,T. Dept. of Physiol., The Tokai Univ. Sch. of Med., 259-11 Isehara Kanagawa

In previous studies by use of our deviced circulation apparatus, we have investigated series of experiments on penetration of amino acids and insulin as activepolypeptides through everted intestine of a rat. In the present study, also using the above apparatus, intestinal penetration phenomena were investigated under each following conditions by use of we devised a new method which can automatically analyze computer program. The obtained experimental results were as follows : 1) In 2 min's pretreatment of surface mucosal membrane with 0.1N HCl, L-Phenylalanine and L-Tryptophane penetration were inhibted, but Insulin penetration were hardly influenced. 2) In 2 min's pretreatment with 0.1N NaOH, L-Phenylalanine and Insulin penetration were enhanced, but L-Tryptophane penetration were inhibited, 3) In 5 min's pretreatment with 5% Sodium Lauryl Sulfate (SLS), intestinal penetration of above all substances were increased in comparison with another pretreatment, 4) As the results it will be considered that selective structural change of such components of intestinal cell membrane as proteins, lipids, mucopolysaccharid layer, could produce their own different penetration features of intestinal wall. It will be also of much interest from the view of pathological physiology.

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REFLEX SWALLOWING ELICITED BY WATER AND CHEMICAL STIMULATION IN HUMANS. SHINGAI, T., MIYAOKA, Y., IKARASHI, R. AND SHIMADA, K. Department of Physiology, Niigata University School of Dentistry, Niigata 951.

Reflex swallowing elicited by stimulation with water and gustatory substances was examined in humans. Subjects were five healthy men. Small amounts of water and chemical solutions were applied to the posterior part of the tongue of the subject reclining on a dental chair. Swallowing was identified visually as laryngeal movement and the latency of swallowing was measured. It was found that water was an effective stimulus in eliciting reflex swallowing and that the laryngeal region was most effective to initiate the reflex swallowing with water. The findings suggested the existence of water receptors in the larynx of man. Gustatory components of test solutions affected the latency of swallowing. NaCl solutions elicited reflex swallowing with longer latencies than that for water. Quinine-HCl and CH_3COOH prolonged the latency of swallowing. In contrast, sucrose and fructose induced reflex swallowing with shorter latencies than that for water.

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THE EFFECT ON BROWN RICE DIET TO ABSORPTION ON NUTRIENTS

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We intended to know the effects of rice dietary fiber on the absorption of nutrients such as protein and lipid in Japanese male students. They were fed daily 560g of brown rice (BR) with some foods, for 2 weeks, then changed the diet containing the well-milled rice diet(WMR) for 2 weeks. Both diets contained 1.2g protein/kg body weight and enough energy for maintaining their body weight. The BR contained the DF approximetry 3 times than WMR. The wet and dry weight of feces of the students fed on BR were about 2 times compaired with those fed WMR. The absorption rate of energy in the students of on WMR was 94% but that was 86% on BR. The absorption rate of protein was 88% on WMR and was 80% on BR, but no significant difference was found in nitrogen balance of both diets. It is noticed that the lipid concentration in the feces was about 2 times higher on the BR. So that the absorption rate of lipid was estimated about 20% less. The blood status were similar in both diets. A little of the DF was degradated on the WMR but the degradation rate was 40% on the BR.

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THE STIMULATION OF THE MEDIAL AMYGDALOID NUCLEUS SUPPRESSED THE GROWTH HORMONE (GH) RELEASE INDUCED BY HUMAN PANCREATIC GH RELEASING FACTOR IN PENTOBARBITAL ANESTHETIZED RATS. KATO, M., and SUZUKI, M. Dept. of Physiol., Inst. of Endocrinol., Gunma Univ., Maebashi 371

The inhibition of GH release from the anterior pituitary is mainly exerted by somatostatin (SRIF). SRIF nerve terminals in the median eminence derived from the hypothalamic periventricular nucleus. The electrical stimulation of this nucleus strongly suppressed the GH release induced by hpGRF (10 μg, intravenous injection) in pentobarbital anesthetized rats. hpGRF augmented plasma GH level from 20±4 ng/ml (mean±SE) to 846±40 ng/ml in 10 min, while the 10 min value was suppressed to 44±20 ng/ml by the periventricular stimulation. The stimulation of the medial amygdaloid nucleus inhibited the GH release to a lesser extent, i.e. the 10 min value was suppressed to 270±48 ng/ml. The lesion of the periventricular nucleus or of the stria terminalis nullified this inhibitory effect. The stimulations of the lateral amygdaloid nuclei, however, did not suppressed the GH release. Moreover, the stimulation of neither the entorhinal cortex nor the subiculum inhibited the GH release. These results indicate that the stimulation of the medial amygdaloid nucleus excites the periventricular SRIF neurons through the stria terminalis and enhances the SRIF release into the hypophysial portal blood which in turn suppresses the GH release induced by hpGRF.

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ACTH, PROLACTIN AND TSH SECRETION AFTER NOXIOUS STIMULI IN THE RATS. HAMAMURA, M., SHIBUKI, K., IKEUCHI, M. and YAGI, K. Dept.of Physiol., Jichi Med. Sch., Tochigi 329-04

Effects of noxious stimuli and hypovolemia on plasma levels of ACTH, prolactin (PRL) and TSH were studied by radioimmunoassay in male rats. Footshocks (FS) with 50-Hz pulses (10 ms, 3 mA) for 3 s were repeated 40 times at every 15 s. Mean plasma levels of ACTH and PRL in shocked conscious rats were signicantly higher than those in unstimulated control rats, while plasma TSH level was not significantly different between both groups of rats. Saphenous nerve stimulation with 4-Hz pulses (1 ms) for 180 s elevated significantly plasma ACTH and PRL but not TSH levels in rats anesthetized with α-chloralose and urethane. Secretions of these hormones were all potentiated after hypovolemia induced in conscious rats by an i.p. injection of 5 % glucose (50 ml/kg) and in anesthetized rats by hemorrhage (10 ml/kg). In addition, plasma levels of the three hormones after hemorrhage were compared with those in the shed blood in each rat and found to be significantly higher than the latter. These data show that noxious stimuli potentiate ACTH and PRL but not TSH secretion, while hypovolemia stimulates secretion of the three adenohypophysial hormones.

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ESTIMATION OF IN VITRO DOPAMINE BIOSYNTHESIS IN TUBEROINFUNDIBULAR DOPAMINERGIC (TIDA) NEURONS BY MEASUREMENT OF DOPA ACCUMULATION IN THE MEDIAN EMINENCE OF HYPOTHALAMIC SLICES. ARITA, J. and KIMURA, F., Dept. Physiol., Yokohama City Univ. Sch. Med., Yokohama 232

A new method for estimation of in vitro dopamine biosynthesis in TIDA neurons was developed by measuring DOPA accumulated in the median eminence after incubation of hypothalamic slices with NSD 1015, an inhibitor of DOPA decarboxylase. The rate of DOPA accumulation in the median eminence was maximal in the presence of 10 mM NSD 1015 and linear up to 120 min. DOPA accumulation in the median eminence was stimulated by increasing tyrosine concentration in medium and was blocked by α-methyltyrosine, an inhibitor of tyrosine hydroxylase. The rate of in vitro DOPA accumulation in the median eminence was 33% of that of in vivo DOPA accumulation. High K+- or veratridine-induced depolarization of membrane potentials caused stimulation of DOPA accumulation in the median eminence in a extracellular Ca2+-dependent manner. The new in vitro method is a useful means to study the regulatory mechanism of dopamine biosynthesis in TIDA neurons.

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DOPAMINERGIC INVOLVEMENT IN ESTROGEN-INDUCED SUPPRESSION OF THE FREQUENCY OF PULSATILE LUTEINIZING HORMONE SECRETION IN OVARIECTOMIZED RATS.
TADOKORO, Y., AKEMA, T. AND KIMURA, F. Dept. Physiol., Yokohama City Univ. Sch. Med., Yokohama, Kanagawa 232

In this work we examined the effects of various catecholamine synthesis inhibitors and receptor blockers on the pulsatile secretion of luteinizing hormone (LH) and on the suppression of the pulse frequency induced by implantaton of estradiol benzoate (EB) in the preoptic suprachiasmatic area (POSC). EB implantation into the POSC suppressed the frequency of pulsatile LH secretion in vehicle-treated rats. Pretreatment of the rat with α-methyl-p-throsine (AMPT), AMPT + threo-dihydroxyphenylserine or pimozide did not affect the basal pulsatile LH secretion but prevented the EB-induced suppression of the LH pulse frequency. In rats pretreated with diethyldithiocarbamate or phenoxybenzamine the basal pulsatile LH secretion was diminished. Further suppression by EB implantation was not clear. Pretreatment of the rats with propranolol did not affect the basal pulsatile LH secretion nor the EB-induced suppression. It appears that the dopaminergic system is involved in the mechanism by which EB implanted in the POSC reduced the LH pulse frequency.

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CORTICOSTERONE(CS) AND GROWTH HORMONE(GH) SECRETIONS DURING LONG-TERM THIOPENTAL ANESTHESIA IN THE ADULT MALE RAT.
MITSUGI,N., PRAPUTPITTAYA,C.,and KIMURA,F. Dept. of Physiol., Yokohama City Univ. Sch. of Med., Yokohama. 232

Recent studies in our laboratory have shown that each of the GH and CS secretions and sleep-wakefulness cycle occurs with common period ultradian rhythms of 1.5 h and its multiples in the rat. Further, a definite phase relationship between the GH and CS secretions was assumed from the findings that CS secretion occurring with a 3.0-hour periodicity began in the late stage of the sleep cycle with the same period rhythm, while GH secretory burst occurred in its early stage.

In the present study, rhythmic characteristics of the GH and CS secretions were examined under the state of continuous sleep due to long-term thiopental anesthesia. They showed ultradian rhythms with the periodicity of 1.5 h and its multiples as well. However, there was observed no definite phase relationship between both secretions. With our earlier findings that a decrease in the amount of sleep did not inhibit the occurrence of GH secretion at the anticipated time in the 3.0-hour period rhythm and that there were minority of animals in which a temporal dissociation among three rhythmic functions was apparent, it was suggested that CS and GH secretions manifest their rhythms based on their own pacemakers.

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NEUROCHEMICAL STUDIES ON THE ISOLATED GROWTH HORMONE DEFICIENT MOUSE (lit).
SUGISAKI, T., NOGUCHI, T., TSUKADA, Y.* Department of Physiology, Toho University School of Medicine, Ohmori-nishi, Tokyo-143, and *Department of Physiology, Keio University School of Medicine, Shinanomachi, Tokyo-160

In 1976, Eicher and Beamer described a new autosomal recessive mutation little (lit) in the mouse that in the heterozygous state produces characteristics similar to the human disorder, isolated growth hormone deficiency Type I.

To define that GH has an independent action on the cerebral development, we used this promising animal model.

Our result is that (A); weights of the two parts of the lit brain were found to be significantly less than those of the normal controls, 81.5% less for the cerebrum, and 81.6% for the cerebellum, (B); the total DNA content was reduced to approximately 80% in the cerebrum and 84% in the cerebellum compared to those of the normal controls, (C); the total RNA content was also reduced in the cerebrum and cerebellum, but it was proportional to the reduction of DNA content, (D); CNPase activity was found to be reduced selectively in the cerebrum of the lit mouse (74.4% of the normal control), and (E); the lit mice exhibited a strikingly reduced level of activity with an indistinct diurnal periodicity.

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CYTOLOGIC ALTERATIONS IN THE hyt ANTERIOR PITUITARY. NOGUCHI, T., SUGISAKI, T., KUDO, M.*, SATOH, I.** Departments of Physiology and Pathology*, and RI Research Unit, General Research Center**, Toho University School of Medicine, Ohmori-nishi, Tokyo-143

The hyt mutant mouse used in this study has a hypoplastic thyroid gland and is characterized by retarded somatic growth, very low to undetectable serum T4, and elevated TSH. Therefore, this congenitally hypothyroid mouse is an ideal model for studying the effects of thyroid hypofunction on the adenohypophysis. The anterior pituitary of the hyt mouse appeared less granular than that of the normal control when viewed by light microscopy, owing to a decrease in the number of somatotrophs. Many cells, in various stages of transformation into "thyroidectomy cells", were recognized by the appearance of the characteristic granules and the dilated rough endoplasmic reticulum. In some cases, the enlarged rough endoplasmic reticulum also contained spherical electron-dense secretory granules. In addition, there were many cells undergoing mitosis. With the electron microscope, these cells were identified as thyrotrophs by their characteristic granules.

The administration of T4 during the first 40 days of postnatal life prevented the abnormal changes in the hyt anterior pituitary. This finding suggests that T4 therapy during early postnatal life might ameliorate the pituitary hypertrophy and enlarged sella turcica seen in cretinous patients.

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THE ROLE OF FETAL OXYTOCIN IN TRIGGERING LABOR IN THE RAT. HIGUCHI, T., UCHIDE, K., HONDA, K. AND NEGORO, H. Dept. of Physiol., Fukui Medical School, Fukui 910-11

Developmental changes of oxytocin (OT) levels in blood and the pituitary gland and of OT responses to OT-releasing stimuli were investigated at perinatal period in rats. OT levels in both blood and the pituitary gland rose gradually from 21-day-old fetuses to 5-day-old infants. There was no difference in serum and pituitary OT contents in newborn pups and unborn littermates on day 22 or 23 of gestation, indicating that there was no increase in OT release around the time of labor. The i.p. injection of hypertonic saline induced a significant increase in serum OT levels at day 5 and later ages, but no effect in 22-day-old fetuses or in 1-day-old infants. The i.p. injection of noradrenaline synthesis inhibitor or phenobarbital rose blood OT levels only in the rats older than 10 days and 20 days of age respectively. The gradual increase in OT levels in both blood and the pituitary gland without an apparent increase in its release and the absence in pituitary response to OT-releasing stimuli during the perinatal period do not support the hypothesis that the increase in fetal OT release triggers labor in the rat.

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VAGINAL DISTENSION INDUCES MILK EJECTION REFLEX DURING SUCKLING IN THE LACTATING RAT. UCHIDE, K., NEGORO, H., HIGUCHI, T. AND HONDA, K. Dept. of Physiol., Fukui Medical School, Matsuoka, Fukui 910-11

In urethane-anesthetized lactating rats oxytocin neurones were identified by means of their antidromic response to stimulation of the neurohypophysis and from a high frequency burst of action potentials displayed 10-15 sec before reflex milk ejection. During suckling the bursts recurred with an interval of about 10 min. Vaginal distension performed by inflating rubber balloon evoked an additional burst of spikes in oxytocin neurones occasionally. The amplitude and duration of the burst were comparable to those of spontaneous ones. The increase in plasma oxytocin immediately after the evoked burst was also analogous to that of spontanous one. During non-suckling period neither spontaneous nor induced burst was observed in oxytocin neurones. These results indicate that signals arising from the vagina may open the hypothesized gate and allow suckling stimuli to generate milk ejection-related burst in oxytocin neurones. This effect of vaginal distension contrasts with the facilitatory effect of omotic stimuli on the burst; the former affects its periodicity and the latter its amplitude.

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EFFECTS OF IMMUNIZATION WITH OPIOID PEPTIDES ON ESTROGEN-ACTIVATED SEXUAL RECEPTIVITY IN THE RAT. KUBO, K. and KIYOTA, Y. Dept. of Bioregulation, Inst. for Med. Genetics, Kumamoto Univ. Med. Sch., Kumamoto 862, Japan.

The spayed rats primed with estradiol benzoate (EB) and progesterone were injected with anti-opioid peptide antisera into the third ventricle either at the time of EB-priming (E) or 47 h after EB (T), and tested for lordosis response to male mounts after 48 h of EB. Lordosis response was inhibited by anti-β-endorphin antiserum injected at E, but facilitated by it at T. Antisera against Leu-enkephalin, Dynorphin and Leumorphin at E facilitated lordosis response. The results were opposite to the effects of each peptide. Passive immunization with Dynorphin and Leumorphin at T failed to affect lordosis response, although injections of the peptides at T facilitated the response. Active immunization with β-endorphin resulted in full activation of lordosis response in the spayed rats with two injections of EB. The data indicate that the opioidergic neuronal systems act to modulate independently both processes of estrogen induction of sexual receptivity and expression of lordosis reflex. Balances between activities of β-endorphin and enkephalins may function in setting background receptivity-level and adjusting lordosis reflex activity.

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THE NUMBER OF GERM CELL OF THE GONAD IN MALE QUAIL EMBRYO ADMINISTERED ESTRADIOL BENZOATE. ITO, Y.and YAMAMOTO, N. Dept. of Physiology, Gifu University, School of Medicine, Tsukasamachi 40, Gifu 500.

Estradiol benzoate (0.02 mg) was injected into yolk of fertilized egg of genetic male quail (sw/+) on the eary stage of incubation. Number of germ cell of both right and left gonad was counted under a microscope and compared with that of control male. That of left testis in the treated group was about 13,000 cells on the nineth day, about 36,000 cells on the eleventh day and about 21,000 cells on the thirteenth and fifteenth days, and showed a decrease of about 60 % as compared with that of the control left testis (about 30,000 cells on the nineth day, about 51,000 cells on the eleventh day and about 58,000 cells on the thirteenth and fifteenth days). On the other hand, that of the right gonad in the treated male was 12,000 cells on the eleventh day and about 8,700 cells on the thirteenth and fifteenth days and showed a great decrease as compared with that of control right testis (about 30,000 cells on the eleventh day, about 42,000 cells on the thirteenth and fifteenth days). the means that estradiol benzoate greatly retarded an increase in number of the gonad during development.

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EFFECT OF THE INTRAVENTRICULAR ADMINISTRATION OF β-ENDORPHIN AND NALOXONE ON THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS IN DOGS. MATSUMOTO, I. AND AIKAWA, T. Dept. of Physiology, Nagasaki Univ. Sch. of Med., Nagasaki 852

The adrenocortical secretion in response to the intraventricular administration of β-endorphin and naloxone was examined in dogs. The drug was infused into the third ventricle at the rate of 15.6 μl/min for 20 min under the pentobarbital anesthesia. The secretion rate of 11-hydroxycorticosteroids(11-OHCS) from adrenal and the concentration of immunoreactive ACTH in peripheral blood were measured. A continuous increase in secretion rate of 11-OHCS attained to a peak at 90 min after the start of the infusion of β-endorphin with a concomitant elevation of ACTH. The simultaneous intraventricular administration of naloxone(100 pmole/ml) for 20 min abolished the 11-OHCS secretory response induced by β-endorphin. On the other hand, a single administration of high dose of naloxone(10 nmole/ml) induced a remarkable increse in the secretion of 11-OHCS and ACTH with two peaks at 10 min and 120 min after the infusion. The adrenocortical secretory profile induced by high dose of naloxone was similar to that induced by Leu-enkephalin(100 pmole/ml, intraventricular) but was different from that induced by β-endorphin.

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TRANSPLACENTAL EFFECTS OF DIMIT (L-T_3 ANALOGUE) ON DEVELOPING BRAINS FROM THYROIDECTOMIZED RATS TAKAHASHI, T., NISHIKAWA, T.*, NAKANO, S., SUDO, S., NAKANO, M. Dept. of Physiol., Kanazawa Med. Univ., Uchinada, Ishikawa 920-02

DIMIT (3,5-Dimethyl-3'-Isopropyl-L-Thyronine) is one of the analogue of L-T_3 and has thyromimetic activities. In the case of infants, which were administered with DIMIT via plcenta during their fetal life, their tubulin contents (per unit DNA) were increased in the cerebrum, cerebellum and hypothalamus at one day old as previously reported. In the present studies, DIMIT (10 ng / g body wt./ day) was administered to radio-thyroidectomized pregnant mother rats from the 7th pregnant day. The tubulin content did not show any change in the three brain areas from one-day-old infants delivered either from the radio-thyroidectomized or from the radio-thyroidectomized and DIMIT-treated mothers. At 11 days old, however, the tubulin content was significantly reduced in the hypothalamus from radio-thyroidectomized rats during the fetal life and rised upto the control level by L-T_4 treatment (200 ng / g body wt./ day) between one and ten days old. After the growth, the female rats, which were delivered from radio-thyroidectomized mother rats, were observed irregular estrus cycles.

Thyroid hormones might control the intracellular tubulin content in the hypothalamus during not only the neonatal life but also the prenatal life. And the prenatally hypothyroid state should prevent normally complete development of brains.

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THE EFFECT OF THYROID HORMONE ON PLASTICITY OF RAT VISUAL SYSTEM OBSERVED IN EVOKED POTENTIAL BY PHOTIC STIMULATION. SUZUKI, M., and KATO, M. Dept. of Physiol., Inst. of Endocrinol., Gunma Univ., Maebashi 371

It has been known that thyroid hormone affects morphological integrity of the visual cortex of developing rats as well as nervous functions including mental states. To find the functional changes in the CNS, caused by thyroid hormone, we applied photic repetitive stimulation to the visual system of rats in various thyroid states. Normal (I), thyroidectomized 3 to 11 weeks prior to experiments (II) and thyroidectomized and T_4 supplemented (III) rats were used. After anaesthesia with Nembutal (55 mg/kg), photic stimulation (2 joules, 5 sec interval, 25 times, 25 cm) was given to the rats. To record evoked potentials (EP) elicited by the photic stimulation, a glass electrode was placed on the dura mater adjacent to the area 17 and/or was inserted into the lateral geniculate nucleus (LGN). Group II rats showed a prolonged latency (+54.3%) and lowered amplitude (-60.8%) of the cortical EP, whereas, T_4 administration (III) recovered the latency to the normal level, and increased the amplitude. In addition to those changes, EPs recorded at LGN also indicated similar changes as the cortical EP. The latency was delayed (+81.4%) and the amplitude was reduced (-50.5%). These results suggest that thyroid hormone is required to maintain the functional integrity of the visual systems.

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VITAMIN D_3 ACTIVATION BY PTH IN RENAL CORTICAL CELLS UNDER PRIMARY CULTURE. KANDA, K., HATANO, T., OGAWA, K., SEO, H. AND MATSUI, N. The Research Institute of Environmental Medicine, Nagoya University, Nagoya, 464

We have developed a primary culture system of renal cortical cells which is suitable to study the effect of PTH and have shown that PTH at physiological concentrations stimulated production of cAMP. In the present report, effect of PTH on vitamin D_3 activation was studied by using the same culture technique. Rat renal cortex was treated with collagenase and dispersed cells were incubated in Dulbecco Modified Medium (DME) containing 16% fetal calf serum (FCS). After 2 days, the medium was replaced with DME (-FCS) containing h-PTH (1-34) and 25(OH)D_3. Production of 1,25(OH)$_2D_3$ was determined by receptor assay using cytosol-chromatin fraction obtained from rachitic chick. Renal cells cultured for 2 days revealed maximum activation of vitamin D_3 as well as adenylate cyclase by PTH. Linear increase in 1,25(OH)$_2D_3$ upto 220 pg was observed in medium by 3 hour-incubation with 100 ng of PTH. There was no production of 1,25(OH)$_2D_3$ in the absence of PTH. PTH addition from 10 to 1000 ng resulted in dose dependent increase in the production of 1,25(OH)$_2D_3$.

This culture system seems to be useful for the elucidation of the mechanism involved in the activation of vitamin D_3 by PTH.

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STIMULATORY EFFECT OF PLATELET ACTIVATING FACTOR ON THE ADRENOCORTICAL SECRETION IN DOGS.

AIKAWA, T., MATSUMOTO, I. and ONOUE, M. Dept., Physiol., Nagasaki Univ., Sch., Med., Nagasaki 852

The left adreanl gland of hypophysectomized-nephrectomized dog was perfused with Krebs Ringer bicarbonate glucose solution saturated with 95% O_2 + 5% CO_2. The secretory rate of cortisol (F), corticosterone(B) and aldosterone (A) were measured before and after the infusion of various concentration of platelet activating factor (PAF) over 10 min. The secretion of F, B and A increased significantly by the infusion of 10^{-9} M 1-o-hexadecyl PAF, but did not increase by that of 10^{-9}M 1-o-octadecyl PAF. The infusion of 10^{-8}M 1-o-hexadecyl PAF or 10^{-6}M 1-o-octadecyl PAF induced a marked increase in the secretion of F, B and A. The secretory response to 1-o-hexadecyl PAF (10^{-8}M, 10 min) was compared with that to ACTH (30 μU/ml, 5 min) and histamine (10^{-5}M, 5 min). The response of F and B to PAF was smaller than that to ACTH, but was larger than that histamine. The response of A to PAF was larger than that to ACTH and was almost equal to that to histamine. PAF may play a role in stimulating the adrenocortical secretion under inflammatory or allergic conditions.

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VASOPRESSIN SECRETION IN RESPONSE TO PHYSICAL AND PSYCHOLOGICAL NOXIOUS STIMULI.

YAGI, K., SHIBUKI, K., IKEUCHI, M., HAMAMURA, M. Dept. of Physiol., Jichi Med. Sch., Minami-kawachi-machi, Tochigi-ken, 329-04.

Footshocks for 30 s significantly elevated VP and ACTH levels in adult male rats. Intermittent footshocks (3-s duration at every 15 s) for 10 min did not potentiate VP but ACTH secretion. In rats anaesthetized with urethane (1.2 g/kg,i.p.), mean plasma levels of both hormones after footshocks for 60 s were not significantly different from those of unshocked group but those after intermittent shocks for 10 min were significantly lower than the control levels. Conscious rats were trained with strobe flashes (CS, 5 Hz) for 6 s, which were associated with inescapable footshocks for 3 s. Training trials were repeated forty times at every 15 s. Three hours after training, testing CS lowered VP and elevated ACTH levels. In rats trained for active avoidance behaviour, VP level was significantly lower and ACTH level was significantly higher than those of untrained control rats. These results suggest that physical but not psychological noxious stress pontentiates VP secretion and that both VP and ACTH secretions are influenced by the neural mechanism of stress analgesia.

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MODULATORY ACTIONS OF TOLBUTAMIDE AND DIAZOXIDE ON GLUCOSE-INDUCED ELECTRICAL ACTIVITY OF MOUSE PANCREATIC B CELLS.

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Modulatory effects of tolbutamide and diazoxide upon glucose-induced electrical activity of mouse pancreatic B cells were studied with intracellular recording techniques. At submaximal stimulatory levels of glucose an increase in glucose concentration of perifusing medium produced prolongation of depolarizing plateau phase, shortening of inter-plateau silent phase and decreased frequency of plateau potentials. The facilitatory effect of glucose was biphasic, namely an initial short-duration activation and a late sustained high activity. Tolbutamide produced an increase in plateau fraction of each activity cycle in similar biphasic fashion as in the case of glucose and a decrease in plateau frequency. On the other hand diazoxide decreased both plateau fraction and plateau frequency. Based on these data it is concluded that tolbutamide facilitates the mechanism of the glucose action, whereas diazoxide inhibits electrical activity of pancreatic B cells by a mechanism distinct to that of glucose action.

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EFFECT OF CHRONIC ISOPROTERENOL-TREATMENT ON RAT ADIPOCYTE.
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The effect of chronic isoproterenol-treatment (2.5 mg/kg body weight /day sc for 3 wk) on rat adipocyte membranes was investigated. Chronic isoproterenol-treatment significantly reduced body weight, the amount of epididymal adipose tissue and lipolytic activity per cell induced by various concentrations of noradrenaline. The maximal number of β-adrenergic receptors in rat fat cell membranes from the treated rats was about 30 % lower than from control rats. Moreover, chronic isoproterenol-treatment reduced the percent of β-adrenergic receptors in the high-affinity state and the ratio of the dissociation constant of the low- and the high-affinity state.

These results suggest that chronic isoproterenol-treatment may reduce the interaction of β-adrenergic receptors with the guanine nucleotide regulatory protein.

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CHANGES IN FATTY ACID COMPOSITION OF LIPIDS DURING EXERCISE.
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Repetitious rat training by swimming for 50 days caused significant decrease or incr -ease of linoleic acid composition of myocardial cardiolipin with swimming times per day. To clarify direct influences of the exercise on the change, statistical analysis has been done at 10th, 20th and 30th of the swimmings. At the 10th of the swimming the linoleic acid composition transiently decreased and at 20th and 30th of the swimmings the composition reversely increased. As thus, during the swimmings the fatty scid composition of myocardial cardiolipin showed a dynamical changes in the composition. To characterize the fatty acid changes of lipids, each rat was sacrificed after each swimmin -g. The linoleic acid composition of myocardial cardiolipin gradually increased and attained at about 70% of the composition. In fatty acid composition of white adipose tissue lipids, despite of constancy of triglyceride fatty acids, free fatty acid and total phospholipids showed antagonistic changes between palmitic and oleic acid compositions. Besides, in liver triglyceride the same dynamical changes were observed during the repetitios swimmings. From these results, fatty acid composition of cardiolipin and other lipids were not stable during the exercise, and fast and dynamical changes of fatty acid composition of lipids directly caused by the swimmings were characterized.

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EFFECT OF A HIGH FAT DIET ON PLASMA AND AORTIC LIPIDS IN YOUNG EXERCISED RATS.
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We studied the effect of a high fat diet, combined with exercise, on the plasma and aortic lipids of young rats.

Female Wistar rats (4 weeks of age) were divided into three groups (NS, HS HE) and were individually caged. One of these groups (NS) was maintained on a laboratory chow and the others (HS, HE) were fed a high fat diet, ad libitum. The NS and HS groups served as controls and the HE was forced to run daily on a treadmill. In the HS group, the estimated values of lipoprotein cholesterol (C) were much greater than those observed in NS. The increment of HDL-C was 43.6 %, while that of LDL-C was 177.9 % ($p<0.005$). The level of plasma triglyceride (TG) in HS decreased by 47.5 % of the values estimated in NS ($p<0.005$). In the HE group, the estimated values of LDL-C and plasma TG were unchanged, as compared to findings in the HS group. In this case, the HDL-C level was reduced by 38.6 % ($p<0.005$). Moreover, the level of HDL-C in the HE group decreased by 11.8 % in comparison with that estimated in NS ($p<0.05$). Aortic TG in HS and HE rats decreased by 61 - 62 % of the level determined in NS ($p<0.005$), whereas, aortic total C and phospholipid were unchanged. There was no close relationship between aortic and plasma lipid levels.

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EFFECT OF MUSCULAR ENDURANCE TRAINING ON REACTIVE HYPEREMIA IN HUMAN FOREARM

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Five male students trained unilateral forearm with hand erogometer using work loads of 1/3 of maximum grip strength 5 days/week for 6 weeks and took a succesive 4 weeks detraining period. During detraining period the subjects maintained their normal, daily pre-trainig activities. Reactive hyperemia in the trained forearm after arresting brachial blood flow for three minutes was measured by means of strain gauge plethysmography before and after training and also after detraining period. After 6 weeks of training, maximum blood flow in the reactive hyperemia (MBF) increased by 16 % and hyperemia decreased by 13 %. These differences were both significant. After 4 weeks detraining, however, the MBF and the hyperemia were not different statistically from the before training values. It is suggested that a repeated muscular contractions may induce the change not only in response to vasodilator substances but also in myogenic response to the changes in vascular transmural pressure with circulatory arrest and reopen. We concluded that the response of reactive hyperemia could be influenced by the muscular activity levels.

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EFFECTS OF ENDURANCE TRAINING TO VENTILATORY CHEMOSENSITIVITIES IN LONG DISTANCE RUNNERS.

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Six male long distance runners were examined by an isocapnic progressive hypoxia test and CO2 rebreathing test, both before and after 2 years of endurance training.

The results of ventilatory and heart rate responses were as follows:

1) Running records improved in every athlete after 2 years of endurance training.
2) Simultaneously, hypoxic ventilatory sensitivity decreased in all the athletes after the training.
3)Hypoxic heart rate sensitivity also decreased in all the athletes except one after the training.
4) However, the training did not elicit significant influence on hypercapnic ventilatory sensitivity.

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IS EMG ACTIVITY CONSTANT DURING AT VARIOUS KINDS OF CONSTANT SPEEDS ?

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In order to study the correlation between EMG activity, respiratory and circulatory responses, the surface EMG of limb muscles, heart rate, respiration rate, ventilation and oxygen-intake were measured during exercise on the treadmill and ground at verious speeds. An apparatus of automated metabolic analyzer, a compact data recorder(4 channel) and telemeter were used for the determination of these items. In general, it is said that EMG activity is constant during exercise. In this resalts, however, the decline of EMG activity at a few minutes after start of exercise were recognized significantly without the decrease of oxygen-intake. Furthermore, these phenomena not only occured the antagonistic muscle, but also were observed on agonistic muscle during maximal and submaximal running.

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ON THE RELATION BETWEEN THE POSTURE AND THE TONIC GRADE OF AUTONOMIC NERVE

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We decided the posture by the distribution of the foot pressure of four divisions. The foot pressure was measured with the measuremental apparatus devised by Sakamoto one of us experimenters. In this measuremental apparatus the microtype mechanoelectro transducer is used, and it has the plan that we can measure the foot pressure in 20 positions on both soles at the interval of one second during 30 seconds. While the decision of the tonic grade of autonomic nerve was tried by the question paper of 21 terms. On this experiment we recognized there is no relation between the posture and the tonic grade of autonomic nerve. Then we recognized the change of foot pressure shows that there are not so many sympathetic nerves tonic people, but many parasympathetic nerves tonic people.

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CHARACTERISTICS OF STEREOTYPED MOVEMENT OF LOWER LIMB DURING WALKING AND RUNNING ON TREADMILL.

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To evaluate the characteristics of stereotyped movement of the lower limb during treadmill walking and running, step lengths and step durations of 200 steps were consecutively determined by means of a computerized system. The subjects, including seven adult men and six adult women, walked at speeds ranging from 60 to 130m/min and ran at speeds from 110 to 200m/min, with an increment of 10m/min. In walking, standard deviation of step length and step duration showed the minima at the speeds which were estimated to be about 90m/min for both male and female. Coefficients of variation of these factors also showed the minima at particular speeds. These results suggest that when the subject is allowed to choose his own step length, there exists the walking speed at which the fluctuation of stereotyped movement of the lower limb shows the minimum. On the other hand, in the case of running, standard deviations of step length and step duration were not related systematically with the running speeds.

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A NEW METHOD FOR ESTIMATING THE LACTATE THRESHOLD AND ITS VALIDITY.

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The lactate threshold (LT) is often estimated by some criteria of gas exchange parameters (GEPs) visually, but the visual inspection contains the subjective variations of intra- and inter-detectors, so its reliability is poor. The purposes of this study are 1) to confirm if the LT measured (LT-meas) is consistent with the LT estimated (LT-est) by the mathematical modeling in each criterion of GEPs to avoid the subjective variations, or not, and 2) if not so, to suggest a new estimation method of LT by GEPs. 11 young male subjects performed an incremental-load ergometer test according to a standard procedure. GEPs was monitored continuously, and venous blood was sampled from a warmed ear lobe to measure LA every 2 min during a test. As a result, LT-est by model were not always consistent with LT-meas. I employed the logistic discriminant function (LDF) analysis to discriminate 2 groups of GEPs at the time of pre- and post-LT. The merit of this method is to stop the test at which work rate reaches the LT level. To examine the validity in the new method, another 10 male subjects performed the same test. LT-est by LDF were consistent with LT-meas. It is concluded that a new method using LDF has two main advantages; 1) as a new method uses not single- but multiple-GEPs, the integrated information of GEPs can be evaluated, and 2) the test for the estimation of LT becomes more safety, because it is not necessary to perform the test until exhaustion.

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THE INFLUENCES OF VIBRATORY STIMULATION ON THE ERROR MAGNITUDE IN FOREARM POSITION SENSE TEST. MIYAMOTO, S., SAWADA, Y. AND *AOKI, M. School of Allied Health Proffessions and *Dept. of Physiology, Sapporo Medical College, Sapporo 060.

To elucidate the mechanism of the error induced by finger pointing task, forearm position sense test was performed using a specially divised protractor in 32 healthy adults and 10 patients with cerebral vascular accident (C.V.A.). In addition, the influences on the error due to vibratory stimulus on the shoulder were examined. The mean error magnitude was within one degree in normal and two degrees in C.V.A. subjects, however, the error magnitude of the latter varied in a wider range ($-10°$ to $+7°$). Although the vibratory stimulus significantly increased the error magnitudes, for the stimulus at 100 Hz the errors shifted downward in the doninant hand and either direction in the nondominant hand as compared with that without vibration. At the varying frequencies (50, 100 and 150 Hz) of the stimulus no consistent tendency of the error magnitude and the direction was observed. It was also recognized that the vibratory stimulus on the muscle belly or the tendon gave rise to no appreciable difference on the errors. The vibratory stimulus of the cutaneous receptors of the shoulder resulted in similar effects shown by vibrating the muscle belly or tendon. In summary, it is possible that the muscle spindle, joint and cutaneous receptors contribute to the accuracy of forearm position sense.

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Effect of intraventricular neurotensin on blood pressure and heat balance in rats.
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The effects of intraventricular neurotensin (NT) at doses of 0.1, 1.0 and 10.0 ug on blood pressure (BP), heart rate (HR), heat production (M), heat loss (H) and colonic temperature (T_{col}) were investigated in conscious rats in a direct calorimeter at 18 and 28°C. At 18°C, 10.0 ug of NT significantly increased BP with prolonged bradycardia. The 1.0 and 10.0 ug of NT significantly reduced M and T_{col}. In sinoaortic denervated rats, a 1.0 ug of NT raised BP and decreased HR. The decreased HR was significantly smaller than that in nerve intact rats, which indicates the occurrence of baroreflex with intraventricular NT. The changes in M and T_{col} in the denervated rats were, however, not statistically different from the intact rats. The baroreflexive suppression of metabolism seems to play a minimum role in the NT-induced hypothermia. H slightly increased for saveral minutes after central NT (1.0 and 10.0 ug) and significantly decreased thereafter. Thermal conductance significantly increased for a longer period of time after NT injection. At 28°C, a 1.0 ug of NT increased H and M. It is concluded that central NT produced hypothermia by reducing M and enhancing H in the cool environment, but not at 28°C.

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Effect of chronically implanted naloxone on wheel running activity and brain cat echolamines in food restricted rats.
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Twelve male Wistar rats were subjected to 2 hr-feeding (12:00-14:00) daily in running wheel cages under a 12:12 LD cycle (light on at 6:00 am) at an ambient temperature of 24°C. The wheel running activity (WRA) was counted with photocells and recorded on digital cassette tapes via computer logging system. After 1 week food restriction, a miniosmotic pump (Alzet, model 2002) containing saline or naloxone (64 ug/ul) was implanted subcutaneously in the back under ether anesthesia. Rats were decapitated at the end of the experiment and brain cathecholamines were determined. 2 hr-feeding progressively increased WRA of rats especially around feeding time. Since rats did not revolve wheels during meal, the WRA showed a biphasic pattern at the light phase. This pattern was established in 5 to 6 days after food restriction and continued thereafter. The chronically treated naloxone seemed to enhance WRA. However, the distribution of WRA in a day was not affected by this drug. The norepinephrine contents of hypothalamus and midbrain were larger in naloxone treated rats than in saline rats. There was no difference between both groups in dopamine contents at all parts of brain.

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EFFECTS OF PHYSICAL EXERCISE ON ETHANOL METABOLISM IN RATS.
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The effect of exercise on ethanol(Et-OH) metabolism in the mammalian species was studied by male SPF Sprague-Dawley 10-wk-old rats(~290g) fasted for 12 and 24 hours. The time courses of plasma Et-OH concentration(P.E.C.) were analyzed at two different temperatures. The exercise used was swimming for 1 hour at 25°C and 35°C. Et-OH doses of 75mg per 100g body weight(as 30% Et-OH) were administered orally via a stomach tube to the rats. The oral administrations were performed immediately before and after swimming, independently. Blood samples were obtained from caudal veins at 20 minute intervals. Plasma samples were prepared by centrifugation. The P.E.C. was determined with gas-chromatograph. We introduced three parameters: Maximum Et-OH concentration(M.E.C.) (A), time arrived to M.E.C.(B) and disappeared time of Et-OH(C). The effects of swimming on the time courses of P.E.C. were influenced by the environmental factors(temperature, degree of fasting time and timing of oral administration). The effects of swimming on parameters varied markedly with changes of the temperature. In 25°C, the swimming increased two parameters(B & C) without changing A significantly. The shivering, piloerection and panting were observed in all rats during and after swimming. In 35°C, swimming increased only A significantly. No three responses seen in 25°C were observed.

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MODIFICATION OF ADIPOCYTE GLUCAGON RECEPTORS IN COLD ACCLIMATED RATS. UEHARA, A., HABARA, Y. AND KUROSHIMA, A. Dept. of Physiol., Asahikawa Medical College, Nishikagura, Asahikawa 078-11

It has been reported that glucagon is possibly responsible for metabolic cold acclimation (Kuroshima et al., 1984). In the present study the changes in glucagon receptors of white adipocytes in the cold-acclimated rats were investigated to know the metabolic role of glucagon in cold acclimation. Wistar strain male rats were kept at 5 °C (cold-acclimated group) or 25 °C (control group) for 4-5 weeks. Isolated white adipocytes were prepared by the method of Rodbell, and the binding of ^{125}I-labelled glucagon to isolated cells was measured. In the first place, optimal conditions for adipocyte glucagon receptor assay were established as follows: the adipocytes ($1x10^5$ /ml) were incubated in 4% bovine albumin K-R phosphate buffer at 25 °C in the presence of bacitracin after 15 min preincubation. It was demonstrated that cold acclimation increased the number of glucagon receptors of white adipocytes: approximately 140% increase per cell, 260% per unit of surface area and 210% per whole tissue. The affinity of binding sites was not changed.

The result suggests that the increased binding sites of glucagon receptors would be involved, at least partly, in the enhanced nonshivering thermogenesis in cold acclimation.

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ADAPTATIVE MECHANISMS TO PROTEIN DEFICIENCY -THE UTILIZATION OF UREA NITROGEN IN RATS-. TANAKA, N., HORI, S. AND YOSHIMURA, H.* Dept. of Physiol., Hyogo Coll. of Med., Nishinomiya 663, *Dept. of Home Economics, Kobe Women's Univ., Kobe 654

In attempt to clarify the physiological role of urea nitrogen in protein deficiency, we experimented using adult rats fed on low protein diet (5 % casein diet) for 5 weeks (LPD group). In LPD groups, increased ammonia nitrogen in small intestine was significant as compared with that in control groups fed on 20 % casein diet after urea administration (0.4 g / 100 g B.W.). The trace of increased ammonia in the intestine was done after administration of ^{15}N-urea (10 mg / 100 g B.W.). The incorporations of ^{15}N into plasma protein and amino acids were significantly greater in LPD groups than in control groups after oral administration of ^{15}N-urea. Furthermore, incorporation of ^{15}N into lysine and other essential amino acids in portal plasma was much greater in LPD groups than in control groups. This uptake of ^{15}N was greatly supressed by antibiotic mixture. ^{15}N retention in the body protein was about 21 % of ^{15}N of urea administered in LPD group and 4 % in SPD group 2 days after oral administration of ^{15}N-urea. These results suggest that ammonia nitrogen converted from urea by the action of intestinal bacteria urease was utilized for the synthesis of essential amino acids in the intestine and for protein synthesis in liver. This urea utilization must be one of the adaptive responses to conserve nitrogen in protein deficiency.

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Na,K-ATPase AND OXYGEN UPTAKE INDUCED BY PILOCARPINE IN DOG SUBMANDIBULAR GLANDS. KOMABAYASHI, T., NAKANO, K., SAKAMOTO, S., ENDOH, S. AND TSUBOI, M. Dept. of Pharmacol., Tokyo Colle. of Pharma., Hachioji, Tokyo 192-03

Pilocarpine dose-dependently stimulated the release of amylase and sialic acid and oxygen uptake in dog submandibular gland slices. Pilocarpine-stimulated secretion and respiration were significantly blocked by oxidative phosphorylation antagonists, antimycin, azide and malonate. In addition, ouabain significantly inhibited the secretion and oxygen uptake by pilocarpine. A Na,K-ATPase was isolated from microsomal pellet of submandibular glands. The activity was dose-dependently stimulated by pilocarpine. pilocarpine-stimulated Na,K-ATPase activity was considerably blocked by ouabain, antimycin and oligomycin. In addition, pilocarpine-stimulated Na,K-ATPase activity was significantly decreased by removing Ca from the medium and the tissues. These results suggest that the activation of Na-pump is dependent on the presence of Ca and is involved in the process of the secretion and of the increase of oxygen uptake.

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RENAL FUNCTION DURING PHYSICAL EXERCISE -THE EFFECTS OF SODIUM BICARBONATE INGESTION ON URINARY EXCRETION OF CHLORIDE ION AFTER EXHAUSTIVE EXERCISE-. IKAWA,S., SUZUKI,M., SHIOTA,M. AND MATSUBARA,S* Dept. Lab. Med., Tokyo Jikei-kai Univ., Sch. of Med., Minato-ku, Tokyo, 105 *Nihon Univ.

The study was designed to assess the effects of $NaHCO_3$ ingestion on urinary Cl^- excretion, and to investigate the acid-base balance during exhaustive exercise. Serum and urinary electrolytes, and urinary HCO_3^-, ammonia, pH and urine volume were observed for 2h after oral administration of $NaHCO_3$ solution(1.78mEq/kg:500ml)without exercise(CE). On exercise experiment(EE), the treadmill exercise was performed untill exhaustion at 30min after the solution ingestion and the same measurements as the CE were observed up to 2h after exercise. Urine pH and urinary HCO_3^- excretion increased significantly and ammonia excretion decreased at 15min after the ingestion, and the levels of these variables were maintained throughout the CE. The Cl^- excretion was not influenced on CE. Urinary ammonia and Pi excretions increased remarkably at 15min and these enhanced levels maintained up to 90min after the exercise. But the urinary Cl^-, HCO_3^- excretions and pH levels depressed at 15min after the exercise, and thereafter recovered gradually to CE levels. It is suggested that the renal anoxia due to a reduction in renal blood flow caused by exhaustive exercise may alter the reabsorption of Cl^- in preference to other ions in urinary tubule.

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SKELETAL MUSCLE MYOGLOBIN (MB) IN COLD-ACCLIMATED RATS. OHNO,T., KUROSHIMA,A.* Division of Nutrition, Hokkaido Educational Coll., Asahikawa 070 & Dept. of Physiol., Asahikawa Med. Coll., Asahikawa 078-11*

It has been suggested by several investigators that cold acclimation leads to increase in the oxidative activity of skeletal muscles. Mb is known to play the role in storing oxygen and facilitating its diffusion inside muscle cells. In the present study effect of cold acclimation on the skeletal muscle Mb was investigated in the rats exposed to cold for 4 wk, 10 wk and reared in the cold for 35 generations. Furthermore, Mb was measured in the noradrenaline (NA)-treated (30 μg/100 g, sc, 4 wk) or in the heat-acclimated (33 °C, 4 wk) rats. Mb content of the muscles was assayed essetially by the method of Reynafarje (1962). Either cold exposure for 4 wk or that for 10 wk did not cause the significant changes in the Mb levels of red as well as white quadriceps, soleus, diaphragma and heart. But, Mb levels in the quadriceps, soleus and diaphragma were significantly elevated in the rats reared in the cold for 35 generations, who had been reported to posess the greater nonsivering thermogenesis as compared with the usual cold-acclimated animals (Moriya et al., 1984). On the other hand, the heat-acclimated rats showed the lower Mb levels in the quadriceps.

There results suggest that the changes in the Mb levels of skeletal muscles are significantly associated with the modification of nonshivering thermogenic capacity.

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SEX DIFFERENCES IN TEMPERATURE AND CIRCULATORY RESPONSES DURING EXERCISE IN A HOT ENVIRONMENT. ASAYAMA, M., OHNISHI, N., MIYAGAWA, T., YAMASHITA, Y., SUGENOYA, J. AND OGAWA, T. Dept. of Physiol., Aichi Med. Univ., Nagakute, Aichi.

We have investigated further the behavior of blood pressure in the exercising male and female subjects as ambient temperature rises. Our previous study showed that diastolic blood pressure significantly decreases progressively during exercise in a hot environment much more in the male subjects than the female. In this study, we extended our observations to clarify the cause of this phenomenon by measuring cardiac output and forearm blood flow of male and female subjects. Eleven male and seven female subjects, aged 18 to 23 years, exercised for 20 min on a bicycle ergometer at 300 kpm at 41 °C with relative humidity of 60%. Rectul and skin temperatures, heart rate, blood pressure, cardiac output and forearm blood flow were measured during rest, 20 min of exercise and 10 min of recovery. During the experiment, males reacted similarly to females except that diastolic and mean blood pressure were significantly lower and forearm blood flow was significantly higher in the course of the experiment. Results suggest that the large progressive decrease in diastolic blood pressure in the male seen during exercise in a hot environment is due in part to larger progressive increments in cutaneous blood flow and volume in the male subjects' than in the male.

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CHANGES IN THE BODY TEMPERATURE AND OXYGEN CONSUMPTION ($\dot{V}O_2$) DURING REPETITIVE IMMOBILIZATION STRESS. YAHATA, T. AND KUROSHIMA, A. Dept. of Physiol., Asahikawa Medical College, Asahikawa 078-11

Repetitive stress imposed as immobilization was previously reported to cause cross adaptive effect to cold through an enhanced thermogenic capacity (Pflüg. Arch. 402:402, 1984). The present study was undertaken to examine the effect of immobilization on body temperature as well as metabolism. Immobilization stress was imposed on the rat in a warm environment of 25°C. Immobilization for 3 hr caused the hyperthermia accompanied by increased $\dot{V}O_2$. The hyperthermic effect of immobilization was accelerated after one week repetition of daily immobilization and was lessened after 2 to 4 week repetition. The magnitude of $\dot{V}O_2$ increase was the same throughout the experimental period of 4 weeks during the immobilization. Immobilization-induced hyperthermia was significantly potentiated in the cold-acclimated rats, while $\dot{V}O_2$ increase did not differ between cold-acclimated rats and warm controls. These results suggest that immobilization stress causes the changes in body temperature through thermogenic and heat-loss mechanisms.

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OXYGEN CONSUMPTION AND HAIR QUANTITY OF MICE RAISED AT HIGH ALTITUDE ENVIRONMENT YANAGIDAIRA, Y. AND UEDA, G. Dept. of Environ. Physiol., Shinshu Univ. Sch. of Med. Matsumoto, 390

Mice raised at Yatsugatake (Y group) for one month in Autumn (mean temperature, MT, 2.3℃) and the control raised at Matsumoto (M group, alt 610 m, MT=19℃) were studied on the thermogenic mechanisms.

The results indicated that the rectal temperatures (RT) were higher in Y than those in M ($p<0.01$). The RTs were 37.45 ± 0.16 in Y and 36.20 ± 0.35 in M under 0 C and 2400 m conditions. This is mainly due to the elevated oxygen consumption in Y than in M ($p<0.05$) during the cold exposure. Oxygen consumption on the 2nd day after return to the 610 m was 6.37 ± 0.37(SE) in Y and 5.38 ± 0.21 in M. The unit is ml/g/hr. This was significant at $p=0.05$. On the 20th day a slight differnce remained , but there was no difference between Y and M under 0 C room condition. However, the increas e of hair amount covering the body surface was also noticed in Y than in M. The quantity of body lipid was lower in Y than in M only immediately after the return to 610 m. From these, not only the thermogenic, but the thermoinsulative mechanisms must be considered in response to chronic cold exposure. Moreover the deadaptation was complete at latest on the 40th day.

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EFFECTS OF CENTRIFUGAL ACCELERATION (-Gz) UPON CARDIAC OUTPUT IN HAMSTERS. SATAKE, H., MIZUNO, Y., ITO, K., UEKI, S., WATANABE, S.[1], URANO, H.[2] and JIJIWA, H.[3] Inst. Equilibrium Res., Gifu Univ. Sch. Med., Gifu 500 [1]Res. Inst. Environ. Med., Nagoya Univ. [2]Ope. Theat., Fukui Med. Sch. [3]Inst. Develop. Res., Aichi Pref. Colony

In our previous observations, hamsters exposed to centrifugal acceleration (G) in each direction of -Gx, -Gz and +Gz showed cardiac output changes differently depending on the exposed axis. This study was aimed to investigate more precise effects of -Gz (foot to head) upon cardiac output and differences between intact and labyrinthectomized hamsters. Experiment was carried out in the same method as the previous report and the magnitude of G were 4G, 6G, 8G and 10G for 10 minutes or more. Cardiac output of a lightly anaesthetized hamster was estimated by impedance plethysmogram on the chest. Both intact and labyrinthectomized hamsters, cardiac output was decreased during -Gz exposure and there was no recognizable difference between two groups. Recovery of cardiac output after the cessation of -Gz was quicker in intact than labyrinthectomized hamsters and the difference between both groups was significant ($p<0.05$). Above results suggest that the decrease of cardiac output in hamsters during high -Gz exposure may be explained by the decrease of venous return resulting from shift of blood distribution to headward. It is difficult to explain about the difference between intact and labyrinthectomized hamsters in recovery process of cardiac output after -Gz exposure.

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HEMODYNAMIC RESPONSES TO HEAD-UP TILT DURING A 7-DAY SATULATION DIVE AT 31 ATA.
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Cardiovascular responses to a 90°head-up tilt were studied in three male subjects on 2 days of 1 ATA predive and on 2nd and 7th days of 31 ATA exposure, using impedance cardiography. As a result of hyperbaric nocturia on 2nd day of 31 ATA (Hong et al, 1984), there were a reduction of stroke volume (SV), tachycardia and an elevation of total peripheral resistance (TPR) suggesting a circulating blood volume; head-up tilt induced a fainting in one subject and the tilt-induced increase in TPR was less in other subjects, as compared with the controls at 1 ATA, indicating a weak orthostatic reflexes in hyperbaria. On 7th day of 31 ATA the negative fluid balance was compensated and there was no tendency for orthostatic intolerance to those at 1 ATA. It is suggested that the major factors modulating orthostatic reflexes in hyperbaria are 1) an inhibitory effects of negative pressure breathing on vasomotor control via cardiopulmonary receptors and 2) a volume depletion as a result of hyperbaric nocturia.

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EFFECT OF DEXAMETHASONE ON ALDOSTERONE RESPONSE TO ALTITUDE EXPOSURE IN MAN. TAMURA,Y., HATANO,T., KANDA,K., SEO,H. AND MATSUI,N. 2nd Dep., Res.Inst.Environ.Med., Nagoya Univ., Chikusa-ku, Nagoya 464

In our previous studies, an increase in serum aldosterone was induced by simulated altitude exposure and supposed to be one of the causes of altitude-induced disorder affecting water and electrolyte metabolism. This increase correlated positively with that of serum cortisol. To clarify whether ACTH suppression results in abolishment of such aldosterone response and in a decrease of symptoms, 16 men and 1 woman were administered 1 mg of dexamethasone per os at 7:00 and exposed to simulated 6,000 m altitude from 11:00 to 17:00 (every 2 hrs of ascent, stay and return). Meanwhile, subjects were ordered to check symptoms questionnaire. 4 blood and 3 urine samples were collected. 5 subjects who had no serum cortisol response did not show any change in serum aldosterone (suppressed (S)- group), while the other showed significant increases in both hormones by the exposure (non suppressed (NS)-group). In S-group, a decrease in serum K and that in urinary Na/K were less dominant and symptoms were less frequent than in NS-group. Plasma renin activity rose and urinary adrenaline increased equally in both groups.

These results demonstrate that dexamethasone, being sufficient to inhibit cortisol increase, suppresses aldosterone response which may cause a decrease of symptoms.

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HEART FREQUENCY(fH), RESPIRATORY FREQUENCY(fR) AND SLEEP PROFILE IN RHESUS MONKEY UNDER HYPERBARIC ENVIRONMENT($He-N_2-O_2$,ρ=27g/lBTPS). SEKI,K., SHIDARA,F., MIZUSHIMA,Y.,TAYA,Y., NAKANO,M., NARAKI,N. and[1]NAKAYAMA,H. Japan Marine Science and Technology Center, Yokosuka 237, [1]) School of medicine, Tottori University, Yonago

The study in adaptation of the organism to the hyperbaric environment lead us to the flourish future posibility to penetrate in the deep sea. We have carried out comparative (rat, cat) research into neurophysiological and respiratory physiological response, physical performance and adaptation under various environmental gases($He-O_2$, $He-N_2-O_2$, N_2-O_2), depth(surface to 500m) and ways of compression and decompression. Under these background, since 1983, technique of polygraphic study in the isolated hyperbaric chamber have been established in the rhesus monkey, In the present study, in order to obtain the information about the processes of response and adaptation, physiological indices in 4 rhesus monkeys were evaluated under saturation dive in $He-N_2-O_2$ to the maximum environmental pressure of 51 bar(PIO_2=30.8bar, PIN_2=20bar, PIO_2=0.2bar). EEG,EOG,EMG,fH,fR and brain temperature were continuously recorded with the chronic implanted eletrodes during the pre-dive, compression, saturation and decompression. 4 monkeys survived and got back to the surface without serious physiological disorder. fH,fR and sleep profile did not change in these hyperbaric environment, though the body weight decreased 4-11% from reduction of food and water intake probably attributed to the hyperbaric stress.

704

DIVING PATTERN AND ENERGETICS OF WET-SUIT DIVING IN TSUSHIMA MALE BREATH-HOLD DIVERS.
SAGAWA, S., KONDA, N., SHIRAKI, K., PARK, Y. S., and HONG, S. K. Dept. Physiol., Sch. Med., Univ. Occup. Environ. Hlth., Kitakyushu 807; Kosin Med. Coll., Busan, Korea; and SUNY at Buffalo, USA.

Ten professional breath-hold male divers in Tsushima participated in the present study to evaluate the thermal balance of male divers during diving work. Body temperatures, energy balance and insulative value were determined during breath-hold diving in four selected divers with wet suits (protected) and swimming trunks (unprotected) in summer and on protected divers in winter. Rectal temperature decreased by 0.4 °C in 120 min (summer) and 0.8°C in 90 min (winter) in protected divers, and 1.3 °C in 60 min (summer) in unprotected divers. Metabolic heat production during diving work increased by 130% (summer) and 103% (winter) in protected divers and by 220% in unprotected divers (summer). The total thermal cost of daily diving work was 425 kcal/m2 in summer and 405 kcal/m2 in winter, which was by 2.4-fold (summer) and 1.7-fold (winter) greater than that of Korean women divers, respectively. Insulative power of the tissue in protected divers increased from 0.028°C·m2·h/kcal in summer to 0.053 °C · m2 · h/kcal in winter ($p < 0.05$), suggesting an extensive vasoconstriction in male divers in winter, which has never been reported in female divers. The insulative value was less comparing the female counterparts, which is a consequent result of a less body fat in male divers of the present experiment.

705

HEAT TORERANCE OF REPETITIVELY STRESSED RATS.
MURAZUMI,K.,YAHATA,T. AND KURASHIMA,K. Dept. of Physiol., Asahikawa Medical College, Nishikagura, Asahikawa 078-11

In order to see the cross-adaptive effect of repetitive stress on thermal adaptation, heat torerance and metabolic response as assessed by oxygen consumption ($\dot{V}O_2$) were investigated in the stressed rats during heat exposure.

Three-hour immobilization stress was forced on the adult male rats by means of restraining them on the wooden board 6 days a week for 4 weeks. The initial colonic temperature before heat exposure was significantly higher in the stressed rats. Heat exposure(38°C, 60min) caused significant elevations of colonic temperature(Tc) in both controls and repetitively stressed rats. The increases of Tc from the initial levels were significantly greater in the controls. In spite of such differences in Tc between the groups, the initial resting metabolism and the changes by heat exposure were similar in both groups.

These results suggest that a repetitively stress can develop cross adaptation to heat, possibly by means of enhanced heat dissipating capacity.

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INFLUENCE OF FORNICAL SECTION ON COLONIC TEMPERATURE UNDER REPEATED IMMOBILIZATION STRESS IN MALE RATS.
MOHRI, M. Dept. of Physiol., Kanazawa Univ. Sch. of Med., Kanazawa 920

Effects of immobilization stress on heat production (M) and colonic temperature (Tcol) were studied in intact and streotaxic fornical sectioned male rats. Both rats were forced to immobilize 7 days each day for 6 hours. 1st immobilization stress remarkably decreased Tcol, however, 7th immobilization stress did not change Tcol in intact male rats. On the other hand, 1st and 7th immobilization stress markedly decreased Tcol in fornical sectioned male rats. M make little differnce in both groups. But M remarkably increased during 7th immobilization stress in both groups.

It is suggested that hypothermia in the fornical sectioned male rats which induced during 7th immobilization stress, regardless of increased heat production, was remarkably increased heat loss.

707

Effect of dehydration on change in body temperature in hot humid environment.
Nunomura, T. and Nagasaka, T. Kanazawa Univ. Sch. Med., Kanazawa 920

Effects of dehydration on change in body temperature (ΔT_b), total sweat rate (TSW), effective sweat rate (ESW), plasma Na^+ concentration ($[Na^+]$) and % change in plasma volume (PV) were studied. 1) Four healthy men exercised intermittently five times, at 45% $\dot{V}O_2max$ by bicycle ergometer for 20 min, at 30°C, 80% R.H., in two hydration states, once with water (37.5°C) replacement of the same amount as body weight loss every 10 min; rehydration (R) and once without water ingestion; dehydration (D). During the last 20 min, TSW and ESW were less in D than in R. ΔT_b and $[Na^+]$ were greater in D than in R. 2) Six healthy men were exposed to heat at 40°C, 80% R.H. three times intermittently, in two conditions, once with water (37.5°C) replacement of the same amount as body weight loss every 20 min (R), and once dehydrated by about 3% of body weight in sauna the night before and without water ingestion (D). During the last 20 min, there were no significant differences in ΔT_b , ESW and PV between the two conditions (R and D). TSW and $[Na^+]$ were less and higher in D than in R, respectively. These results suggest that dehydration does not affect the change in T_b and ESW at environment extreamly inefficient of sweat evaporation, though it does at relatively efficient environment, such as 30°C, 80% R.H.

708

EFFECTS OF 11 BAR He-02 ON EEG AFTER ADMINISTRATION CI-581 AND THIOPENTAL IN CATS. IWASAKI,M.*, MIZUSHIMA,Y**., SHIDARA,F**., SEKI,K.**, TAKEUCHI,H *. * Dept.Med., Gifu Univ.,Gifu-shi,Gifu 500. ** Japan Marine Science and Technology Center. Yokosuka-shi, Kanagawa 237.

The effect of CI-581 and thiopental on the Motor Cortex and Hippocampus power spectra at 1-6 bar air, 1-11 bar He-02 were investigated. The spontanous EEG with chronically implanted electlodes were simultanously recorded with bipolar leads through eight channel data recorder. Digitalized EEG data were divided 35 x 60 samples of 10 sec of EEG and power spectra calliculated for each sample by ATAC-450 using the fast fourier transform. Within one or two minutes after administration of CI-581, high frequency, low amplitude waves changed to low frequency, high amplitude waves at all of experimental conditions,1-6 bar air and 1-11 bar He-02. Significant differences in the sleep time of CI-581 (5 mg/kg, 1 ml/min) were found between 1 bar air, 6 bar air and 11 bar He-02. CI-581 produced a peak at 1.8- 2.1 Hz in MC at 1-6 bar air,1-11 bar He-02 environments. Recovery from CI-581 took about 5- 18 minutes after injection. No significant differences in sleep time of thiopental (5 mg/kg, 0.2 ml/min) were found between 1 bar air and 6 bar air environments.

709

EFFECTS OF CENTRIFUGAL ACCELERATION UPON RESPIRATORY FUNCTION. MIZUNO, Y., SATAKE, H., UEKI, S., WATANABE, S.,* URANO, H.,** JIJIWA, H.*** Dep. of Neurophysiology, Inst. of Equilibrium Res., Gifu Univ. Sch. of Med. Gifu 500. *RIEM, Nagoya Univ., **Fukui Med. Coll., ***Aichi colony.

Respiratory responses of intact (IN) and bilateral labyrinthectomized (LA) hamsters were investigated during centrifugal acceleration exposure. Seventy-five hamsters were used and anaesthetized lightly with Nembutal throughout the experiment. Hypergravity by centrifugal acceleration was loaded on the hamster in -Z direction (from tail to head; -Gz), which were given in magnitudes of 4,6,8 and 10G. Respiratory responses were analyzed by an impedance plethysmogram, from which the tidal volume and minute volume were estimated. Results were summarized as follows: 1) During high -Gz exposure, the tidal volume was markedly reduced in IN group as well as LA one, and the pattern of pneumogram was changed conspicuously i.e. in same cases the double inspiratory and rapid expiratory patterns, etc. 2) LA group revealed a prolonged recovery of reduce minute volume of respiration, which was mainly caused by decreasing of respiratory rate. 3) From these results, it was suggested that the centrifugal force of -Gz at first acts mechanically on the relatively movable abdominal viscera which put pressure upon the chest cavity, and second, the labyrinthine influence on respiratory function may serve on the recovery of respiratory rate after removable of hypergravity.

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DEADAPTATION OF HIGH-ALTITUDE INDUCED RIGHT VENTRICULAR HYPER-TROPHY IN WILD WOOD MICE, APODEMUS ARGENTEUS SAKAI, A. UEDA G. AND YOSHIDA Y* Dept. of Environ. Physiol. Shinshu Univ. Sch. of Med. and Allied Med. Sci* Matsumoto, 390

Chronic high-altitude exposure is known to bring about right ventricular hypertrophy in various animals. This was studied in high altitude (alt. 2400 m) native mice (H group, n=5). The second is a transferred one to a lower level (alt. 650 m), (HL group, n=4) and was reared there for 18 months. The third was born from H-group parents at a lower level and grown up there (HL-F1 group, n=6). The fourth was a lower-altitude native mice (L-group, n=8). The results were as follows : * is significant at p=0.05.

(1) The whole and right ventricular hypertrophies in H were found in muscle weight as well as in the fiber cross sectional area (CSA).

(2) In HL, the hypertrophy recovered to the normal levels of L.

(3) In HL-F1, the whole (WVW) and the right ventricular weight (RVW) were similar with those of the L even in the level of fiber CSA. WVW for H is 114.5± 15.0 mg and 101.0 ± 14.0 mg for L, *. RVW for H is 31.6± 4.2 and 24.1± 2.9 for L ,*. RVW for HL and HL-F1 are 23.5 ± 5.3 and 21.0± 2.9. the cross section diameter(μm^2) is 46.8 ± 4.9 for H and 27 ~ 31 for other groups. The mean± SD was shown.

711

EFFECTS OF NON-THERMAL STIMULI TO PREOPTIC THERMOSENSITIVE NEURONS IN THE ALERT MONKEY.
KIYOHARA, T., HORI, T., OOMURA, Y.†, NISHINO, H.†, & AOU, S.† Department of Physiology Saga Medical College, Saga 840-01 and †Department of Higher Humoral Control, National Institute of Physiological Sciences, Okazaki 444.

Responsiveness of 214 monkey preoptic neurons (thermosensitive(TS) units - 43 warm units and 37 cold units - and 134 non-TS units) to non-thermal emotional stimuli was investigated while rewarding (juice, food, water) and aversive (5% NaCl, toy snake, air puffer) objects were shown and/or given. Of these, 109 (51%) units changed the activity to such non-thermal stimuli. About 37.5% of TS units and 16.4% of non-TS units changed the acitivity when juice and NaCl filled in the syringes of different colors were shown and/or were tasted. The majority of such units showed the same direction of changes in activity to both rewarding and aversive objects. About half of units tested changed the activity when the monkey opened the mouth and protruded the tongue in an effort to obtain food or to pick up solid foods, indicating the response was not simply related to a specefic movement. This reponse was found most frequently among warm units (19/27 units). All these three types of responses appeared to be dependent upon the emotional changes aroused by stimuli. The results raise a possibility that preoptic TS neurons, besides their postulated thermoregulatory functions, may be involved somehow in non-thermal emotional behaviors controlled in the hypothalamus.

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EFFECTS OF CAPSAICIN AND SUBSTANCE P ON THE ACTIVITY OF PREOPTIC THERMOSENSITIVE NEURONS
SHIBATA, M., HORI, T., KIYOHARA, T., NAKASHIMA, T. AND YAMASAKI, M. Dept. of Physiol., Saga Medical College, Saga 840-01.

Systemic and intrahypothalamic administration of capsaicin (Caps) induces hypothermia by its action on warm sensors. Caps selectively releases and subsequently depletes the substance P (SP) in primary sensory neurons. The preoptic area (POA) contains immunohistochemically active SP. The question arises therefore whether intraPOA Caps affects thermoregulation by the release of SP at POA. Single-unit activities of POA neurons were recorded in urethane-anesthetized (1.2 g/Kg, i.p.) rats and the effects of Caps and SP applied electrophoretically to the neurons were observed with a 8-barrelled microelectrode. Caps increased the activity of 16 (60%) of 27 warm units, and decreased that of 12 (71%) of 17 cold units. There was no effect on 35 (58%) of 60 thermally insensitive units. Of these, 91 units were also tested by SP. SP increased the activity of 3 (14%) and decreased one (5%) of 21 warm units, and increased 2 (15%) and decreased one (8%) of 13 cold units. In contrast, 23 (40%) of 57 insensitive units responded to SP. Immunohistochemical SP in the dorsal horn of Caps-treated rats was drastically reduced while no change was found in the POA even after a local application of Caps. It is concluded that the hypothalamic SP is not involved in thermoregulatory changes observed after Caps injection into the POA.

713

RAT ENDOGENOUS PYROGEN AND FEVER.
MORIMOTO, A., WATANABE, T. AND MURAKAMI, N. Dept. of Physiol., Yamaguchi Univ. Sch. of Med., Ube, Yamaguchi 755

Rat endogenous pyrogen (EP), prepared from the leukocytes of LPS-pretreated rats, produced a fever in both rabbits and rats. The effects of human, rabbit and rat EP on the body temperatures (Tre) of rabbits and rats were investigated. The time to onset and the time to peak of fever induced by rat EP in rabbits and rats were greater than those induced by human and rabbit EP. The maximum elevation of Tre induced by rat EP in both animals was almost identical to those by human and rabbit EP. Comparing the febrile responses to various EPs, the time to fever onset, time to peak and the maximum elevation of fever in rats were approximately half the times those observed for rabbits. The threshold dosage to induce a fever response in rats was greater than that necessary for rabbits.

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RESPONSES OF THE THERMORESPONSIVE NEURONS IN THE HYPOTHALAMIC SLICES TO ENDOGENOUS PYROGEN AND PROSTAGLANDIN E_2. WATANABE, T., MORIMOTO, A. AND MURAKAMI, N. Dept. of Physiol., Yamaguchi Univ. Sch. of Med., Ube, Yamaguchi 755

In this study, effects of endogenous pyrogen (EP) and prostaglandin E_2 (PGE_2) on the hypothalamic thermoresponsive neurons were investigated using in vitro slice preparations. Crude EP was partially purified by the ultrafiltration for excluding many substances other than EP, outside the range of molecular weight 10,000-50,000. Such partially purified EP had no effect on the warm-responsive neurons when applied in close proximity to the neurons through the micropipet attached to the recording electrode. Both bath-applied EP and PGE_2 facilitated a-third of the warm-responsive, cold-responsive and thermally insensitive neurons. In addition, when EP and PGE_2 were bath-applied to the same neuron, all of ten neurons examined changed their firing rates by EP and PGE_2 in the same manner. These results suggest that bath-applied EP caused the neuronal response by stimulating the production and the release of PGE_2 in the slice, which in turn directly effects on the neuron examined.

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INFLUENCE OF HYPOTHALAMIC TEMPERATURE ON THE ACTIVITY OF THALAMIC NEURON RESPONDING TO PERIPHERAL WARMING. SAKATA, Y. AND MURAKAMI, N. Dept. of Physiol., Yamaguchi Univ. Sch. of Med., Ube, Yamaguchi 755

Based on the results obtained from the experiment, using the [^{14}C]-deoxyglucose technique, we examined electrophysiologically if the activity of the VB neurons responding to peripheral warming is affected by the preoptic/anterior hypothalamic (PO/AH) temperature.
The result runs as follows.
1) Most of the VB neurons showed step-like increase of firing rate when average skin temperature exceeded 36.8°C.
2) When the PO/AH temperature was cooled or warmed, neuronal responses of the VB neurons responding to peripheral warming were classified into three types; warm-type of the neuron (15/44) increased the firing rate with a rise in the PO/AH temperature, cold-type (19/44) decreased with a rise in the temperature and insensitive type (10/44) did not show any change.
3) The response curve of the VB neurons to alteration of the PO/AH temperature showed non-linear relation with a threshold temperature for the PO/AH temperature.

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EFFECTS OF CARBON DIOXIDE INHALATION ON PREOPTIC THERMOSENSITIVE NEURONS. TAMAKI, Y., MATSUMURA, K., AND NAKAYAMA, T. Dept. of Physiol., Osaka Univ. Med. Sch., Kita-ku, Osaka 530

Carbon dioxide (CO_2) inhalation has been known to influence body temperature regulation : the downward shift of shivering threshold and the increase in sweating rate , etc. The purpose of this experiments is to examine the effects of rebreathing and inhalation of 4,7 and 10% CO_2 on the preoptic thermosensitive neurons in anesthetized rats. By the time when CO_2 concentration increased to 10% and O_2 concentration decreased to 5%, 54 out of 60 units were influenced. In most cases, unit activities increased to the maximum at 6-8% CO_2 and then decreased with a further increase in CO_2 regardless the neuronal responses to local temperature. During inhalation of 4,7 and 10% CO_2, 35 out of 62 units showed higher firing rate and 15 out of 62 units showed lower firing rate compared with the control level. Thermosensitivity to local brain temperature was significantly reduced in warm-sensitive neurons which increased the steady state discharge by inhalation of 10% CO_2.

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THERMALLY INDUCED SALIVA SPREADING BEHABIOR AND SALIVA SECRETION IN RATS. TANAKA, H., KANOSUE, K., NAKAYAMA, T., AND KAMINAGA, T. Dept. of Physiol., Osaka Univ. Med. Sch., Kita-ku, Osaka 530

Saliva spreading behavior is one of the major responses of heat stressed rat. Present study examined effects of thermal stimulation of various part of the body on saliva spreading behavior as well as on saliva secretion. In one series of experiments,we observed behavior of unrestrained rats with recording of hypothalamic temperature (T_{hy}) at ambient temperature (T_a) of 24,28,32,36 and 40°C. Amount of time spent for saliva spreading behavior increased with increase in T_a. The higher the T_a, the lower the T_{hy} at which saliva spreading behavior started. In the other series of experiments, saliva secretion was measured in urethane anesthetized rats (1.0g/kg). Rectal temperature (T_{re}) was raised slowly (2°C/hour) by using water jacket. Saliva secretion occurred when T_{re} reached 40.0 - 40.8°C. Hypothalamus, face, scrotum, and abdomen not including the scrotal area, were locally warmed. When T_{re} was low, saliva secretion was not produced by warming of any regions. When T_{re} was over a certain value, usually around 40°C, warming of each region, especially the scrotum and face, elicited saliva secretion.

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EFFECTS OF SCROTAL THERMAL STIMULATION ON ELECTRICAL ACTIVITY OF SUPRAOPTIC NEUROSECRETORY CELLS. MATSUMURA, K. AND NAKAYAMA, T., Dept. of Physiol., Osaka Univ. Med. Sch., Osaka 530

In anesthetized rats, the influence of scrotal temperature (T_{scr}) was studied on supraoptic neurosecretory (NS) cells identified antidromically. T_{scr} was kept constant between 27° and 32°C in a normal state and changed between 25° and 42°C using a Peltier device. In response to scrotal warming, 10 out of 11 intermittently bursting NS cells (B cells) and three of 38 continuously firing NS cells (C cells) decreased their firing activity. Three of the 10 B cells increased the activity again at T_{scr} over 38°C. The threshold T_{scr} for the decrease of neuronal activity was different among the cells but mostly ranged between 30° and 40°C. The changes in the activity were not transient but lasted while keeping T_{scr} constant at a certain level. Only one C cell showed transient increase of the activity. One B cell, 34 C cells and 6 silent cells were not influenced by T_{scr}. Increases of arterial blood pressure were not essential to the inhibitory influence of T_{scr} on the NS cells. These results indicate that antidiuretic hormone secretion might be suppressed by scrotal warming.

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TRANSMITTER SENSITIVITY OF THERMALLY CHARACTERIZED SINGLE UNITS IN THE HYPOTHALAMUS OF CONSCIOUS DUCKS. SATO, H. and Simon, E.*, Dept. of Physiol., Nagoya City Univ., Sch. of Med., Mizuho-ku, Nagoya 467, *Max-Planck-Institut für Physiologische u. Klinische Forschung, W. G. Kerckhoff-Institut, D-6350 Bad Nauheim, F. R. G.

Multi-barrel carbon fiber microelectrodes were used to record the extracellular unit activity of thermo-sensitive neurones in the hypothalamus of conscious Pekin ducks, and to apply 5-hydroxytryptamine, acetylcholine, noradrenaline and γ-aminobutylic acid, by microiontophoresis. 357 units were tested for responsiveness to local thermal stimulation, which was applied by the thermode chronically implanted in the hypothalamic region. 134 of these units were identified as locally thermo-sensitive, 64 warm and 70 cold. In some experiments, whole body cooling was performed by colonic thermode to examine the relation between extrahypothalamic thermal input and transmitter sensitivity. The majority (67%) of 27 cold units were facilitated by 5-HT. 64% of 34 warm units were depressed by NA. More than 50% of both type of units were facilitated by Ach. A half of warm units were facilitated and the rest depressed by 5-HT. Therefore, this type of units were cathegorized into two groups depending on the action of 5-HT. The warm units facilitated by 5-HT exhibited a close resemblance to the cold units with respect to responsiveness to Ach and NA. It was suggested that 5-HT would be related to the transmission of cold signal in the hypothalamic thermoregulatory network of ducks.

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NEURONAL CONTROL OF BAT THERMOGENESIS AND LIPID METABOLISM. MINOKOSHI, Y. AND SAITO, M. Dept. of Biochem., Ehime Univ. Sch. of Med., Shigenobu, Onsen-gun, Ehime 791-02

Roles of the ventromedial hypothalamus (VMH) and sympathetic nerves on brown adipose tissue (BAT) thermogenesis and lipid metabolism were studied in rats. Electrical stimulation of the VMH induced a rapid rise in BAT temperature, which was almost abolished by severing the sympathetic nerves entering the BAT. A quite similar response was observed when the sympathetic nerves were stimulated, suggesting that sympathetic innervation of BAT is the primary factor involved in VMH-induced BAT thermogenesis.

Electrical stimulation of the VMH greatly activated lipid synthesis in BAT. That is, it enhanced incorporation of ^{14}C from ^{14}C-glucose into both fatty acids and glyceride glycerol. The stimulatory effect of the VMH on BAT lipogenesis disappeared after surgical denervation, but was mimicked by electrical stimulation of the sympathetic nerves. Pretreatment of propranolol suppressed the increase in glyceride glycerol synthesis but not that in fatty acids synthesis. It was suggested that the VMH may control BAT lipid metabolism through activation of two different mechanisms of the sympathetic nerves.

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EFFECTS OF THERMAL STIMULATION OF THE SPINAL CORD ON THE CARDIOVASCULAR FUNCTIONS IN THE BULLFROG (Rana catesbeiana). NAGAI, M. and IRIKI, M. Dept. Physiol., Med. Univ. Yamanashi, Yamanashi 409-38

Recent observations have shown that ectothermic vertebrates possesses the same central mechanism as that of homeotherms for initiating temperature responses. In the present experiments, we investigated the effect of thermal stimulation of the spinal cord on cardiovascular functions in the summer bullfrog(Rana catesbeiana) under urethane-anesthesia. Blood pressures of the truncus arteriosus and conus arteriosus were measured. To effect thermal stimulation of the spinal cord, hot or cold water was perfused through a U-shaped cannula inserted into the vertebral cavity. Results: 1) Warm stimulation of the spinal cord caused an increase in systolic pressure of the truncus, and cooling caused a decrease. This indicates that peripheral vascular resistance in systemic and carotid circulation changes during thermal stimulation. 2) Spinal cord warming caused an increase in systolic and pulse pressures of the conus, and cooling caused a decrease. This indicates that stroke volume changes as well as peripheral vascular resistance.

These results showed the temperature perceptibility of the spinal cord and a relationship between spinal cord temperature and autonomic functions in the frog as in other endothermic and ectothermic species.

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THE DYNAMIC PROPERTY OF THE THERMOREGULATORY SYSTEM IN SPINAL RATS IN RESPONSE TO THE CYCLIC CHANGE OF AMBIENT TEMPERATURE. ASAKI, Y. and IRIKI, M.* (Dept. of Physiol., Tokyo Metropol. Inst. of Gerontol., Tokyo 173, *Dept. of Physiol., Med. Univ. of Yamanashi, Yamanashi 409-38)

The fluctuations of rectal and tail skin temperatures in spinal rats in response to the cyclic change of ambient temperature were measured. The spinal rats were used for experiments after two days, since they had been sectioned between C5 and C7 of the cervical vertebrae. The ambient temperature was controlled to fluctuate in a sine wave centering at 30 °C. The cyclic interval was one hour and its amplitude was 5 °C. The mean levels during a 2-hour experiment in rectal(Tr), tail skin(Ts) and ambient temperatures were calculated, respectively (mean Tr, mean Ts and mean Ta).

In spinal rats as compared with intact rats, 1. the fluctuation of Tr significantly increased , 2. the mean Tr significantly decreased, and 3. the fluctuation of Ts tend to decreased. 4. The fluctuation of Tr in spinal rats significantly larger than that in dead bodies.

The decrease of the thermoregulatory function in spinal rats was demonstrated by the dynamic property due to thermal disturbance. Resulting from the spinal and dead bodies, the effect of blood flow with respect to heat transfer in the body can be considered.

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STUDIES ON THERMOREGULATORY MECHANISMS IN HEAT-ACCLIMATED RABBITS. FUJIWARA, M., OHWATARI, N. & KOSAKA, M. Dept. of Environment. Physiol., Inst. for Trop. Med., Nagasaki Univ., Nagasaki

The effects of heat acclimation on thermoregulatory responses were examined on 3 groups of rabbits (A: control; in 25°C, 60%, B:heat-acclimated; in 30°C, 60% for 0.5 month, C: heat-acclimated; in 30°C, 60% for 6 months). After beginning of heat exposure, rectal temperature (Tr) of groups B and C were slightly raised, but these differences disappeared in 3 or 4 days , and after that period, there were no significant differences in Tr among A,B and C. Increase rate of body weight in B and C were slower than A. Respiratory frequency in both B and C was twice compared that of A. Vaso-dilation in ear skin of A already occured at thermo-neutral zone, whereas that in group B and C was observed at 5°C or 6°C higher temperature than A. During general whole body cooling (40°C - 10°C), temperature of beginning of vaso-constriction was 12.8°C in A. But in heat acclimated groups, it began 32.3°C for B and 31.8°C for C, respectively. Slope of changes in Tr against the ambient temperature (Ta) became steeper according to heat acclimation. This fact suggested that in heat acclimated rabbits, sensitivities against the cold stimulus was raised.

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CARDIOVASCULAR RESPONSES TO THERMAL STIMULATION IN CHRONICALLY SPINALIZED RATS. TSUCHIYA, K. Dept. of Environment. Physiol.,Inst. for Tropical Medicine,Nagasaki Univ., Sakamoto-machi, Nagasaki

In chronically spinalized rats, effects of scrotal skin heating and selective thermal stimulation of spinal cord were investigated. After spinal transection at the level of C8/Th1, rats were reared more than two weeks in the warm room (30°C) before experiment. Rats were anesthetized with sodium pentobarbital. Scrotal skin was heated selectively by a water-perfused thermode, of which temperature was changed stepwise from 30°C to 42°C (2°C/3 min). An increase in heart rate was observed during the scrotal heating. This result is the same as those observed in decerebrated and in acutely spinalized rats. On the other hand, when thoracic or lumbosacral cord was stimulated thermally by means of a spinal thermode of U-shaped polyethylene tube, an increase in heart rate was induced by cooling or heating of the thoracic cord. But definite change in heart rate could not be observed during lumbosacral cooling. It was already reported that effect of thermal stimulation of spinal cord vanished in acutely spinalized rats (K. Tsuchiya, J. Physiol. Soc. Japan 46, 564, 1984). These results suggest that spinal function concerned with this response partially recovered in chronically spinalized rats.

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CIRCULATING BLOOD VOLUME CHANGE FOLLOWING FLUID INTAKE IN THE CONSCIOUS RAT. NOSE, H., MORITA, M., YAWATA, T, and MORIMOTO, T. Dept. Physiol., Kyoto Pref. Univ. Med., Kamigyo-ku, Kyoto 602, Japan.

Recovery from thermal dehydration was assessed by continuous monitoring of blood volume and fluid intake. At least 5 days prior to the experiment, rats were splenectomized and cannulated into jugular vein and descending aorta. After the dehydration (6% of body weight), the catheters were connected with the continuous monitoring system of blood volume, and the blood volume change and drinking rate were recorded at 30 sec intervals for 2 hrs providing tap water or 0.45% NaCl ad. libitum. As soon as fluid became available, the rat took a large draft (2-3 ml/100g b.w.) and then continued to drink about 1 ml/100g b.w. at 30-40 min intervals. Blood volume increased gradually up to 15-30 min after each draft and then decreased untill the following fluid intake. The retention ratio of ingested fluid in vascular space was about 10-20% on an average in both groups. In 2 hrs after the fluid supply, the rats regained about a half of the lost water and of the depleted blood volume in tap water group, while almost all in 0.45% NaCl group. The rehydration for 2 hrs decreased plasma Na concentration to a control level in tap water group, while in 0.45% NaCl group, the level was sustained high. The results suggest that the low fluid intake in tap water group is due to a defense mechanism against hypoosmolality and that blood volume regulation gives priority to osmo-regulation in the restitution from thermal dehydration.

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MODULATION OF HOMEOSTATIC REGULATION OF HEAT PRODUCTION BY β-ENDORPHIN.
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The effects of intra-third ventricular injection of β-endorphin (EP) on rectal temperature (Tre), oxygen consumption and heat escape operant behavior were studied in warm-acclimated rats under acute exposure to various ambient temperatures (Ta). EP induced a rise in Tre with an increase in oxygen consumption at Ta above Critical Ta estimated between 10°C and 16°C, while it induced a fall in Tre with a decrease in oxygen consumption at Ta below Critical Ta. The mean oxygen consumption-level either during hyperthermic or hypothermic response to EP reached an equal level which was equivalent to a maximum level of non-shivering thermogenesis induced by the systemic norepinephrine infusion. Behavioral responses, bar press and heat escape against heat stress were not affected by EP. Any behavioral compensation for EP-induced hyperthermia and hypothermia was not observed. The data indicate that EP acts primarily to suppress homeostatic regulation of heat production in each environment, but may not affect behavioral responsiveness to thermal stress. EP may function to modulate "adaptability-level" of the heat balance.

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MECHANISMS OF SWEATING FACILITATION FOLLOWING HEAT ACCLIMATION BY CONTROLLED HYPERTHERMIA.
SUGENOYA, J., OGAWA, T., ASAYAMA, M., MIYAGAWA, T., YAMASHITA, Y., OHNISHI, N. AND KIHARA, M.* Dept. of Physiol. and 4th Dept. of Int. Med.*, Aichi Med. Univ. Nagakute, Aichi.

Characteristics of frequency of sweat expulsion (Fsw) before and after the heat acclimation accomplished by controlled hyperthermia (CH) was analyzed. Each of 4 unfit subjects underwent two 10-day series of heat exposure, one by a sauna box (dry heating) and the other by a Blanketrol (wet heating). Each exposure was carried out for 90 min per day by adjusting heat load so as to maintain Tre at 38.0 ± 0.1 C. Before and after a series of heat exposure, forearm sweat rates and weight loss were recorded continuously at rest in various hot environments (Ta, 32 - 43 °C; rh, 40%). Except a few cases where no significant increase of sweat rate was observed following the heat exposures, the regression line of Fsw in relation to body temperature shifted to the left following the acclimation, while the regression line of total sweat loss in relation to Fsw shifted to the left in some experiments but hardly shifted in the others. This shift appeared to be more conspicuous in dry heating than in wet heating. It was suggested that for the increase of sweating capacity following the heat acclimation by CH the central mechanism is predominant over the peripheral mechanism.

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EFFECTS OF THYROTROPIN RELEASING HORMONE ON HUMAN THERMOREGULATORY RESPONSES.
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Thyrotropin releasing hormone (TRH) with a concentration of 1×10^{-5} was administered intravenously to 3 volunteers at a rate of 10ml/min. In hot environments (Ta, 35-45 °C; rh, 40%), TRH was infused for a period of 20 min. TRH caused immediately an increase in local sweat rate (recorded on forearms using capacitance hygrometry) followed by a fall of Tcore; sweat rate reached its peak in 3 to 4 min; subsequently it began to decline and sustained thereafter a level lower than that before the infusion of TRH, while Tcore maintained the low level. The regression line obtained from the plots of frequency of sweat expulsion (Fsw) during the last 10 min of TRH infusion against mean body temperature shifted to the left of the control during the TRH infusion. The regression line obtained from the plots of sweat rate against Fsw hardly changed. During the TRH administration in a thermo-neutral zone (Ta, 25 °C; rh, 40%), an increase of skin blood flow and a slight elevation of skin temperature were observed by fingertip plethysmography, thermography and thermistor thermometry. It was suggested that TRH reduces the set-point temperature in the human, contrary to the results on animals reported by others.

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HUMAN ECCRINE SWEAT GLANDS ACTIVITIES STUDIED BY LOCALIZED ELECTRICAL FIELD STIMULATION
OHARA,K, HASHITANI,T, KUMAZAKI,M. 2nd Dept. Physiol., Nagoya City Univ. Med. Sch., Nagoya 467

Sweat glands activities, especially upon neuro-glandular transmission of the secretory nervous impulses, were studied by electrical field stimulation. For this study, a double cylinder sweat capsule was newly developed. Stimulating current was applied between skin surface electrode mounted underside of the outer capsule (inn.dia. 20 mm) and a intradermal indwelling needle which was placed in the skin under the inner capsule (inn. dia. 10 mm) and coated for electrical isolation except for 2 mm of the tip. The inner capsule served as the sweating measuring capsule, sweat volume being recorded as usual using dry N_2 gas flow and VAISALA hygrometer method. Test drugs were administered intradermally 0.05 ml through the indwelling needle. Current pulses of 20 Hz, 3 msec duration were applied with polarity reversal for each 5 pulses. Pulse amplitude up to about 1200 μA arose any remarkble pain. The current strength - response relation curves were obtained with high reproducibility. Threshold current strength was used as the index of the effect of test drugs. It was reconfirmed that adrenergic agonists facilitated the neuro-glandular transmission as reported before in the generalized sweating reflex in a hot room.

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REGIONAL DIFFERENCES IN SWEAT RATE OF MEN AT REST. TSUJITA, J., HORI, S., MAYUZUMI, M.* AND MEREN, J.* Dept. of Physiol., Hyogo Coll. of Med., Nishinomiya 663, *Dept. of Physical Education, Mukogawa Women's Univ., Nishinomiya 663

Observation of sweat rate on different parts of the body surface was made on four young men in winter. Each subject, dressed in shorts only, sat in a chair in the room of 20 °C - 22 °C with 70 % relative humidity for 30 min. Then each subject in the sitting-resting position was exposed to a hot environment for 2 hrs. physiological responses to heat were measured under four different environmental conditions, 35 °C and 50 % R.H., 35 °C and 70 % R.H., 40 °C and 50 % R.H., and 40 °C and 70 % R.H. Samples of local sweat from the forehead, forearm, chest, abdomen and thigh were collected for a 5 min. period using the filter paper method. The greater the weight loss, the greater was local sweat volume despite considerably individual variations in the ratio of weight loss the body weight. There were great individual variations in patterns of local sweat distribution in four conditions. Peak sweat rate on the forehead was markedly greater than those on the trunk and extremity. Hidromeiosis occurred earlier under humid condition. Local sweat rate on the forehead showed earlier onset of hidromeiosis in comparison of those on the extremity and trunk in all conditions. Hidromeiosis tended to occur earlier in extremity than in trunk.

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COMPARISON OF HEAT LOSS ABILITY BETWEEN TROPICAL INHABITANTS AND JAPANESE. OHWATARI, N., FAN, Y., YE-WIN, IWAMOTO, J., KOSAKA, M. Dept. of Environment. Physiol., Inst. of Tropical Medicine, Nagasaki Univ., Sakamoto-machi, Nagasaki

The sweating onset time on chest and abdomen of seven tropical inhabitants and two Japanese were determined by the use of thermography in environmental chamber (28°C,60%). The use of thermography enables to detect mean skin temperature of specified area without physical contact, irrespective of skin color and area size. The lower limbs were immersed in 43°C circulating water for half an hour while the oral temperature, skin temperature at fore-arm, upper arm, back and room air temperature were continuously monitored and recorded every one minute. The sweating onset on chest and abdomen were determined by use of thermography pictures. The duration between starting heat load application and sweating onset were found to be longer in tropical subjects compared to that of Japanese subjects. The rate of rise of oral temperature in tropical subjects were higher than that of Japanese subjects. Individually, the mean skin temperature on abdomen during sweating is always lower than that on the chest. The abdomen tends to sweat faster compared to chest. After terminating heat-load, the chest skin mean temperature instantly rises whereas the abdominal skin mean temperature showed a little but definite dip, the physiologic significance of which is a task for further investigation leading to solution of the problem of heat-acclimatization.

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HUMAN TYMPANIC MEMBRANE TEMPERATURE DURING 5% CO_2 INHALATION.
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The tympanic membrane received its blood supply from internal branches of the carotide artery as does the hypothalamus. This study examined how common carotide artery blood flow affects the responsiveness of the tympanic membrane temperature(Tty). Common carotide artery blood flow(CCAF), common carotide artery diameter(CCAD) and common carotide artery flow velocity(CCAV)was measured by Doppler's method. The results were as follows: 1. During 5% CO_2 inhalation CCAF increased 30-60%, CCAD and CCAV likewise increased , and the degree is not large than CCAF. Among CCAF, CCAD and CCAV, it was observed that the liniear relation was same. 2. Tty decreased 0.12-0.16 °C during CO_2 inhalation and after then, Tty decreasing 10 min. 3. The skin temperature of the forehead decreased on the whole, after then, it's temperature as befor. In case of hyperventilation CCAF, CCAD and CCAV had inclination to decrease. Tty hardly changed. One of causes which Tty decrease was thought that aortic blood temperature which is lower than common carotide artery blood temperature flows in intracranial region. This matter is the result of quickning a heatloss mechanism when hypothalamus temperature decreased. During hyperventilation(respiration rate: 20/min) decreased CCAF 23-33%, CCAD and CCAV somewhat, but Tty decreased 0.07-0.19 °C.

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A PORTABLE DEEP BODY THERMOMETER WITH A NEWLY DESIGNED FLEXIBLE PROBE.
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A portable monitor with a light, thin and flexible probe was developed for measuring the whole day variation in the deep body temperature(DBT). The probe consists of a thermistor, a heat flow sensor and a heater. The heater functions to compensate the heat flow from the tissue so as to insulate the skin surface against the ambience. The diameter and thickness of the probe are 46 and 3mm, respectively, and the weight is about 5g. It is light and flexible enough to contact well with the skin. By a 2-kbyte RAM built in the monitor, maximally 34-hour DBT variation can be stored. This has been applied on the forehead, the chest wall, the abdomen and the calf. It was found that the DBT in the trunk during the sleep was low and stable, while that in the calf was high and stable, compared with those during the day.

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CONTINUOUS RECORDINGS OF SWEATING RATE BY USE OF A HYGROMETER OF ELECTROSTATIC CAPACITY TYPE.
SAKAGUCHI, M., OHHASHI, T# AND AZUMA, T.##
Dept. of Elect. Eng., Nagano Tech. Coll., Nagano 380 and #Dept. of Physiol., Sch. of Med., Shinshu Univ., Matsumoto 390 and ##Juntendo Univ., Tokyo 113

We have designed and constructed a simple apparatus for continuous recordings of sweating rate utilizing a new hygrometer which can change electrostatic capacity with changes in vapor tension of the ambient air. An acryl skin capsule was equipped with the hygrometer on the internal surface of the roof. A thermometer using silicon transister of microdisc type was placed on the skin area covered with the capsule. Dry N_2 gas at a fixed flow rate in order to diffuse quickly the vapor produced by sweating in the capsule and adjust the level of vapor tension to the dynamic range of the hygrometer. A linear relationship between the amount of water loss in the capsule and the output voltage was obtained within a range of water loss from 4 to 50mg/5min. A few results of the preliminary application of this apparatus to human beings were also presented. Thus, mental calculation deep brething, and talking caused a significant increase of the sweating rate from the palmer area, respectively.

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CHANGES IN THE TURNOVER RATE OF CATECHOLAMINES IN THE RATS BRAIN TISSUES DURING EXPOSURE TO COLD ENVIRONMENT. KONDO,Y., YAMAZAKI,S., SAKAGUCHI,E. & YURUGI,Y. Dept of Hygiene National Defense Medical College, Tokorozawa, Saitama 359.

Effect of cold exposure(5°C for 3 weeks) on turnover rate(TOR) of noradrenaline(NA) and dopamine(DA) in the rats brain were studied in conjunction with observation of food intake, body weight gain, hormone sensitive lipase(HSL) activity in the brown adipose tissue(BAT), TOR of NA in the BAT and lipids content in the blood. NA and DA content were measured by liquid chromatography technique. TOR was assessed after blockig catecholamine synthesis with α-methyl para-tyrosine(α-MPT). NA and DA contents varied with location of the brain, whereas showed no significant change by the cold exposure. However, TOR of hypothalamic NA exhibited marked elevation during first two weeks of cold exposure and levelled thereafter. In the mean time, increased food intake and antipodally depressed weight gain were observed soon after starting the cold exposure. Wet weight of intrascapular BAT and its total HSL activity were progressively increased during first 9 days of cold exposure and levelled thereafter. Regardless of the same levels of NA content in BAT in both warm and cold groups, its TOR was markedly elevated in cold exposure group. Peculiar changes were also observed in the levels of blood lipids after injection of α-MPT and cold exposure. From these results, hypothalamic NA TOR was presumed to cope in some extent with the change observed in peripheral organs.

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SEASONAL VARIATIONS IN EFFECTS OF ACUTE COLD EXPOSURE ON HUMAN BLOOD PARAMETERS. OHNO, H., YAHATA, T. AND KUROSHIMA, A. Dept. of physiol. Asahikawa Medical College, Nishikagura, Asahikawa 078-11

Seven healthy male students voluntarily underwent acute cold exposure at 10 °C for 60 min in summer (Jun.) and in winter (Jan.-Feb.), respectively. In summer blood pyruvate and lactate concentrations showed significant ($P<0.05$) decreases during cold exposure, while they did not vary substantially in winter. In the pre-exposure values in winter there were significant ($P<0.05$) increases in α_2-macroglobulin, sodium, and chloride of plasma and in zinc of erythrocytes; in contrast, there were significant ($P<0.05$) falls in ceruloplasmin and lactate dehydrogenase activity of plasma and in iron of erythrocytes.

It was considered that the seasonal changes in these blood parameters might be the outcome of climatic acclimatization, although the mechanism remains to be explained.

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SUDDEN DEATH OF STARVED ANALBUMINEMIC RATS (NAR) AND ITS ABOLISHMENT BY URIC ACID ADMINISTRATION KAWAGUCHI, T., SHIMODE, M., MATSUSHITA, H. AND NAGASE, S. Dept. of Physiol. Wakayama Medical College, Wakayama 640 and Dept. of Chemistry, Sasaki Institute Tokyo..

NAR, Wister, S.D. Charles River rats were kept without food at 5°C or 24°C. In all conditions, NAR died fastest than others, especially it survived only 12 hr at 5°C without food. Sudden death of NAR is considered to be due to a lack of some substance present in control SD rat blood. Therefore several small molecular fractions of SD rat serum were administered to NAR kept without food at 5°C. When Fraction 5 was administered , NAR survived 30 hr: NAR with other fractions lived only 12-14 hr. This fraction was proved to be uric acid. Administration of uric acid to fasted C57BL/6J-ob/ob mouse extended its survival time from 7days to 30 days. Administration (oral and injection) of it to ob/ob mouse with freely access of food and water made it lean as a control mouse: it bore baby.

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ACTIVATION OF THERMOREGULATORY-LIKE RESPONSES BY ELECTRIC STIMULATION OF VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS IN RABBITS. ONO,T.,MORIMOTO,A. AND MURAKAMI,N. Dept.of Physiol.,Yamaguchi Univ.Sch. of Med., Ube, 755

It has been revealed in our laboratory that the metabolic activity of the ventromedial nucleus of the hypothalamus was markedly affected by cold stimulation of the whole skin or the preoptic/anterior hypothalamic area in rats, using the [^{14}C]deoxyglucose technique. Hence, we examined in rabbits if electric stimulation of ventromedial nucleus of the hypothalamus could induce heat conservative and heat productive responses that were developed when exposured to cold environment.
The results runs as follows: 1) During electric stimulation for 30 min, oxygen consumption increased to the maximum (13.0 ± 0.03 ml/kg·min) 20 min after the onset of stimulation. Ear skin temperature fell rapidly down and returned to the initial level after the completion of stimulation. In consequence, the rectal temperature gradually increased reaching the maximum of 0.61 ± 0.08°C. 2) With a pretreatment of propranolol (1 mg/kg, i.v.) 20 min before the stimulation, responses of O_2-consumption, ear skin temperature and rectal temperature to the stimulation were significantly reduced.

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EFFECTS OF BOMBESIN AND NEUROTENSIN ON THERMOSENSITIVE NEURONS IN PREOPTIC AND ANTERIOR HYPOTHALAMUS IN THE RAT. YAMASAKI, M., HORI, T., KIYOHARA, T. and SHIBATA, M.
Dept. Physiol., Saga Medical College, Saga 840-01, Japan.

Bombesin (BBS) and neurotensin (NT), hypothalamic peptides, have been reported to exert potent effects on the central thermoregulation, in addition to their CNS-mediated effects on blood glucose regulation, food intake and nociception. Both peptides, when given into the preoptic and anterior hypothalamus (PO/AH), elicit poikilothermia, i.e., hypothermia in a cold environment and hyperthermia in a warm environment. Single-unit activities of PO/AH neurons of urethane-anesthetized rats were studied for their responsiveness to electrophoretically applied BBS and NT by the use of 8-barrelled microelectrodes. Of 144 PO/AH units (41 warm-units, 22 cold-units and 81 thermally insensitive units) tested, BBS decreased the activity of about half of units (54.0% of thermosensitive units and 49.4% of thermally insensitive units) and excited 13.9% of units. NT produced similar effects on 128 PO/AH units (50.0% of units were inhibited and 19.5% were excited). Long-lasting (2-10 min after termination of drug application) inhibition and excitation, besides short-lasting responses (less than 30 sec), were observed after application of both peptides. The poikilothermic responses to intraPO/AH BBS and NT may be explained, at least in part, by the depressing effects of two peptides on PO/AH thermosensitive neurons.

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PROTECTIVE EFFECT OF PROSTACYCLIN(PGI_2) ON THE BRAIN ISCHEMIA. YONEDA, K and OKADA, Y. Department of Physiology, School of Medicine, Kobe University, Kusunoki-cho, Chuo-ku, Kobe 650, Japan

To investigate the effect of PGI_2 on the survival time(duration of ischemic period for the complete functional recovery) of the ischemic brain, PGI_2(Ono 47241) was injected(2-4mg/kg,i.c.) to guinea pig.One hour after the injection, animals were decapitated to make the brain completely ischemic. The head was then left at room temperature for 10, 20, 30, 40 and 60 min and thereafter the skull was opened and the hippocampus was excised. Thin hippocampal slices were prepared and incubated in glucose containing(10mM) Krebs-Ringer solution for 30 min. To test the functional recovery of the slices, the postsynaptic field potential was recorded in CA3-4 region of pyramidal cell layer.The level of ATP and creatine-P was determined in each slice. In the PGI_2 treated animals, electrical activity could be recorded in the slices even after 30 min complete ischemia whereas in the control slice no activity was observed after 20 min ischemia, although the level of ATP and Cr-P recovered similarly in both PGI_2-treated and control slices. Thus the PGI_2 prolonged the survival time of the ischemic brain tissue.

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LONG-TERM DEPRESSION OF PARALLEL FIBER-PUKINJE CELL SYNAPSES IN VITRO. SAKURAI, M., Department of Physiology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

Long-term depression(LTD) of parallel fiber(PF)-Purkinje cell(PC) synapses induced by conjunctive PF and climbing fiber(CF) stimulation has been studied so far in decerebrate rabbits by extracellular recording. For further investigation of its cellular and molecular mechanisms, studies in vitro preparation should offer advantages over those in vivo. In the present study, it is shown that LTD is also induced in slice preparation by intracellular recording technique. Parasagital slices were prepared from guinea pig cerebellar vermis and intracellular recordings were made from PCs mainly from their dendrites. PFs were stimulated through the electrode placed near the pial surface, while CFs were activated by white matter stimulation. Picrotoxin was added into the medium to eliminate IPSPs. Concurrent stimulation of PFs and CF at 4 Hz for 25 sec resulted in appreciable reduction of PFs-evoked EPSPs. This reduction lasted for 20-60 min as long as intracellular recordings continued stably. On the other hand, when PF stimulation was associated with white matter stimulation just subthreshold for activating CF, such depression was not observed. There were no associated changes in membrane potential and input resistance.

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＊印は非会員を示す

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〔会　報〕

日本生理学会昭和60年度第1回常任幹事会議事録要旨

日　時：昭和60年3月27日(水)　午後2時～6時
会　場：萃香園ホテル
出席者：加藤正道，広重　力，田崎京二，西山明徳，新島　旭，本郷利憲，本間三郎，三浦光彦，伊藤正男，酒井敏夫，島津　浩，竹内　昭，塚田裕三，古河太郎，星　猛，菊地鎌二，入沢　宏，富田忠雄，江橋節郎，御手洗玄洋，久野　宗，中馬一郎，中山昭雄，藤本　守，岡田博匡，中山　沃，大村　裕，後藤昌義，西　彰五郎(当番幹事)，望月政司(次回当番幹事)，加藤宏司(次回当番幹事)
欠席者：永坂鉄夫
議　長：纐纈教三(当番幹事)

Ⅰ．報　告

1. 庶務報告（伊藤庶務幹事）

◦会員の異動（昭和60年3月現在）．入会128名，退会68名，会員総数3,319名（一般会員3,055，評議員1,006，特別会員28，名誉会員 3，準会員233）である（表1）．

◦科学研究助成．第11回日産学術研究助成に神野耕太郎氏が採択された．昭和60年度山田科学振興財団研究援助に久野　宗氏と酒井敏夫氏を推薦した．

◦協賛．第1回ニューロエソロジー国際学会を協賛する旨報告され了承された．

◦日本学術会議．会員候補者に本間三郎氏，推薦人に伊藤正男氏を推薦した．

2. 会計報告（星会計幹事）（表2）

◦日本生理学会昭和59年度決算について報告，説明がなされ，了承された．この決算については古河，島津両会計幹事の監査を受け，承認を得た．

3. 日本生理学雑誌編集報告（酒井編集幹事）

◦昭和60年度47巻1～4号についての発刊と編集状況及び昭和59年度編集報告（表3）がなされ，論文表題集原稿の締切りを従来より1ヶ月延期して4月末日とすることが報告された．

◦昭和59年度編集委員の交代について．
新島　旭委員，永坂鉄夫委員，田中励作委員は，4月より本間信治氏，小野武年氏，真野範一氏と交代になる．

4. J. J. P. 編集会計報告（中山委員長）

◦昭和59年度決算報告（59年4月より60年3月まで）と，60年度予算（見込）が了承された（表4）．

◦J. J. P. 刊行状況．昭和59年度の配布状況は表5のとおりである．

5. 会則委員会報告（島津委員長）

委員会が1回開催され，日本生理学会会則第9条について討議した．

6. 教育委員会報告（中馬委員長）

委員会が3回開催された．

7. 研究費委員会報告（新島委員長）

委員会が1回開催された．

8. 選挙管理委員会報告（竹内委員長）

委員会が2回開催された．J. J. P. 編集委員の改選の件について評議員による投票の結果，酒井敏夫，大村裕，星　猛，本田良行及び広重　力の以上5氏が選出されたとの報告があった．

9. 評議員選考委員会報告（本間委員長）

昭和60年度の日本生理学会評議員候補者は，56名（表6）であり，全員が推薦された．

10. 生理学用語委員会報告（酒井委員長）

『新版生理学用語集』の出版を機に委員会を解散する旨報告がなされた．但し，日本医学会の『医学用語集』の改訂の為に酒井敏夫氏が日本生理学会側の窓口となることが提案され了承された．

11. 国際生理科学連合報告（伊藤 IUPS 理事）

昭和60年7月にパリで開催される理事会の席上，ヘルシンキ大会の国際プログラム委員選出が審議される予定であると報告された．

12. 生理科学研究連絡委員会報告（本間委員長）

委員会が1回開催された．

13. 第63回（昭和61年）日本生理学会大会の準備状況について（望月次回当番幹事）

◦会期は，昭和61年4月2日(水)～4日(金)．場所は山形大学医学部．10会場程度で口演発表を主体にする

予定であると報告された.

14. 第62回（昭和60年）日本生理学会大会に関する報告（纐纈当番幹事）

一般演題として742題の申し込みがあり，それ以外に教育シンポジウムも含めて7つのテーマについてのシンポジウムが開かれる予定である．参加者は，約1,500名が見込まれていると報告された.

15. 生理学学生実習設備改善に関する報告（三浦常任幹事）

群馬大学医学部では昭和60年度国立大学概算要求に際し，特別設備費の項目で生理学学生実習装置約3,000万円を申請していたがこのほど承認されたことが報告された.

Ⅱ. 議　題

1. 前回議事録について

変更なく承認された.

2. 昭和60年度予算案（表7）が了承された.

3. 評議員推薦について.

56名の候補者が適格であるとの評議員選考委員会の判定が承認され，評議員会に提出することになった.

4. 常任幹事辞退の件

後藤昌義氏が常任幹事を辞退された.

5. 教育委員会昭和60年度活動計画

日本生理学会より教育委員会の活動費として20万円を支出することになった.

6. 日本生理学会会則第9条について

会則第9条文中の『当番幹事は大会の開催を引受けた評議員であって，大会の一切の事務を行なう，大会終了後次回当番幹事に事務引継を行なって任期を終わる，この任期中は常任幹事会の一員に加わる』の解釈について討議され，『当番幹事は，常任幹事会に出席して意見を述べることができる』という意味であると理解することに合意された.

7. 第65回（昭和63年）大会の当番校として和歌山県立医科大学が適当と決議され，評議員会・総会に計ることになった.

第62回日本生理学会評議員会・総会議事要旨

日　時：昭和60年3月29日(金)　午後4時15分～5時40分
会　場：久留米大学商学部御井学館
出席者：約200名
議　長：纐纈教三，西　彰五郎

議長は開会にあたり，恒例にしたがい評議員会，総会を並行して行うことを提案し，了承され，議事進行が行われた.

Ⅰ. 評議員会・総会報告

1. 庶務報告（伊藤庶務幹事）

◦会員数. 昭和60年3月現在，会員総数3,319名，特別会員28名，名誉会員3名である（表1）.

◦科学研究助成. 第11回日産学術研究助成に神野耕太郎氏が採択された. 昭和60年度山田科学振興財団への推薦は2名であった.

◦九州地区常任幹事の後藤昌義氏が常任幹事を辞退された旨報告された.

2. 会計報告（星会計幹事）

会計監査（2月19日）にて適正と判断を受けた昭和59年度決算報告（表2）が承認された.

3. 日本生理学雑誌編集報告（酒井編集幹事）

47巻1号，2号は発刊済み. 3号は印刷中で，4号は編集済みである事が報告された.

4. J. J. P 編集報告（中山委員長）

◦会計報告.（表4）.

◦編集委員会報告. 昭和59年4月～60年3月までの論文受理数は114件で，掲載数は92件であった.

5. 教育委員会報告（中馬委員長）

16名で構成され，年3回委員会を開催した.

6. 研究費委員会報告（新島委員長）

文部省科学研究費に関するアンケート調査の結果，分化細目をふやしてほしいとする要望が多数あった旨報告された.

7. 生理学用語委員会報告（酒井委員長）

『新版生理学用語集』の出版を機に委員会を解散するとの報告がなされた.

8. 国際生理科学連合会報告（伊藤 IUPS 理事）

昭和60年7月の理事会で1989年 IUPS ヘルシンキ大会でのプログラム委員選出に関する審議が行われる予定であるとの報告がなされた.

9. 生理科学研究連絡委員会報告（本間委員長）

委員会の発足，役員の選出及び活動方針について報告された．

10. 第63回（昭和61年）日本生理学会大会の準備状況（望月次回当番幹事）

・日生誌46巻5号に第一報を出し，詳細は46巻7号に第二報を出す予定であるとの報告があった．

11. 第62回（昭和60年）日本生理学会大会の報告（当番幹事）

申込み演題総数は742題であり，その他，2題の特別講演，7つのテーマに関するシンポジウムも行われているとの報告があった．参加者は，1,502名であった．

Ⅱ．評議員会・総会議題

1. 昭和59年度予算

説明の後，評議員会の承認を経て総会に付議し，承認された（表7）．

2. 新評議員推薦

選考委員会推薦の新評議員候補者は全員，評議員会総会にて承認された（表6）．

3. 第65回（昭和63年）日本生理学会大会

和歌山大学で開催予定の旨承認された．

評議員会総会終了後，前大会当番幹事より，今大会の各幹事に謝辞が述べられた．

表1　日本生理学会庶務報告

（昭和59年12月末現在）

会員	
一般会員	3,055名
特別会員	28名
名誉会員	3名
準会員	
学校図書館	121部(119件)
研究所書店	126部(71件)
寄贈及び交換	
外国	21部
国内	15部
合計	3,369部

特別会員(28名)

朝比奈一男　井上　清恒　伊藤　真次
伊藤　龍　勝　義孝　勝木　新次
勝木　保次　久保　秀雄　黒津　敏行
斎藤幸一郎　瀬尾愛三郎　高木健太郎
戸塚　武彦　問田　直幹　冨田　恒男
名取　禮二　長嶋　長節　西丸　和義
福田　邦三　福田　篤郎　福原　武
藤森　聞一　松田幸次郎　三田　俊定
簑島　高　吉井直三郎　吉村　寿人
若林　勲

名誉会員(3名)

Sir John Eccles　Dr. Ichiji Tasaki
Dr. Chandler McCuskey Brooks

表2　日本生理学会昭和59年度決算報告

（自　昭和59年1月1日　至　昭和59年12月31日）

収　入

	59年度予算	59年度決算	増減	備考
(1) 前年度繰越金	2,923,175円	2,923,175円		
(2) 昭和59年度収入	25,190,000	26,872,608		
(内訳)				
会費	17,280,000	17,095,000	−	
購読料	1,740,000	2,242,440	+	
論文掲載料	1,500,000	1,398,362	−	
広告料	1,100,000	1,640,000	+	11件
会誌分冊売	50,000	33,600	−	
預金利子	130,000	82,247	−	第一勧業銀行
日本医学会奨励金	100,000	200,000	+	1件
印税	500,000	369,674	−	生理学実習書，用語集

論文表題集	1,600,000	1,905,000	+	
雑収入	50,000	544,085	+	教育委員会他
生理学教室史上巻	1,140,000	1,200,000	+	
余外集，試道集	—	162,200		
(1)+(2) 合計	**28,113,175**	**29,795,783**		

支出

(内訳)	59年度予算	59年度決算	増減	備考
会誌印刷代	11,500,000	11,250,046	−	45巻12号～46巻11号
会誌発送代	2,700,000	2,776,053	+	同上
編集会議費	120,000	120,000		日本生理学雑誌
校正料	250,000	216,000	−	同上
原稿査読料	30,000	18,000	−	同上
大会援助費	500,000	500,000		久留米大学
事務室使用料	2,231,394	2,231,394		12ヶ月
事務室光熱費	300,000	316,731	+	同上
通信費	850,000	878,160	+	
事務費	800,000	868,404	+	
備品	80,000	230,000	+	電子和文タイプライター
会合費	350,000	263,484	−	常任幹事会，委員会
旅費	1,500,000	1,279,540	−	同上
人件費	3,100,000	3,130,072	+	アルバイト含む
職員健康保険	100,000	75,480	−	
職員退職金積立	100,000	100,000		
論文表題集	1,600,000	1,266,704	−	
国際交流基金	250,000	250,000		
雑費	100,000	146,200	+	名誉会員証他
予備費	1,651,781	200,000	−	教育委員会
余外集，試道集	—	546,050		
合計	**28,113,175**	**26,662,318**		
昭和60年度繰越金総額		3,133,465		
合計		**29,795,783**		

表3　日本生理学雑誌46巻(59年度)編集報告

46巻1～12号(8・9号合併)発行11回

46巻総頁数　815頁

＜内訳＞	編	頁数	(45巻総頁数：824頁)
原著	5	64	
短報	3	10	
総説	6	82	
口演演題		290	
学会抄録	(9)	93	
会報，その他(目次，索引含)		127	
広告		95	
特集	(13)	54	

尚，業績表題集は別冊として刊行.

表4　JJP昭和59年度決算および60年度予算(見込)　　(昭和60年3月16日)

支出	59年度 予算額	59年度 決算額	59年度 摘要	60年度 予算額	60年度 摘要
製作費	13,139,000円	13,109,000円	1,146頁, 1,600部×6回	13,980,000円	1,200頁, 1,600部×6回
審査・英文校閲料	1,720,000	1,757,000		1,800,000	
旅費・会議費	1,076,000	883,200		950,000	
通信・消耗品費	1,389,000	1,389,000		1,389,000	
送料(外国)	2,920,000	3,192,000		3,320,000	
送料(国内)	2,886,000	2,932,000		2,932,000	
人件費	2,060,000	2,060,000		2,160,000	
雑事務費	809,000	809,000		850,000	
支出合計	**25,999,000**	**26,131,200**		**27,381,000**	
収入					
国内会員購読料	7,200,000	7,157,920	913名×8,000円×98%	7,157,920	
国内機関購読料	2,600,000	2,600,000	130名×20,000円	2,600,000	
外国購読料	7,300,000	7,581,000		7,920,000	
別刷・バックナンバー	3,729,000	3,561,800		3,700,000	
文部省助成金	5,670,000	5,840,000		5,840,000	
繰越金	△406,155	△406,155		203,365	
収入合計	**26,092,845**	**26,334,565**		**27,421,285**	
差引繰越	93,845	203,365		40,285	

表5　昭和59年度配布状況

国内個人購読	913	1,053部	外国機関購読	399	414部
国内機関購読	130		外国会員購読	8	
国内寄贈, その他	10		外国寄贈, その他	7	
			合計		1,467部

表6　昭和60年度日本生理学会評議員名簿

計56名 (五十音順)

氏名	所属, 現職
アルバート・シンプソン	昭和大, 医, 生理, 研究生
天野仁一朗	九州歯大, 口腔科学, 教授
石川　巧一	群馬大, 内分泌生理, 助手
稲沢　實	九州大, 医, 生理, 助手
今井　英雄	日本大, 医, 生理, 助手
岩月　矩之	東北大, 医, 生理, 助教授
碓井　外幸	東京慈恵医大, 保健体育, 助手
海野　修	東京女子医大, 生理, 助手
枝重　則治	高知学園短大, 衛生, 教授
榎本　純男	東京医歯大, 歯, 口腔生理, 助手
加藤　和雄	東北大, 農, 家畜生理, 助手
彼末　一之	大阪大, 医, 生理, 助手
川喜多健司	明治鍼灸大, 生理, 助教授
河野　憲二	東大, 医, 脳研生理, 助手
小西　真人	東京慈恵医大, 生理, 助手
小松由紀夫	京都府医大, 生理, 講師
坂井　泰	昭和大, 医, 生理, 講師
酒井　哲郎	東京医歯大, 医, 生理, 助手
酒井　鉄博	近畿大, 医, 生理, 助手
渋木　克栄	自治医大, 生理, 助手
鈴江　俊彦	東京医歯大, 医, 生理, 助手
鈴木　秀次	杏林大, 医, 生理, 助手
関　邦博	海洋科学技術センター, 副主幹
田内　雅規	生理学研, 神経情報, 助手
田之倉　優	大分医大, 生理, 助手
高井　章	名古屋大, 医, 生理, 助手
高橋　智幸	京大, 医, 生理, 講師

竹口　紀晃　富山医薬大，薬，物理薬剤学，教授
津田　泰夫　九州大，医，内科，講師
徳納　博幸　名古屋大，医，生理，助手
永雄　総一　東大，医，生理，助手
永田　　徹　兵庫医大，生理，助手
根本　哲朗　香川医大，医，体育，教授
橋本　正史　大阪大，医，生理，助手
蓮尾　　博　久留米大，医，生理，助手
広田　秋彦　東京医歯大，医，生理，助手
福田　正治　富山医薬大，医，生理，助手
藤井　志郎　東京医歯大，医，生理，助手
藤田　一郎　生理学研，研究員
升田　吉雄　松戸市立病院，外科医長
松井　秀樹　香川医大，生理，助手
松村　京子　滋賀医大，教育学，講師
丸山　芳夫　東北大，医，生理，助手
宮側　敏明　愛知医大，生理，助手
宮沢多恵子　日本大，医，生理，助手
森　　昭雄　日本大，歯，生理，講師
森田喜一郎　東京医歯大，難研，自律生理，助教授
矢島　幸雄　兵庫医大，生理，助手
安原　昭博　関西医大，生理，助手
八木　　寛　富山大，工，教授
柳沢　輝行　東北大，医，薬理，助手
山口　隆美　国立循環器病センター，脈管病態生理室長
山田　誠二　産業医大，応用生理，助教授
山元　大輔　三菱化成生命研究所，副主任研究員
山本　哲朗　福井医大，生理，助教授
泉井　　亮　東北大，医，応用生理，助手

表7　日本生理学会昭和60年度予算

（自　昭和60年1月1日　至　昭和60年12月31日）

収　　入

(1)	前年度繰越金	3,133,465円	
(2)	昭和60年度収入	25,010,000	
	（内　訳）		備　　考
	会　　費	17,520,000	（会費 6,000円×3,162×0.9 臨時会費含む）
	購　読　料	1,690,000	（準会費 8,000円×235×0.9）
	論文掲載料	1,500,000	
	広　告　料	1,500,000	
	会誌分冊売	50,000	
	預金利子	100,000	（第一勧業銀行）
	日本医学会奨励金	200,000	
	印　　税	500,000	（生理学実習書，用語集）
	論文表題集	1,600,000	
	雑　収　入	50,000	
	余外集，試道集	300,000	
(1)+(2)	合　　計	**28,143,465**	

支　　出

（内　訳）		備　　考
会誌印刷代	11,500,000	（46巻12号～47巻11号）
会誌発送代	2,800,000	（同　上）
編集会議費	120,000	（日本生理学雑誌）
校　正　料	252,000	（同　上）
原稿査読料	20,000	（同　上）
大会援助費	500,000	
事務室使用料	2,275,512	（12ヶ月）
事務室光熱費	320,000	（同　上）

通信費	900,000	(会誌外通信)
事務費	850,000	(印刷物，事務雑費)
備品	50,000	
会合費	350,000	(常任幹事会，委員会)
旅費	1,500,000	(同 上)
人件費	3,200,000	(アルバイト含む)
職員健康保険	100,000	
職員退職金積立	100,000	
論文表題集	1,600,000	
国際交流基金	250,000	
雑費	100,000	
予備費	1,355,953	
合計	**28,143,465**	

〔お知らせ〕

生理学教育委員会報告の一部訂正について

日本生理学雑誌第47巻第6号「医学教育における生理学および臨床生理学・病態生理学に関するアンケート調査報告」の p. 247 図3生理学実習項目パターン (1971と1985の比較) (大学別) の最下段，1971と1985の比較の図に間違いがありましたので，次の如く訂正致します．

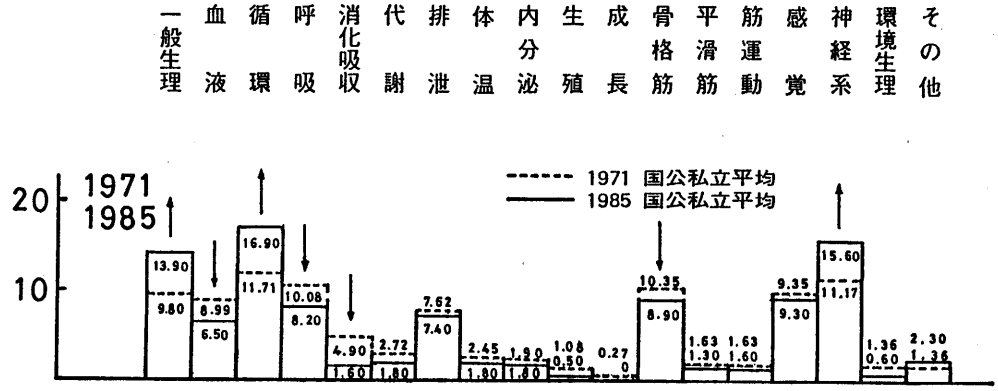


図3　生理学実習項目パターン (1971と1985の比較) (大学別)

日本生理学会特別会員　大阪大学医学部名誉教授　久保秀雄君は，昭和60年7月15日ご逝去されました．ここに謹んで哀悼の意を表します．

日本生理学会評議員　大阪大学基礎工学部教授　塚原仲晃君は，昭和60年8月12日ご逝去されました．ここに謹んで哀悼の意を表します．

〔編集後記〕

史蹟に富む久留米で開かれた第62回生理学大会の抄録集をお届けします．各抄録の著者にとって一語一句に深い思いをこめて書かれた貴重な文章と思います．久留米の学会を思い出す良き資料でもあり，また，来春の山形での学会へ向けての反省材料ともなりましょう．この抄録集の編集は全て当番幹事の額縁・西の両先生が労をとられました．両先生の御苦労にここであらためて御礼申しあげます．

さて，今度の久留米の学会にかぎらず，最近数年来の生理学会の傾向として，特別講演やシンポジウムの比重が次第に重くなってきている印象を受けます．当番幹事の先生方の意欲的企画による結果であり，それはそれなりに立派な成果をあげていると思います．しかし，学会の生命はやはり一般発表でどれだけ重要な新知見が発表されるかに在るのではないでしょうか？特別講演やシンポジウムと聞きたい一般発表とが時間的にぶつかり，どちらの会場に行くべきかと迷う場合がしばしばあるのは私だけではないと思います．プログラム編成の際に当番校で最も苦心される点とは思いますが，今後の生理学会のあり方に対する一つの問題点として指摘させていただきたいと思います．生理学会会員の皆様の御意見を本誌のブルーページにお寄せいただければ幸いです．

編集後記をここまで書いたところで塚原仲晃先生(阪大)御搭乗の日航機墜落の悲報が入りました．つい1ヶ月程前にはNIHのE. V. Evarts先生が心筋梗塞で急逝の報に接し，愕然としていたところです．両先生とも現代の神経科学界を代表する独創的業績をあげられ，第一線で御活躍中の御急逝でした．この夏，世界の学界が失ったものは計り知れない大きなものであります．ここに慎しんで両先生の御冥福をお祈りいたします．

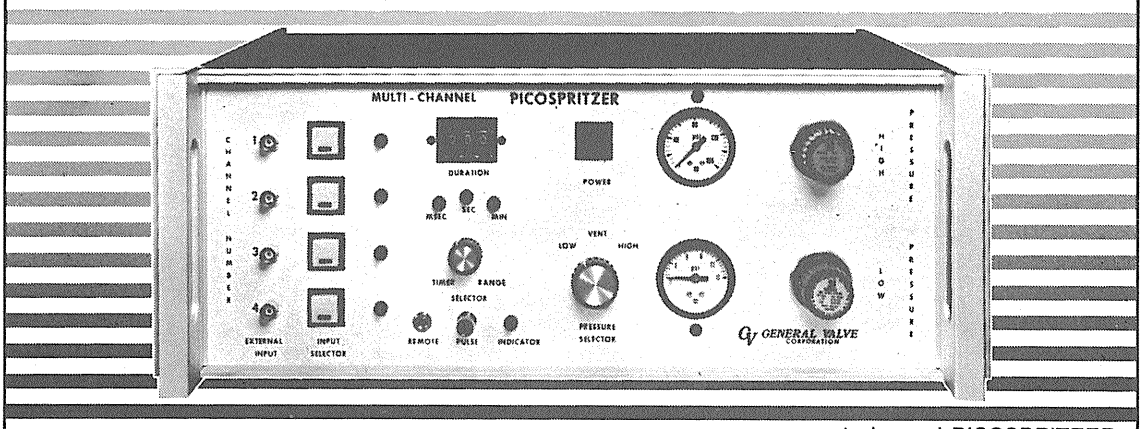
ところで，私はこの4月から田中励作先生の後任として編集委員を仰せつかりました．特にこれと言った抱負はありませんが，若い学会員の皆様のパイプ役になれればと考えております．日生誌についてお気付きの点がありましたら何んなりと気軽に御意見をお寄せ下さい．　(真野範一)

編集委員

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4 channel PICOSPRITZER

PICOSPRITZER は標準ラックに取り付ける事が出来ます。
繰り返し連続使用が可能で、駆出量は設定時間と圧力調整に依り任意に変える事が出来ます。
PICOSPRITZERに依る圧力駆出装置はイオン泳動法に依る注入方法に比較して神経組織に対する電気的な影響を心配する必要が全くありません。
本装置は御使用に際し直ちに稼動出来ます様必要な物は全て用意されて居り、亦廉価で経済的に御使用預けます。
PICOSPRITZERにはSingle channel用、multi channel用があります。

■仕　様

電　　源：115 V A.C.·50, 60 Hz
電　　流：1 Amp. max
消費電力：15 watts. max
電 源 コ ー ド：8 feet
操 作 圧 力 範 囲：0-100 PSIG
圧 力 パ ル ス 信 号：2 ms～999 ms
タイムマークシグナル：1 ～30 mv

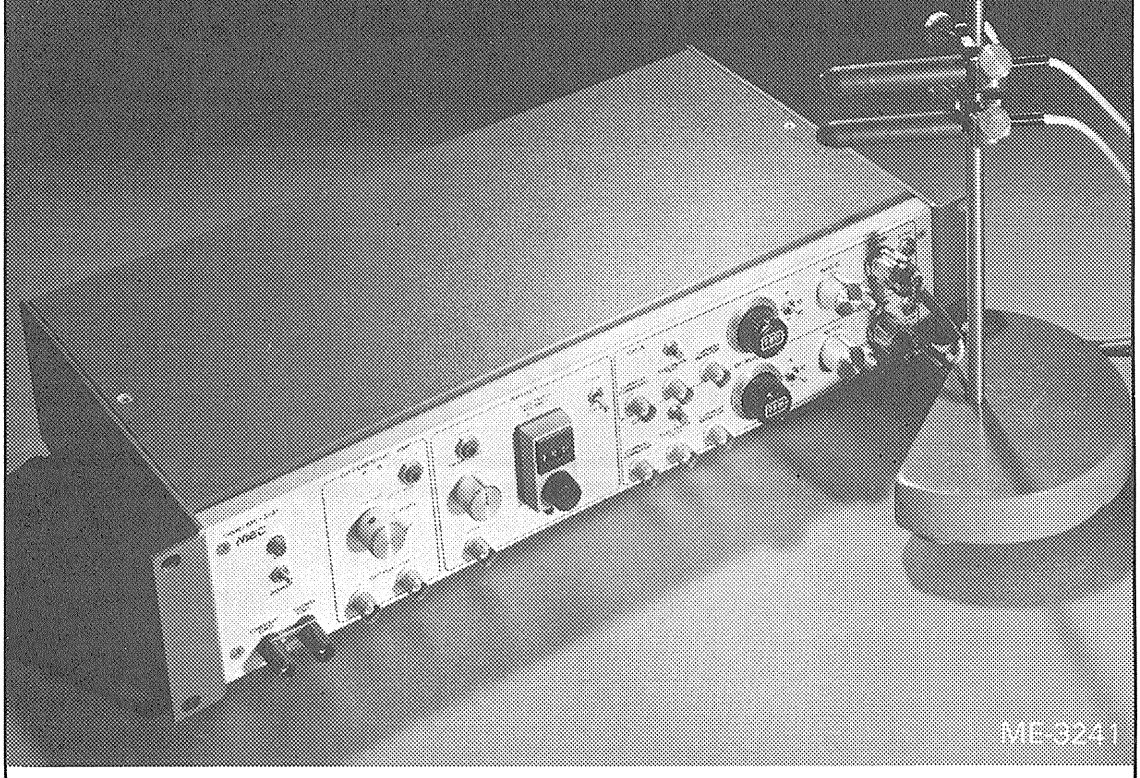
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高度化する細胞電位の研究に

MEC細胞電位計測システム



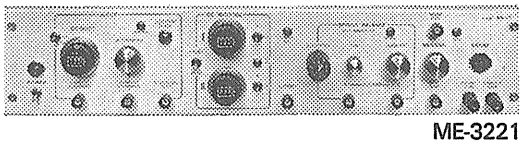
ME-3241

ガラス電極など微小電極をもちいた各種細胞電位の研究に，高い精度と使いやすい機能をもつ機器ラインをそろえています。

2点間の電位差をダイレクトに示す

差動型微小電極用増幅器

ME-3241 差動増幅器内蔵 デジタル直読 刺激通電機構つき

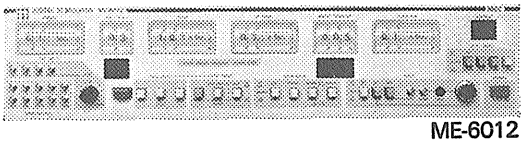


ME-3221

色素注入も可能な高性能タイプ

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ME-3221 DCシフト 2chDCバッキング 刺激通電機構つき



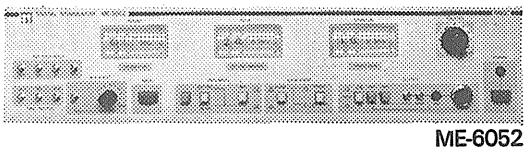
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高い精度をもたらすデジタル設定

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alzet®ミニ浸透圧ポンプは、マウス、ラット、イヌ、サル等の実験動物の皮下、あるいは腹腔内に埋め込むことができ、厳密に制御された流量で薬物を体内に連続注入できる小型自動ポンプです。埋め込み後は体液の浸透圧により、一定流量で連続的に動物体内の全身系又は、脳内、脊髄、静脈等の局部へ目的の薬物をデリバリーできる画期的なポンプです。

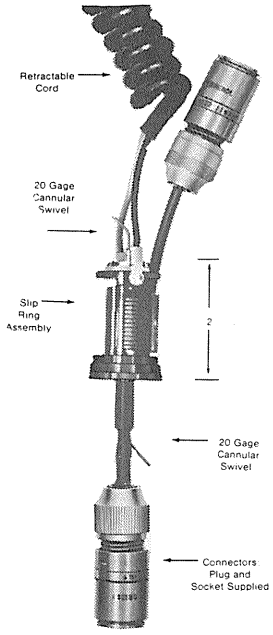
モデル		2001	2002	2ML1	2ML2	2ML4
輸液流速(μℓ/hr at 37°C)		1	0.5	10	5	2.5
リザーバー容量 (μℓ)		200	200	2000	2000	2000
サイズ	長さ (cm)	3.0	3.0	5.1	5.1	5.1
	直径 (cm)	0.7	0.7	1.4	1.4	1.4
総容量 (mℓ)		1.0	1.0	6.5	6.5	6.5
重さ (g)		1.1	1.1	5.1	5.1	5.1

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ELECTRO-CANNULAR SLIP RING



エアーフライト社のカニューラ・スリップ・リングは、動物の脳波を連続的に記録したり、体液を抽出することができ、動物がケージの中で動き回っても、動物に接続されているリード線やプローブはねじれたり、もつれたりすることはありません。
このスリップ・リングは、脳波研究、慢性的カニュレーション、EEG記録、リーション作成、および、これらの関連研究に最適のものです。

●電気的特性 電　流：1μA～1A/ring
電　圧：1μV～115V/ring
絶縁抵抗：500MΩ以上(500V DCの時)

●機械的特性 トルク：
12circuit assembly is less than 1/2in-oz.
including 20 gage swivel
寿　命：10,000,000回転以上
重　量：57グラム以下（リトラクタブルコードを付けない時）

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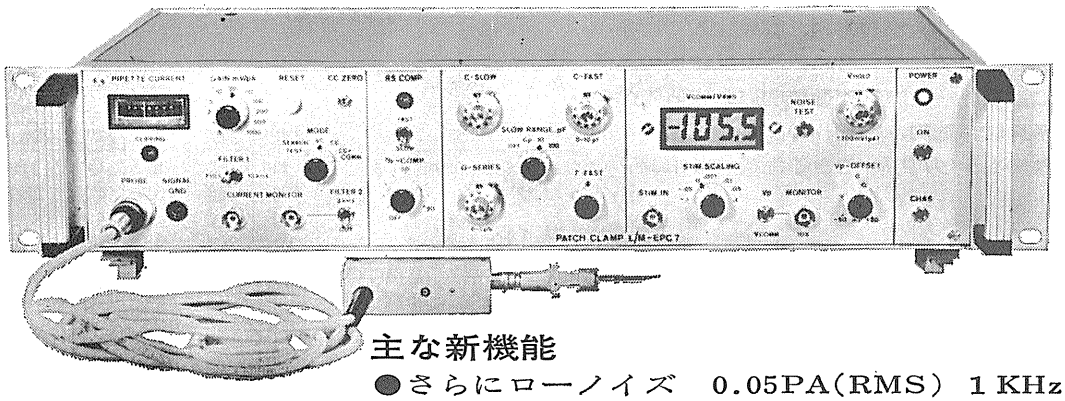
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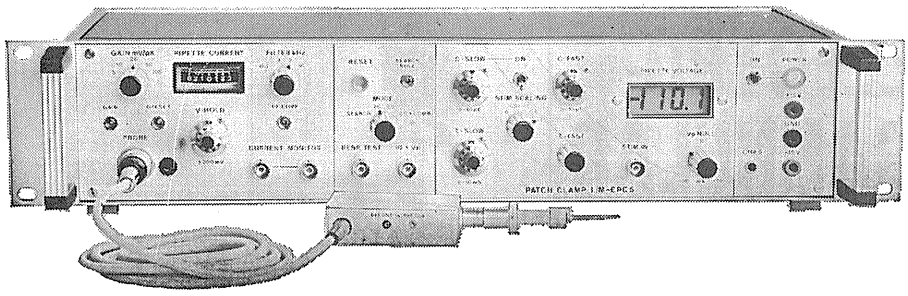
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パワフルなハード 充実のソフト

多チャネル高速処理で定評のある7Tシリーズの最高機種7T17は発売以来多くのユーザーにご使用いただいております。その実績から優れたアプリケーションプログラムが次々と生まれ、オンライン処理プログラムを作成できるSignal BASICと共にさらに完成度を高めました。

■入力は広帯域(DC～100KHz)4ch、中帯域(DC～8KHz)16chを装備

■エディタ機能の充実したSignal BASICは多チャネルのオンライン処理プログラム作成に威力を発揮

■ゆとりある実装メモリ容量512KByte、4MByteに増設可能(本体内蔵)

■プログラムやデータのファイルに便利なフロッピィディスクを内蔵

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豊富なアプリケーションプログラム●16chアベレージ、16chパワスペクトル(標準付属)●パワスペクトルアレイ処理(周波数帯域別分類付)●脳波等電位分布図処理(周波数帯域別分布図)●誘発電位分布図処理●パルス処理プログラム(PST、CORRELATION、INTERVAL)●聴性誘発反応処理(L-Hカーブ)●筋電図処理(運動単位電位、干渉波)●ニスタモグラフ処理(自発、視運動性、温度性、滑動性、サッケード眼振)●重心動揺検査プログラム(平衡神経科学会検査基準に準拠)

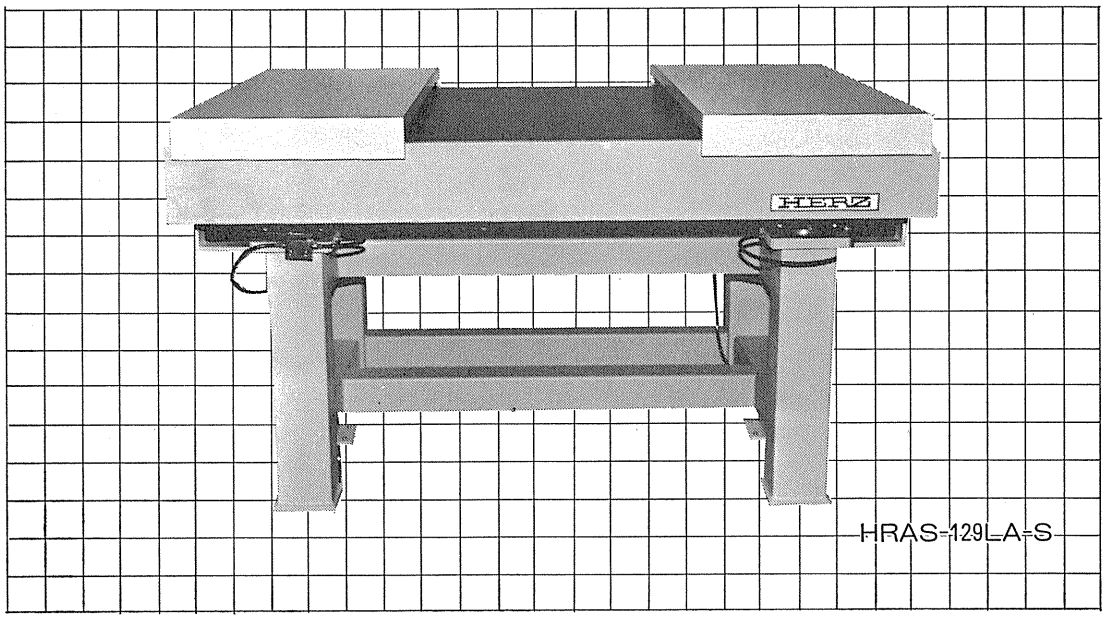
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HERZ

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ヘルツ大形空気ばね式防振台は、生理学・薬理学の分野に多く使用されている「微小電極装置」を搭載する為に開発されました。

防振性能はレーザー機器用空気ばね式防振台と全く同一であり性能において変わらず、また操作性についても「搭載盤保護枠」を設け、さらに防振性能を損なわぬよう「肘当台」を具備しております。機器配置による「水平アンバランス」は「自動水平レベルセンサー」により自動的に水平を保ちます。



HRAS-129LA-S

仕様

寸法mm

項目＼形式	HRAS-107LA-S	HRAS-129LA-S
固有振動数	約1.7Hz	
防振方式	HERZ空気ばね	
制振方式	オルフィスによるエアーダンピング	
搭載盤寸法	1000×700	1200×900
外形寸法	1060×800×750	1260×1000×750
搭載可能重量	200kg	
全体重量	130kg	155kg
付属品	肘当台および保護枠（本体に取付）	
その他	空気源は御客様にてご用意ください。	

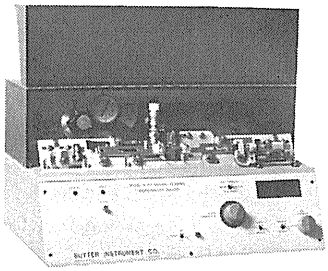
ヘルツ工業株式会社

〒252 神奈川県藤沢市遠藤1980

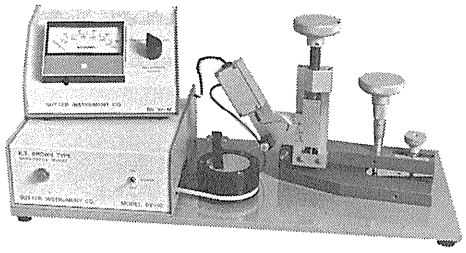
TEL.0466-88-1301(代)　FAX.0466-88-3273

Sutter Instrument

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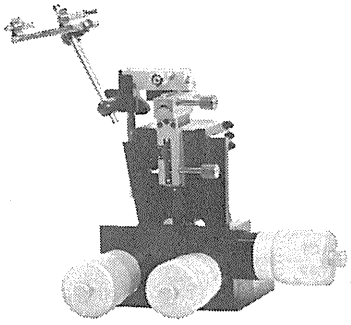
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MICRO—ELECTRODE PULLER
P—77B型　(本体価格：180万円)



K.T.BROWN TYPE
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特徴

- ウルトラ・ファインな動き
  最小目盛2ミクロンのマイクロメーター
  ＋1/10リダクションレバー
- 優れた操作性
  全て板バネによるメカニズム
- バックラッシュフリー

価格：70万円(右・左手共)

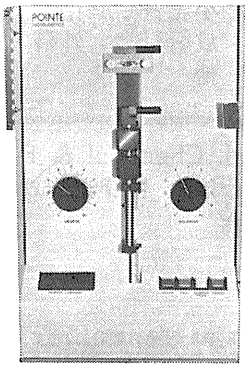
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本体価格：69万円

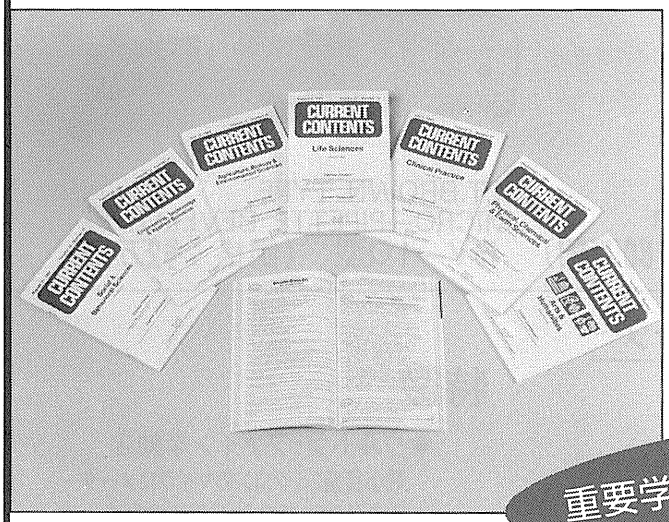
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Clinical Practice

対象誌数 810以上

対象分野 アレルギー 麻酔 泌尿 外科 放射学 公衆衛生 予防医学 精神病 物療及びリハビリテーション 看護学 産婦人科 内科 核医学 神経 ガン 病理 病院管理 足病学 眼科 職業病 皮膚学 開業医 耳鼻咽喉科 法医学 肛門 臨床心理 整形外科 胸部外科 他

- Physical, Chemical & Earth Sciences
- Engineering, Technology & Applied Sciences
- Agriculture, Biology & Environmental Sciences
- Social & Behavioral Sciences
- Arts & Humanities

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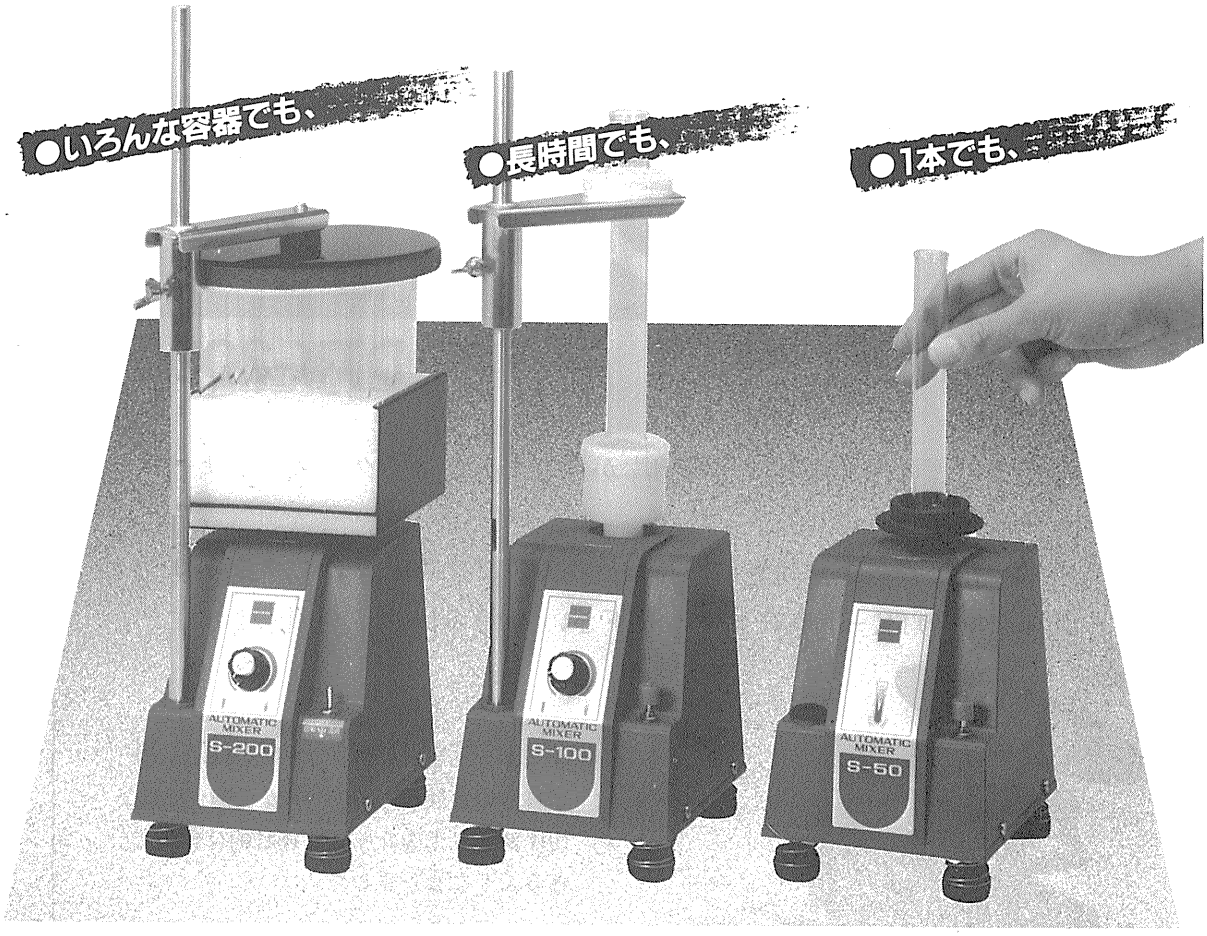
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「ラットの脳を生のまま薄切します。」

末凍結切片作製装置

マイクロスライサー

DTK-3000

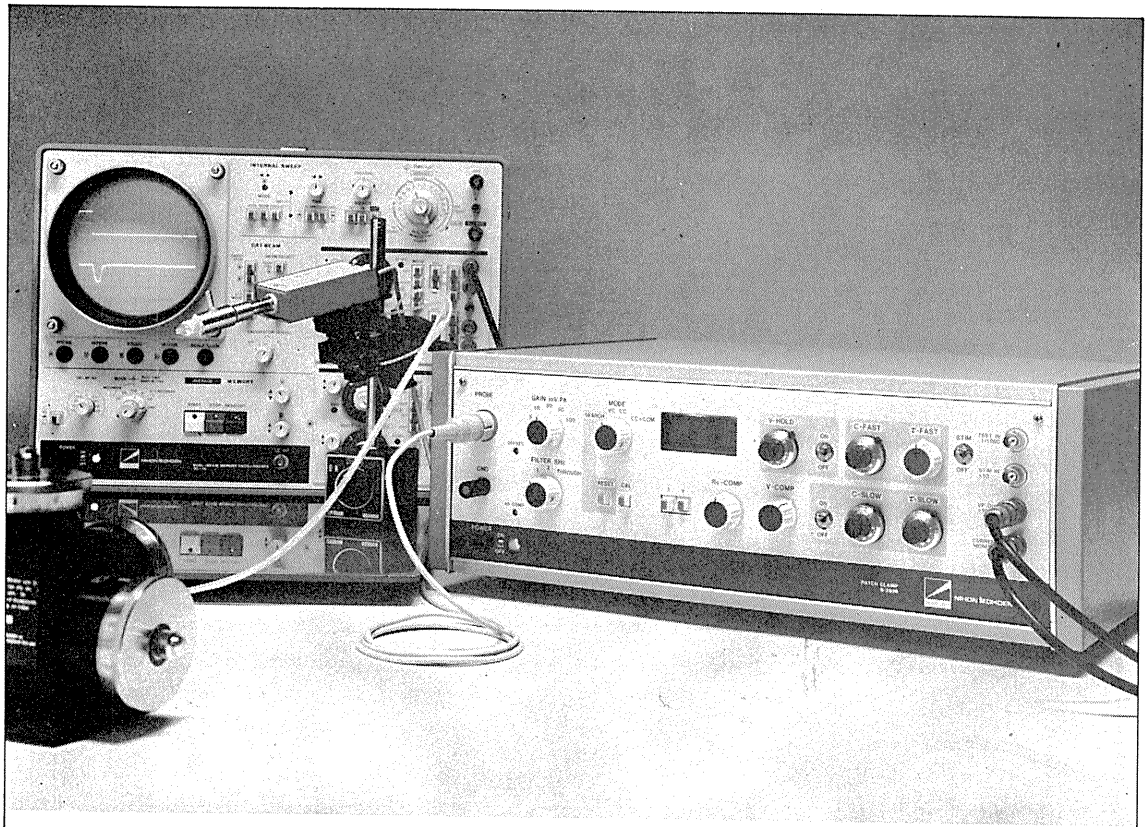
凍結や包埋の操作なしに組織切片が作製できるマイクロスライサーが全自動になりました。
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