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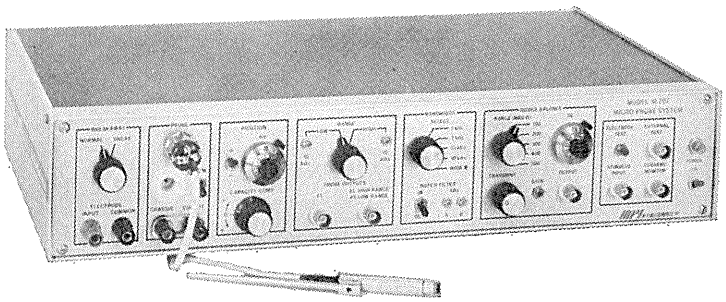
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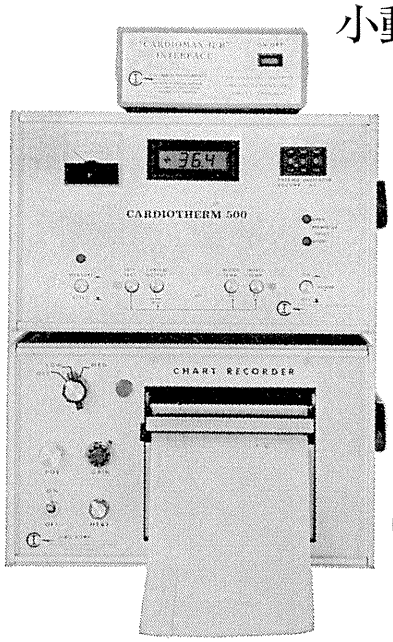
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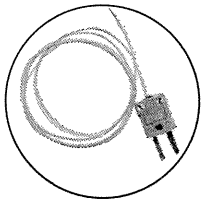
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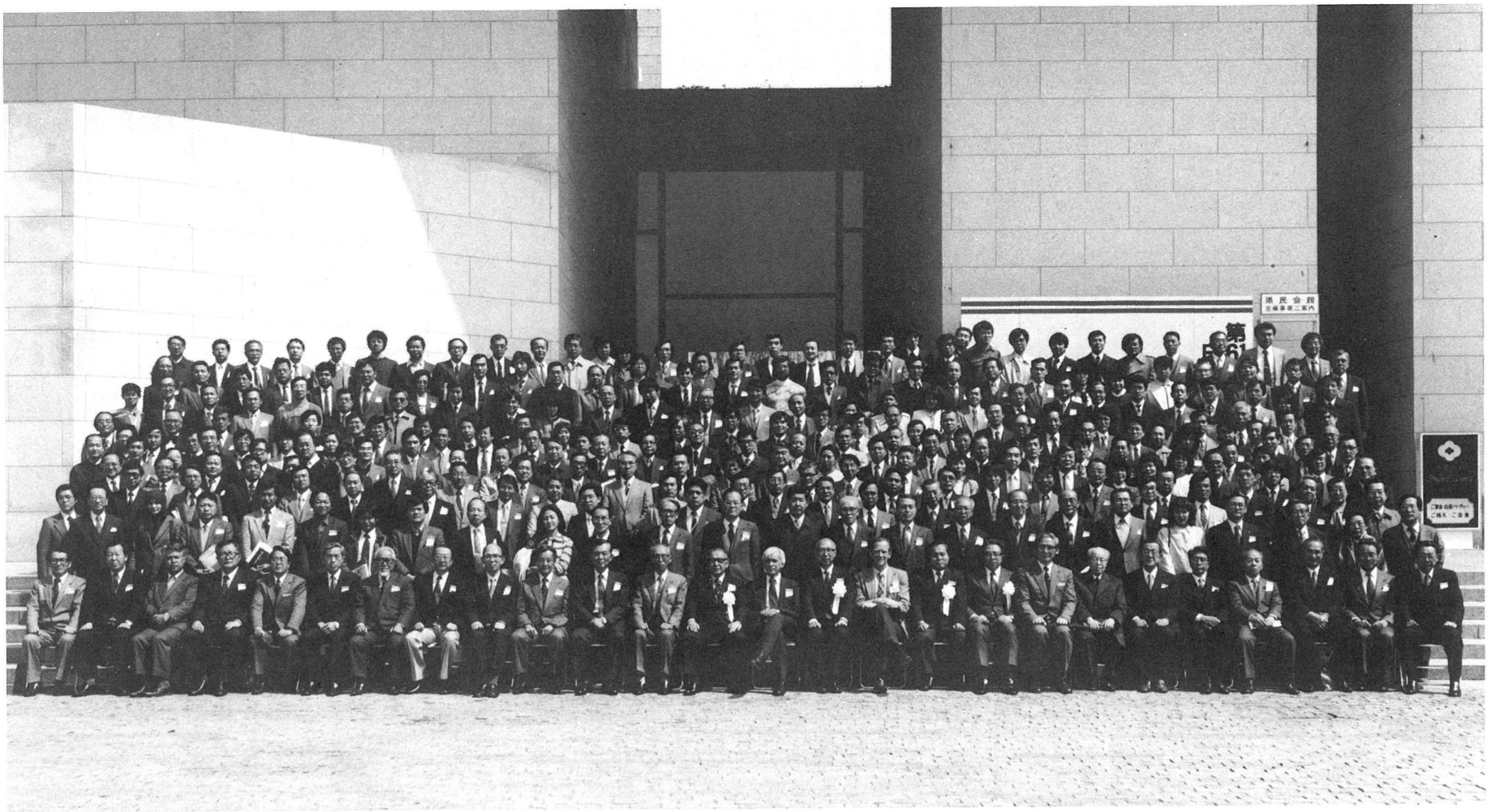
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第61回　日本生理学会大会　　昭和59年 3 月28～30日　於 群馬県民会館

第61回日本生理学会大会を終えて

第61回日本生理学会大会
当番幹事 高木貞敬・三浦光彦
平尾武久・鈴木光雄

第61回日本生理学会大会は昭和59年3月28日から30日までの3日間，群馬県民会館と前橋商工会議所会館との2会場で10セクションに分かれて開催されました．今年は殊の外，厳しい冬が長くつづいたので，当日の天候への影響が心配されましたが，前橋名物の空っ風も吹き荒れることもなく無事に終了できました．

今大会では演題応募を無制限とし，ただし口演料を徴収するという新企画を打ち出しました．幸いに，演題数は過去最高の735題を数えました．これを3日間に納めるためにプログラムの作成に苦心しました．

分類された演題の内訳を見ますと，分子生理18，細胞生理35，能動輸送21，興奮性膜33，シナプス・終板41，脊髄・脳幹26，小脳12，視床下部・大脳辺縁系23，視床・大脳皮質28，筋運動とその制御15，行動・生体リズム20，神経化学33，網膜39，視覚12，聴覚18，体性・化学感覚40，骨格筋24，平滑筋21，心筋35，自律神経19，循環47，血液・腎・体液調節33，呼吸29，消化・吸収13，内分泌・生殖24，体育・労働生理16，環境・エネルギー代謝16，温熱生理44．特に，神経生理の分野と温熱生理の分野の発表演題の増加が目立ちました．今回は研究方法，その他の分類を削除し，中枢神経系は機能別分類ではなく，研究対象の形態的分類を行い，視覚系は51題あったので2つに分けました．前回まで少数派であった神経化学が大幅に増加しているのは，世界的な傾向を反映しているようです．

技術面では，予稿集を分冊にせずに1冊にまとめ，表題集の部分を黄色紙に大きな活字を用いて印刷し，見やすく，眼が疲れないように工夫しました．

次に懇親会を敢えて行わず，増設された研究別グループディナーで親睦を深めていただきました．

今大会は基本的には前大会（大阪）で取り入れた省力化の精神を踏襲し，大会準備をいたしました．

次に，今大会では実行できませんでしたが，大会準備委員会で話題となった貴重な意見を記して置きます．

1) 事務手続きの簡素化のため，参加申し込み書をマークシート方式にして，コンピューターを導入する．

2) 抄録はタイプではなく，フロッピーディスクに換える．

3) 討論を活発にするため，座長を複数にして，座長に必ず的確な質問を義務づける．

4) 1演題当たり15分という枠を取り払い，座長の宰領及び個性に任せて，各セクション独自の運営をお願いする．

5) 大会の財源確保のため，各演題に記載されている共同研究者全員から発表料を支払っていただく，などなどでした．

次に大会の経験から，申し継ぎしておきたいことを付け加えます．

1) 各座長はそれぞれの会場の受付へ必ず事前に立寄り受持ちの確認をして頂きたいこと．

2) 分類された演題数の多少に比例して会場の大小を割当てたが，演題数とその会場への聴衆の数とが比例しないケースがあった．たとえば興奮性膜の場合，演題数が少なかったので，小さい会場を用意したが，聴衆が非常に多く，入り切れない人が出て大変迷惑をかけた．

3) グループディナーは大会終了後が効果的である．

最後に，今大会では大会運営に占めるサービス業務を思い切って減らしましたが，特に苦情もなく終了できましたことは，ひとえに会員各位の御理解の賜物と感謝いたします．

今後も人手不足でさらに省力化を進めざるを得ないことになるでしょうが，生理学会大会が，ますます盛会となりますことを希望して止みません．

THE 61st ANNUAL MEETING
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THE PHYSIOLOGICAL SOCIETY OF JAPAN

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1

THERMODYNAMICAL PARAMETERS OF HELIX-COIL TRANSITION.

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Newly devised method of conformational analysis of biological macromolecules which takes account of the solvent effect is reported. When an atom of macromolecule is exposed from interior of the molecule to the solvent being accompanied with the conformational change of the molecule, the free energy change of the atom (Δg) is plausibly assumed to be proportional to the increase of the accessible surface area (ASA) of the atom and the free energy change of the molecule (ΔG) is simply to be accumulation of Δg. Thus once the proportional coefficients of Δg to ASA are determined, ΔG of any molecule is able to be calculated from the increase of ASA of composing atoms. Classifying the atoms of macromolecules into five types, the proportional coefficients of each atom type were determined as functions of temperature as to give the best fitting to the experimental data of Zimm-Bragg's parameter s of helix-coil transition of peptide molecules. Since these coefficients express the free energy change of atoms due to exposing their unit ASA to the solvent, it is possible to calculate the entropy and enthalpy changes of the atoms from the temperature dependency of the coefficients. Obtained entropy and enthalpy changes and the coefficient itself explain qualitatively well the character of each atom type showing usefulness of the method proposed here.

2

ASSESMENT OF SHORT-RANGE INTERACTION OF PEPTIDE CHAIN.

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For the estimation of peptide conformation by empirical force field method two requirments of which one gose against the other are to be met. These requests are that the method can search all allowed conformation with energy minima and that the computer time for the calculation is short as possible. For these requirements to meet we devised the method to calculate the probable ϕ, ψ main chain dihedral angles and their clustered probability using potential map and freedom of side chain dihedral angle is diminished as few as possible. As C_α and C_β is tetragonal carbon, X_1 dihedral angle (N-C_α -C_β -C_γ) is restricted to 60°, -60° or 180° corresponding to gauche or trans respectively. X_2 dihedral angle (C_α -C_β -C_γ-C_δ) for the aromatic amino acids, aspartate, aspargine and histidine are restricted to 90° or -90° as C_γ-carbon is trigonal for these amino acids. For the other amino acids X_2 are restricted to 60°, -60° or 180°. Other side chain dihedral angles and ω main chain angle are fixed to 180°. Thus ϕ and ψ of each amino residue are fitted to most probable values calculated by method described above one by one changing X_1, X_2 to above described values if necessary. The fitting continues untill conformatinal energy decreases any more. These method applied to angiotensine, the octapeptide and satsfied result are obtained.

3

ON THE RNA CODON

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In order to clear the significans of RNA codon of amino acid, we paid the attention to the special case of UUA→ leu under the consideration of the matrix $S_2=\begin{pmatrix} * & U \\ U & A \end{pmatrix}$. S_2 is the matrix of the linear transformation of coordinate axis, that is $X'= S_2X$. The molecular weight of U is 112 and that of A is 135 respectively. The characteristic equation, the characteristic value λ and the characteristic vector e {l,m} can be estimated respectively, when the value of * is given. If the value of * is 517, the matrix is expressed as following, $S_2=\begin{pmatrix} 517 & 112 \\ 112 & 135 \end{pmatrix}$.

From this matrix, we get $\lambda= 547.4159$ as the suitable proper value. Further, we get the values of the components of the characteristic vector as follows, $\cos\theta= l= 112G$ and $\sin\theta= m= 30.4159G$. Where G is the constant. The rotational angle of the coordinate axis is estimated as 15.1934°. This value is strictly coincidence with $[\alpha]_D^{20}$(6N,HCl)=15.2° of leu.

4

ATP-DEPENDENT UNCOUPLER OF OXIDATIVE PHOSPHORYLATION IN MITOCHONDRIA.
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The effect of 5,7-Dichloro-8-quinolinol(5,7diCl8Q) on the mitochondrial function was studied. Addition of 5,7diCl8Q to state 4 mitochondria accelerated the respiration(i.e. uncoupling action). The uncoupling action increased in the presence of ATP(100~500 μM). Although , the addition of ATP had little effect in the absence of Phosphate. The mesurements of the electrical conductance of lipid bilayer membrane showed that the ATP enhanced uncoupling by 5,7diCl8Q did not occurred in a lipid domain of the membrane. Incubation of mitochondria with oligomycin and atractyloside, inhibitor of mitochondrial energy conversion system and adenine nucleotide translocator respectively, inhibited the enhancing effect of ATP completly. From these results, we could find out that the interaction of ATP and its transport system and/or energy conversion system enhanced the uncoupling action by 5,7diCl8Q.

5

On the structures of D-amino acid oxidase reaction intermediates.
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When D-amino acid oxidase reacts with D-lys or D-pro, the purple complex appears in anaerobic and the green complex does in aerobic. We measured the resonance Raman spectra of these complexes using isotopically labeled compounds. In purple complex, the 1675 cm^{-1} band was assigned to the C=N stretching mode of Δ^1-piperideine-2-carboxylate derived from D-lys. The 1369 and 1339 cm^{-1} bands are associated with COO^- of the imino acid. The 1609 cm^{-1} band is derived from reduced flavin. In green complex, the 1579, 1544 and 1348 cm^{-1} bands are associated with oxidized flavin, judging from the isotopic shifts using labeled FAD's. The 1388 cm^{-1} band are associated with COO . The 1646 cm band has no shift with lys-ε-^{15}N. The assignment of the band which disappears using lys-U-^{13}C is not until clear, but the band is associated with lysine derivative.

purple complex(1200-1750cm^{-1})

lys	1-^{13}C	ε-^{15}N	lys,D
1675	1676	1660	1667
1609	1609	1609	1607
1369	1365	1369	1370
1399	1314	1342	

pH 8.3 D, D_2O pD 8.3

green complex(1200-1750cm^{-1})

lys	1-^{13}C	U-^{13}C	ε-^{15}N	lys,D	1-^{13}C,D	U-^{13}C,D
1646	1646		1646	1630	1628	
1579	1579	1578	1580	1578	1578	1577
1544	1543	1546	1544	1541	1539	1543
1388	1370	1356	1389	1408	1364	1354
1348	1349	1350	1348	1371	1350	

6

STUDY ON THE LOHMANN REACTION IN FROG MUSCLE BY NUCLEAR OVERHAUSER EFFECT OF 31-PHOSPHORUS NUCLEAR MAGNETIC RESONANCE.
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The gamma position of phosphorus in ATP molecule and that of creatine phosphate are exchanged by creatine kinase (Lohmann reaction). The behaviors of magnetization of these atoms can be described by Bloch equation and chemical relaxation time or flux of the enzyme reaction can be obtained by saturation transfer method using nuclear Overhauser effect. The frog sartorius is inserted in the sample tube and perfused by Ringer solution, and is measured by 31-phosphorus nuclear magnetic resonance (24 C). The concentration of ATP and creatine phosphate are measured 5 and 24 mM, respectively. By the prolongation of the selective irradiation time on the gamma phosphorus of ATP, the signal intensities of creatine phosphate are decreased. This decay is the sum of reciprocal of longituidinal relaxation time and of chemical relaxation time. This decay is obtained as 2.9. When the signal of creatin phosphate is irradiated selectively, the decay is obtained as 1.0. From these results, the fluxes are 2.9 and 2.6 mM/sec/kg for inverse and reverse reaction, respectively. Thus the gamma phosphorus of ATP and that of creatine phosphate are considered to be in equilibrium.

7

INTRAMOLECULAR SULFHYDRYL-DISULFIDE EXCHANGE REACTION OF BOVINE PLASMA ALBUMIN — N-A ISOMERIZATION. KUWATA, K., ERA, S., NAGAOKA, S., SOGAMI, M., INOUYE*, H. AND SAKATA*, S. Dept. of Physiol., *3rd Dept. of Int. Med., Sch. of Med., Gifu Univ., Gifu 500

Bovine mercaptalbumin (N) when incubated at alkaline pH isomerizes to a new component (A). The reaction is sulfhydryl (CYS-34) catalyzed and reversible (Sogami et al. (1969) and Nikkel et al. (1971)). The influence of ionic strength on the N-A isomerization was studied by a moving boundary electrophoresis. The N-A isomerization was supressed by an increase of the ionic strength generally and especially by the small amount of Ca^{2+} due to chelate formation (Saroff et al. (1962)). The measurement of the relaxation time of the N-A isomerization in 0.15 M KCl at pH 8.6 at 35°C reveals that the life span of bovine plasma albumin in vivo was controlled by the N-A isomerization. The N-A isomerization was the multi-step reaction. In glutathione oxidoreduction buffer (GSH/GSSG = 0.3 mM/0.03 mM) without added salt at pH 8.6 at 25°C in air, the rate of the N-A isomerization was notably increased and at an early state of the reaction two peak of the A-form was observed in a moving-boundary electrophoresis. In oxidoreduction buffer system, the rate of the N-A isomerization was more strongly dependent on the oxidoreduction potential than the concentration of the reduced glutathione.

8

THE EFFECT OF Ca^{2+} ON THE N-B TRANSITION OF BOVINE PLASMA ALBUMIN (BPA). ERA, S., ASHIDA*, H., NAGAOKA, S., KUWATA, K. AND SOGAMI, M. Dept. of Physiol., Sch. of Med., Gifu Univ., Gifu and *Dept. of Physiol., Defence Med. Col., Tokorozawa

Harmsen et al. (1971) and Zurawski et al. (1975) have reported that in the presence of Ca^{2+} the N-B transition of BPA (0.15 M KCl, pH 7.0 — 9.0) observed by $[\alpha]_{313}$ steepens and shifts to lower pH side. The effect of Ca^{2+} on the N-B transition of BPA was studied by CD. The changes of CD-resolved secondary structures in the N-B transition were less than that in the N-F transition and 2 — 4 mM Ca^{2+} had almost no influence on the change of CD-resolved secondary structures in the N-B transition. Furthermore, in 2 — 4 mM Ca^{2+} the changes of $[\theta]_{262}$ and $[\theta]_{268}$ indicated the two-step change ($N\text{-}B_1$, $B_1\text{-}B_2$), though in the absence of Ca^{2+} the one-step change corresponding to the N-B transition. These results indicate that the N-B transition might be the two-step conformational change and in the presence of Ca^{2+} the change of $[\alpha]_{313}$ is correlated exactly with the initial part of the N-B transition.

9

^{1}H-NMR STUDIES ON VARIOUS CONFORMATIONAL STATES OF BOVINE MERCAPTALBUMIN (BMA). NAGAOKA, S., ERA, S., KUWATA, K., SOGAMI, M., 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 600

BMA has various conformational state, such as E(pD 3.02), F(pD 4.03), N(pD 4.5 ~ 7.4), B(pD 9.4) in 0.10 M NaCl. An increase of BMA concentration in the F-form showed ^{1}H-NMR spectral changes (360MHz) in parts of aliphatic and histidin signals which were substantiated by the gel formation but no in the N-form. In saturation transfer experiments by irradiation either at -7,18 ppm or 2.40 ppm from HDO, the intensity measurements were carried out at the peaks of $-CH_3$, LYS and aromatic resonances. In the N-B transition, no significant changes in the cross relaxation times (T_{IS}) were obwerved. T_{IS} values of the F- and E-forms were 3~4 folds greater than those of the N- and B-forms. These results indicated that a partial loss of the secondary structure in the N-F transition leads to increase the mobility of side chains (side chain melting). In contrast to ^{1}H-NMR spectra of the F- and E-forms, those of the N- and B-forms showed the typical shape being ascribed to the inhomogeneous rigid internal structure.

10

^{1}H-NMR STUDIES ON THE WATER STRUCTURE IN GEL OF MODIFIED BOVINE PLASMA ALBUMIN (BPA*).
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Bovine plasma albumin Fr. V (BPA) has known to contain a small amount of proteolytic enzyme. The enzyme cleaves the peptide bonds of BPA around pD 4.0 between GLU-390 and CYS-436 in one or two portions. The resultant BPA (BPA*) has a tendency to form clear gel above 8 %. The water structure inside of the gel was studied using 360MHz ^{1}H-NMR by measuring T_1, T_2 and cross relaxation time of the saturation transfer method. The gel showed a similar spectrum to that of the F-form except for slightly broad HDO signal. The saturation transfer experiment from the irradiated protein moiety to HDO revealed the strong cross relaxation between the protein and the surrounding water molecules which may be strongly immobilized. Both T_1 and T_2 of HDO in the gel were remarkably shorter than those of proteolytic enzyme-free bovine mercaptalbumin solution and supported the results of the cross relaxation experiments. From the gel concentration-dependent T_1 change, the hydration of the gel state BPA* was calculated to be more than 8g/g BPA* which was 6 folds higher than that of BMA solution.

11

NONMERCAPT-MERCAPT CONVERSION OF HUMAN SERUM ALBUMIN (HSA) BY HEMODIALYSIS.
SOGAMI, M., NAGAOKA, S., ERA, S., KUWATA, K., MIURA† K., INOUYE† H., SUZUKI† E., NOGUCHI*† K. AND HAYANO***† S. Dept. of Physiol., *3rd Dept. of Int. Med., Sch. of Med., Gifu Univ., Gifu 500, **Tech. Develop. Lab., Asahi Chemical Industry Co., Ltd., Kawasaki 210 and ***Hayatoku Hosp., Gifu 500

HSA is the mixture of mercaptalbumin (HMA) and nonmercaptalbumin (HNA). The present authors recently developed a new high-performance liquid chromatography (HPLC) system for the separation of HMA and HNA at neutral pH (PGP 2000 (Asahi Pak GS-520H), 0.75 X 200 cm). Using a new HPLC method, the present authors found the nonmercapt-mercapt conversion (HNA $\longrightarrow$ HMA) during hemodialysis and mercapt-nonmercapt conversion (HMA $\longrightarrow$ HNA) after hemodialysis in hemodialysis patients, indicating HMA being " a covalent carrier protein " for sulfa-containing amino acids, such as cysteine. Mercapt-nonmercapt conversion might decrease the concentration of oxidized form of sulfa-containing amino acids in blood.

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC STUDIES ON PATHOLOGICAL HUMAN SERUM ALBUMIN.
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Using a high-performance liquid chromatography on PGP 2000 column (0.75 X 200 cm) at neutral pH, human serum albumin was resolved into two components, human mercaptalbumin (HMA) and nonmercaptalbumin (HNA). Mean values of [(HMA)/(HMA + HNA)], $\bar{f}_{HMA}$ of healthy male adult serums and umbilical cord serums were 0.76 ± 0.025 (n = 10) and 0.71 ± 0.018 (n = 6), respectively. In hemodialysis patients, $\bar{f}_{HMA}$ before dialysis and mean value of [(f_{HMA} after dialysis)-(f_{HMA} before dialysis)] were 0.45 ± 0.077 (n = 112) and 0.16 ± 0.054 (n = 100), respectively. In chronic renal failure (n = 10), peritonical dialysis (n = 7) and renal transplantation (n = 4), ranges of f_{HMA} were 0.32 ~0.60, 0.30~0.62 and 0.52~0.72, respectively. In nephrotic syndrome, $\bar{f}_{HMA}$ values were lower than that of healthy adult and were strongly affected by prednisolone medication. In Addison's disease (n = 1), f_{HMA} was 0.47 before medication. By cortisone medication, f_{HMA} was extremely decreased and then increased up to 0.69. In liver cirrhosis (n = 10) range of f_{HMA} was 0.25~0.56.

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INTERACTION OF CHLOROTETRACYCLINE(CTC) WITH ALBUMIN TROUGH Ca AND ITS FLUORESCENCE CHANGES.

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The tetracycline antibiotics are known to increase its fluorescence by chelation to divalent cations, especially to Ca bound with proteins. We reported that CTC fluorescence shows prominent sigmoidal increase depending on Ca concentrations in Ca-binding protein solutions(Nishiye and Sassa, 1983). In this study, we clarified the interactions of CTC and human serum albumin(HSA) under the presence of Ca by its fluorescence changes and absorption spectrum changes. In the solution of HSA, CTC fluorescence increased significantly and plateaued at around 3 mM Ca. The CTC fluorescence which is emitted by the excitation wavelength for proper protein fluorescence, is defined as the coupled CTC fluorescence. This fluorescence also showed the sigmoidal increase depending on Ca concentrations. These sigmoidal changes of fluorescences in HSA solution were completely disappeared by addition of EGTA. The CTC fluorescence was prominently decreased by addition of haemin to HSA solution. The binding of haemin to HSA was measured by changes of absorption spectrum of haemin(Beaven et al., 1974). In the presence of CTC, the binding of haemin to HSA was significantly decreased. It is strongly suggested that CTC binds with HSA through the chelation to Ca bound on HSA and its binding site is very close to the site(s) for haemin binding.

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MEASUREMENT OF REAL TIME FLUOLESCENCE SPECTRA OF HEMATOPORPHYRIN DERIVATIVE (HpD).

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Hematoporphyrin derivative (HpD) is excited from the ground state to the excited singlet state at irradiation of B or Q absorption band. HpD on the excited state in phosphate buffer saline solution pH7.2 decay to the ground state with emitting the fluorescence from 600-750nm. The fluorescence of HpD in flow chanber is focussed on the input slit of streak camera by a lens after passing through the polychrometer. The fluorescent image on the transfere the electrical image on the photocathode. It's image is sweeped in the streak tube by the high speed - high voltage electric pressure. The electrical image sweeped at 600 pico second / one flame scanning is superimposed on the phosphor, which has been high intensive sensibility. The light intensity on the phosphor is detected by a two dimension silicon-intensified-target visicon tube (SIT camera) devided the ten track for 600 pico second. From the decaying fluorescence profil displayed on the TV monitor the fluorescence lifetime is determind. Fluorescence lifetime of HpD in phosphate buffer saline solution pH 7.2 have been a fast (pico second order) and a slow (nano second order) component. Non effective porphyrin to the cancer therapy have not a fast lifetime component. Real time fluorescent spectra of fast lifetime component have been three peakes at 607, 627 and 674 nm. The fluorescent lifetime at 607, 627 and 674 nm are determined to be 77.3, 58.5 and 89.0 pico second.

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SPECTROFLUOROMETRY FOR MEASURING HEMATOPORPHYRIN DERIVATIVES RETAINED IN TISSUES.

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Hematoporphyrin derivatives (HPD) distribute selectively in tumors and also play a role of photosensitizer to yield cytotoxic singlet oxygen under illumination of visible light. The both features brought a favorable phototherapeutic technique to cancer. Using spectrofluorometer we made a basic and preclinical experiment on tumors and some organs of golden hamsters to quantify the amount of HPD retained in tissues. HPD showed distinctive, double humped spectra superposed on those of tissues. The estimation was achieved by referring tissue spectra to compensate possible errors (excitation light intensity, optical alignment, so on) in quantification. Measurable concentration was ablove 0.2 μg/ml which satisfactorily covered medicative level of retained HPD. The method was very effective to evaluate retentivity and selectivity of porphyrins or pheophorbids in tissues. Some types of pheophorbid dimers presented superior selectivity to HPD.

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ISOLATION AND PROPERTIES OF MOUSE MYOGLOBIN (Mb). NAKATANI, A., ENOKI, Y. AND KAWASE, M. Second Dept. Physiol., Nara Med. Univ., Kashihara, Nara 634

Mb was isolated from skeletal muscles of JCL-ICR mice by heat denaturation-gel filtration combined with ion exchange chromatography or chromatofocusing by which the isoelectric point was estimated as 7.50±0.10. The Mb was homogeneous by gel electrophoretic and ultracentrifugal analysis. The molecular weight by sedimentation equilibrium was essentially identical with those by the iron analysis and the amino acid composition in which one residure of cysteine was found per molecule. Spectral properties of the various derivatives were determined in visible, Soret and UV regions. pK' of acid-alkaline transition of the met-Mb was estimated as 8.57±0.30(30°C) from the pH-dependent spectral change. The oxygen equilibrium studies revealed complete absence of such allosteric properties as heme-heme interaction, anion effect and Bohr effect. Oxygne tension for the half-oxygenation was 0.69 torr(20°C) and its temperature-dependent change gave the ΔH^0 of -14.1 Kcal/mole.

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HEAT OF CALCIUM-BINDING TO CALMODULIN IN THE PRESENCE OF TRIFLUOPERAZINE. TANOKURA, M. AND YAMADA, K. Dept. of Physiol., Medical College of Oita, Oita 879-56

Microcalorimetric titrations were carried out at pH 7 in order to study the enthalpy changes (ΔH) of the Ca^{2+}-binding to bovine brain calmodulin (CaM) in the presence of various amounts of the inhibitor of CaM, trifluoperazine (TFP). In the absence of Mg^{2+}, the reaction of Ca^{2+}-binding to CaM was endothermic (ΔH=16kJ/mol CaM) in the TFP-free solution but was exothermic (ΔH=-75kJ/mol CaM) in the presence of 8-fold molar excess of TFP at 25°C. The ΔH value of CaM in the presence of TFP is similar to that of skeletal troponin (Tn) C-I complex. Because TnC is similar to CaM on the primary structure but is much different from CaM on the physiological function, these findings must reflect the Ca^{2+}-dependent conformational changes responsible for the function.

CaM and TFP make a Ca^{2+}-independent complex under the present conditions. Ca^{2+}-dependent conformational changes were analyzed in terms of the entropy changes arising from the hydrophobic effect (ΔS_{hyd}) and intracellular vibrational modes (ΔS_{vib}). Both ΔS_{hyd} and ΔS_{vib} were gradually increased following Ca^{2+}-binding in the presence of 5 mM Mg^{2+}.

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FLUORESCENCE SPECTRA OF ANM-LABELED GLYCERINATED RABBIT PSOAS MUSCLE FIBERS CHAEN, S., SUGI, H. AND SHIMADA, M. Dept. of Physiol. and Central Lab., Sch. of Med., Teikyo Univ. Tokyo

Fluorescence spectra of ANM-labeled, glycerinated rabbit psoas muscle fibers were measured in rigor, relaxation and contraction. SDS polyacrylamide gel electrophoresis of ANM-labeled muscle fibers showed that myosin heavy chain was selectively labeled with ANM. During the transition from rigor to relaxation or contraction, there was a blue shift (about 5nm) of the emission spectrum of the dye. The influence of ANM-labeling on the contraction characteristics of muscle fibers were also investigated. Labeling in a relaxing solution containing ATP led to the marked decrease of isometric tension and unloaded shortening velocity. Under a rigor or in the presence of PPi, isometric tension was not largely suppressed. A fluorescence enhancement and a blue shift (about 5nm) of the emission maximum were observed in HMM + MgATP or HMM + MgATP + F-actin in comparison with HMM + F-actin. These results suggest that fluorescence spectrum of ANM-labeled muscle fibers reflect a part of conformational changes in actomyosin ATP hydrolysis.

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CELL CYCLE ANALYSIS BY PERCOLL ® ITANO, T., TOKUDA, M., OHMURA, Y., DOI, A., and HATASE, O., Dept. of Physiology, Kagawa Medical School, Miki-cho, Kagawa, 761-07

Cell cycle analysis is one of the most basic and important techniques for investigation of cell physiology. We used Percoll ®, a kind of silica beads coated with polyvinyl pyrolidone, to analyse cell cycle and to isolate the subset populations of the cyclating cells. The densities of the Percoll media for discontineous gradient were 1.085, 1.066, 1.063, 1.63 and 1.052 respectively. The experimental suspensions were overlayerd on the top of Percoll media and the centrifuge tubes were spun at 3000rpm (X1700g) for 10 minutes. The number and volume of the cells were analysed by a Coulter Counter with channel analyser. The volumes and densities of the cells changed during cell cycle. The averaged volumes were 1240 um^3 in G_1 phase, 1790 um^3 in S phase and 1930 um^3 in G_2 and M phase. The cells in S phase were concentrated in the layer of 1.052 and the G_1 cells in 1.063, the G_2 ones in 1.063 and the M ones in 1.066, respectively. Discripancy between the data of the volume analysis and density might be influenced by the physicochemical properties of Percoll. However we easily and simply separated the cell population of each phase.

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FLUORESCENT PROBE ANALYSIS OF COTRANSPORT IN ISOLATED CHICK ENTEROCYTES. KINOSHITA,Y. and IRIMAJIRI,A. Dept. of Physiol., Kochi Med. Sch.,Nankoku, Kochi 781-51

The applicability of the fluoroprobe technique to cotransport in enterocytes was assessed using a cyanine dye, diS-C_3-(5). The major difficulty involved was a long-lasting decline in the basal fluorescence Fo; this effect, however, was effectively cancelled by the use of a differential mode fluorometry that enabled detection of fluorescence changes solely due to added solutes. D-glucose, D-galactose, α-methylglucoside and 3-O-methylglucose (all 2 mM, final concn.) induced sizable increases (10-20%) in ΔF/Fo with potencies decreasing in this order. Also confirmed was a linear relationship between $(\Delta F/Fo)^{-1}$and $[Glc]^{-1}$or $[Na]^{-1}$, a finding in accord with cotransport involving a saturation kinetics. D-fructose and D-mannose evoked no such ΔF's. Although 2-deoxyglucose as received evoked a spurious increase in ΔF, this was totally attributable to contaminating glucose. Addition of phloridzin elicited varying degrees of quenching and red shift in the spectra depending on [Phl] and cell protein. Through a check by L-alanine that is not phloridzin inhibitable, we reconfirmed the usefulness of 'ΔF/Fo' as a measure for transport even in the presence of phloridzin. It was thus found that the phloridzin inhibition, in enterocytes, of D-glucose-evoked 'ΔF/Fo' was dose dependent with half-maximal and full-inhibitory concentrations of 0.02 and 0.5 mM, respectively. These results strongly support the view that this method would provide a new approach to the elucidation of cotransport phenomena in isolated epithelial cells.

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ELECTRIC PULSE-INDUCED CELL FUSION: EXPERIMENTAL CONDITIONS TO ATTAIN A HIGH FUSION YIELD AND HIGH CELL VIABILITY.
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Mouse lymphoma cells (L5178Y) were fused by a technique of electric pulse-induced cell fusion (electrofusion). A booster amplifier, which enables to reinforce the output voltage and reduce the output impedance of a pulse generator, was employed to achieve electrofusion even in a highly conductive electrolyte solution. After the cells were brought into close contact under dielectrophoresis by applying an alternative electric field (0.8 kV/cm, 100 kHz), cell fusion was induced by a train of four electric pulses (5-8 kV/cm, 20 μsec) applied through a pair of extracellular platinum electrodes. At low temperature (especially below 10 °C), the cell fusion was strikingly suppressed. Discontinuities in the Arrhenius plot suggested an indispensable role of the fluidity of membrane lipids in electrofusion. The external calcium ions (more than micromolar concentrations) were found to be prerequisite for the cell fusion and cell viability after exposure to pulses. The cell fusion was facilitated by the treatment of the cells with proteolytic enzymes. In the presence of Ca^{2+} (0.1 mM) and dispase (10 μg/ml), most of the cells were viable and partook in cell fusion at 25-30 °C. Intercellular pulse application through microelectrodes impaled into two adjacent fibroblastic L cells was also found to induce cell fusion.

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SEX DIFFERENTIATION AND NUMBERS OF GERM CELLS OF RIGHT GONAD IN FEMALE QUAIL EMBRYO. ITO, Y. and YAMAMOTO, N. Dept. of Physiology, Gifu University, School of Medicine, Tsukasa-machi 40, Gifu 500.

Gonads of quail embryos asymmetrically differentiate in the genetic female, that is, the left gonad is developed into ovary but the right gonad become atrophied. In this right gonad, to investigate the degeneration process during gonadgenesis, numbers of germ cells were counted in six stages of incubation and the change of germ cell number with regression of gonad was studied.

Fertilized eggs (genetic male sw/+, genetic female sw/o) were incubated and genetic female embryos from the fourth to the fifteenth day were fixed with Bouin's solution. Sections of gonad were stainned with hematoxylin and eosin. Number of germ cells in the right gonad counted under a microscope, were about 1,700 cells on the average at the fourth day. They attained to a maximum of 3059 cells at nineth day and thereafter, gradually decreased. The ratio of germ cell number of right gonad reached the maximam of $18 \times 10^4/mm^3$ at the fourth day and afterward diminished progressively with incubation age.

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DIELECTRIC MONITORING OF EMBRYOGENESIS: I. TELEOSTEAN AND AMPHIBIAN EGGS. ASAMI,K. and IRIMAJIRI,A. Dept. of Physiol., Kochi Med. Sch., Nankoku, Kochi

We used the dielectric dispersion technique to monitor embryogenesis of the teleost, Oryzias latipes, and the frog, Xenopus laevis, both having different cleavage patterns, viz., meroblastic and holoblastic cleavage, respectively. The results were: (1) The uncleaved eggs of these species in 10 % physiological salines showed Debye type dispersions with the characteristic frequencies of 3-20 kHz. (2) The dispersion for the Oryzias egg remained unchanged at early cleavage stages up to blastula stage, being sensitive to epiboly in which the enveloping layer encompasses the yolk sphere at gastrula stage. This suggests that the dispersion, prior to the onset of epiboly, was attributed not to the blastmeres but to the yolk sphere occupying most of the egg volume. (3) In contrast with the Oryzias egg, the dispersion for the Xenopus egg was drastically reduced in the cleaveage process until morula stage at which a lower-frequency dispersion took place to superpose on the previous dispersion. This dielectric behavior could be qualitatively simulated by the following two models: (I) The cleaved egg up to 32-cell stage is composed of a concentrated suspension of shelled spheres as models for the blastmeres ('suspension model'). (II) For the subsequent stages, the 'suspension model' is covered with a spherical low conductive shell corresponding to the layer of the outermost cells tightly connected.

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CELL FUSION INDUCED BY INSEMINATION IN GOLDEN HAMSTER EGGS. KAWAKAMI, T*, URABE, M*., IGUSA, Y. AND MIYAZAKI, S. Dept. of Physiol., Jichi Med. Sch., Tochigi, 329-04.

We found that two zona-free hamster eggs in contact each other fused after insemination, resulting in a larger egg cell. The fusion occurred in 6.8 % of egg pairs, mostly within 50 minutes after insemination. Almost all the other egg pairs that failed to fuse adhered firmly each other. We examined continuously this fusion process with microelectrodes placed in each egg in contact. Each egg showed periodic hyperpolarizing responses (HRs; Miyazaki and Igusa, 1981) at one's own constant intervals prior to the occurrence of cell fusion. Suddenly, the HR as well as a potential step by a current pulse in one egg spread to another egg, indicating the appearance of electrical coupling. The coupling ratio reached 1.0 within 20 minutes (only 2 minutes in one case). At this period a partition between the two eggs was still visible. Then, each HR in the two eggs became to synchronize and completely fused to a single HR. Since the HR has been shown to be based on the Ca-induced K conductance and to reflect Ca release from intracellular stores (Igusa and Miyazaki, 1983), the synchronization of the HRs indicated that two different eggs became to behave as a single cell. Several minutes later the eggs became a spherical egg. On the other hand, the eggs that only adhered to adjacent eggs showed neither electrical coupling nor synchronized HRs.

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EXTRACTION AND PURIFICATION OF HUMORAL FACTORS POSSESSING THYMIC HORMONAL ACTIVITY. KINOSHITA, Y., KIMURA, S., HASHIMURA, H., NIN, T., OKIMOTO, T. and OZAKI, Y. Dept. of 2nd. Physiology and Urology, Osaka City University Medical School, Abeno-ku, Osaka 545

The factor(cTHF) extracted from calf thymus was,further,separated into 14 polypeptides using High Performance Liquid Chromatography(HPLC) attached gel filtration column. Endowment of rosette-forming and concanavalin A-responding capacities to undifferentiated lymphocytes was employed as the parameters of thymic hormonal activity(THA). Hormonal secretion of thymus epithelial cells was reduced in the rats treated with BBN(chemical carcinogen). This reduction induced an impairment of T cell differentiation and maturation. The 9th and 13th fractions from HPLC which repaired the impairment were suggested to possess THA. Furthermore,both fractions were purified with HPLC attached anion-exchange column. The result was that the 9th fraction was separated into 5 fractions and the 13th into 3 ones. And then the effect of each fraction on the undifferentiated cells was examined. It was found that the part which has relatively high anion charge in the elution pattern was more effective than the others.

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DEGRANULATION OF RAT MAST CELLS INDUCED BY ANTIGEN-ANTIBODY REACTION OR COMPOUND 48/80. OKADA, T., FUJISAWA, M., KINOSHITA, Y., SASAKI, T., SUGIYAMA, M., TANII, T. AND SHIBATA, T. Dept. of Physiol., Otorhinolaryngol. and Dermatol., Osaka City Univ. Med. Sch., Osaka 545 and Osaka Prefectural Inst. of Public Health, Osaka 537

Rat mast cells purified with a modified coil planet centrifuge retained the binding activity of IgE receptor. Dynamic records of cytological changes with a time-lapse cinematography showed the responses of mast cells to various stimuli.

1. Anti-rat mast cell antiserum caused complete degranulation due to cytolysis.
2. One third of sensitized mast cells, challenged with antigen (DNP-Ascaris), showed granule extrusion within two minutes and 30 minutes later, degranulated mast cells regain contractile movements and their original shape.
3. Compound 48/80 induced palisading of granules and damaged the cells 40 minutes after the stimulation.
4. Calcium ionophore A23187 induced nearly complete disruption with cytoplasmic and nuclear edema within 10 minutes.

Calcium is essential in all these reactions, but morphologically, each stimulus seems to exert different effect on the degranulation process of mast cells.

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RECEPTOR-MEDIATED HYPERPOLARIZING RESPONSES TO SEVERAL COMPLEMENT COMPONENTS IN MOUSE AND HUMAN FIBROBLASTS. OKADA, Y., *OIKI, S., OHNO-SHOSAKU, T., **TSUCHIYA, W. AND ***YADA, T. Dept. Physiol., *Dept. Anesthesiol. and **Dept. Dermatol., Fac. Med. Kyoto Univ., Kyoto 606, and ***Dept. Physiol., Tokyo Med. Dent. Univ., Tokyo 113

Mouse fibroblastic L cells respond to whole complement with a slow hyperpolarization. The reversal potential of responses was close to the equilibrium potential for K^+. Deprivation of external Ca^{2+} or administration of a Ca^{2+} channel blocker inhibited the complement response. A Ca^{2+}-activated K^+ channel blocker abolished the hyperpolarizing response. Thus, it appears that the hyperpolarizing response is brought about by an increase in the intracellular Ca^{2+} due to Ca^{2+} influx which, in turn, stimulates Ca^{2+}-activated K^+ channels. Among the complement components, Clq and C5 were found to be effective, but C2, C3 and C4 were not. Trypsinized C3 (C3a,b) and C5 (C5a,b) were also effective. Similar hyperpolarizing responses to whole complement and Clq were observed in human normal diploid fibroblasts (Flow 1000). After successive application of the complement components, the cell membrane became unresponsive. Such desensitization to one of the effective components could not prevent the responses induced by other components. Therefore, it is concluded that fibroblasts have independent receptors for these components. Using the agarose well method, L cells were found to exhibit chemotactic activity to whole complement.

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COURSE OF ATP UTILIZATION IN BRIEF CONTRACTIONS OF FROG SKELETAL MUSCLES AS STUDIED BY ^{31}P NMR. YAMADA, K. AND TANOKURA, M, Dept. of Physiol., Medical College of Oita, Oita 879-56

The course of changes of the creatine phosphate (PCr) and inorganic phosphate (P_i) during the recovery period after brief (2 s) isometric contractions at 4°C was studied at the time-resolution of 16 s by ^{31}P nuclear magnetic resonance (NMR). To improve the sensitivity of ^{31}P NMR we have used a 25 mm diameter chamber designed to study spectra from 16 sartorius muscles of bullfrogs. Accumulation of eight signals obtained by applying RF pulses at intervals of 2 s were taken as a unit of observation (time-resolution 16 s). The factors needed for correcting for "saturation" were 1.33 for PCr and 1.49 for P_i. RF pulses were applied exactly at 2 s intervals by triggering the spectrometer with external pulse generator. In separate experiments, RF pulses were applied at intervals of 4 s. All the results indicated the post-contractile PCr (i.e. ATP) splitting; the level of PCr continues to decrease for a few min after the muscle has relaxed from tetanus, ATP being utilized mainly in the post-contractile period in 2 s tetanus at low temperature, whereas P_i released is maximal at the end of contraction. The above results also indicated that the spin-lattice relaxation times (T_1) of PCr and P_i in muscle cells do not change significantly during contraction.

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A ^{1}H-NMR STUDY OF LACTATE PRODUCTION IN ANAEROBIC CONTRACTION OF FROG SARTORIUS MUSCLE. SEO, Y., YOSHIZAKI, K., NISHIKAWA, H. (Dept. Physiology, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602)

The isolated sartorius muscle of frog was contracted isotonically under an anaerobic condition (ca. 26°C) and the contribution of the Lohmann reaction and the glycolysis were analyzed from the consumption of creatine phosphate, and also from the production of lactate. The concentrations of high energy phosphorus compaunds and of lactate were measured by using ^{31}P-NMR and ^{1}H-NMR, respectively. The work of muscle was calculated from the length of shortening and the load.

The production of lactate increased linearly when the work of muscle was increased. The consumption of creatine phosphate was observed as the work intensity was increased, and then, with the depletion of creatine phosphate, a levelling off of creatine phosphate consumption and the consumption of ATP were observed. The total energy production increased linearly with the increment of the work intensity. The contribution of the Lohmann reaction was larger than that of the glycolysis at the lower intensity of work (ca. 30 J/kg muscle). The contribution of the energy production from glycolysis was increased with the increment of work intensity and exceeded a half of total energy production at the medium intensity of work (ca. 100 J/kg muscle).

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REDUCTION OF CYTOCHROMES AND BURST CONCOMITANT ELECTRICAL ACTIVITY IN THE BULLFROG BRAIN INDUCED BY ELECTRICAL STIMULATION OF THE DORSAL ROOT OF SPINAL CORD. KANNO, T., SAITO, T. AND *SAITO, A. Dept. Physiol., Fac. Vet. Med., Hokkaido Univ., Kita-ku, Sapporo 060 and * Dept. Vet. Physiol., Obihiro Univ. Agri.& Vet. Med., Obihiro 080

Bullfrog(body weight;400-500g)brains were perfused with HEPES Ringer solution via cannula inserted into intracarotid arteries with the aid of a roller pump at a constant flow of 2 ml/min at a room temperature. Redox states of cytochromes(Cyt.) in the cerebral hemisphere was measured with a scanning organ spectrophotometer and electrical activity of the brain was recorded with a conventional polygraph by unipolar lead via stainless steel electrode placed on the surface of the cerebral hemisphere. Electrical stimulation(25 mA,20 Hz,5 msec in duration,for 2 min) of the right 3rd or 4th dorsal root in the spinal cord caused a transient and parallel reduction of Cyt. $a(a_3)$, b and $c+c_1$, which coincided with burst potential. Both the redox and electrical response were abolished when 1 μM TTX was added to the perfusing solution. These results show that cytochrome reduction closely correlate with electrical activity in the brain.

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REDUCTION OF CYTOCHROMES INDUCED BY ELECTRIC STIMULATION OF EXCESS K IN NEUROHYPOPHYSIS OF GUINEA PIG. HARADA, E. AND KANNO, T., Dept. Physiol. Fac. Vet. Med. Hokkaido Univ. Sapporo 060

Redox states of cytochromes were recorded continuously with a scanning organ spectrophotometer in the isolated perifused neurohypophysis of guinea pig. Electrical stimulation (5mA, 5ms, 15Hz) induced immediate and parallel reduction of cytochrome a(a_3), b and c + c_1. Continuous stimulation with excess K (17, 34 and 60 mM) induced dose-dependent reduction of the cytochromes. The reduction abolished when NaCl in the medium was replaced with LiCl, but remained unchanged in a Ca-free environment containing 2 mM EGTA. The reduction was decreased by addition of TTX (3 uM) or DNP (0.5 mM). Anoxic treatment always caused reduction of cytchoromes. The cytochrome reduction may not be due to Ca influx, but to Na influx. The reduction may not directly involved in stimulus-secretion coupling of the neurosecretory cells in the hypophysis.

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STIMULUS-SECRETION COUPLING AND THE REDOX STATE OF CYTOCHROMES IN THE PANCREATIC ACINAR CELL. MATSUMOTO, T., KANNO, T., SHIBUYA, I.* Dept. Physiol., Fac. Vet. Med., Hokkaido Univ., Kitaku, Sapporo 060, *Dept. Physiol., Fac. Med., Yamagata Univ., Yamagata 990-23

The effects of CCK-OP and VIP on the redox state of cytochromes in the isolated perfused pancreas of rats was examined with scanning organ spectrophotometer. CCK-OP induced both the secretory responses and the reduction of cytochromes dose-dependently. However, CCK-OP at a low concentration(10 pM) increased the secretory responses without changing in the redox state of cytochromes. VIP(1 nM) induced a slight increase in the secretory responses and no change in the redox state of cytochromes. Combined stimulation of 10 pM CCK-OP and 1 nM VIP, however, significantly increased the secretory responses comparable to those to 100 pM CCK-OP. The combined stimulation caused no reduction of cytochromes, whereas the stimulation with 100 pM CCK-OP reduced cytochromes.

The time course of the secretory responses induced by 200 pM CCK-OP coincided with that of the reduction of cytochromes in the presence or absence of Ca^{++} in the perfusion solusion.

These results are compatible with a view that, in the pancreatic acinar cell, the reduction of cytochromes induced by CCK-OP at high concentrations may coincide with the mitochondrial uptake of cytoplasmic Ca^{++}.

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EFFECTS OF GLUCOCORTICOID ON PHOSPHORUS COMPOUNDS IN CHONDROCYTE.
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Glucocorticoid is known to inhibit matrix production and cellular proliferation of chondrocyte. In order to know the mechanism of these inhibitions, the metabolic effects of glucocorticoid on sugar phosphates and high-energy phosphate compounds in ear cartilage slices of young rabbits were studied by means of ^{31}P-nuclear magnetic resonance and high performance liquid chromatography. By administration of glucocorticoid, the concentrations of sugar phosphates, GTP and UTP in chondrocyte decreased to 56%, 49%, 31%, respectively, compared with those in controls. While, creatine phosphate, ATP and nucleocide diphosphates did not show any significant change. Sugar phosphates are not only intermediate metabolites in glycolytic pathway but also are used as materials for matrix production. GTP and UTP are energy sources for matrix synthesis and they are also used as materials for DNA or RNA synthesis. Therefore, the decrease of these compounds in chondrocyte which is induced by the administration of glucocorticoid might be closely related to the inhibitory effects of the hormone on matrix production and cellular proliferation.

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ROLE OF Zn^{2+} IN PROLIFERATION OF ADULT RAT HEPATOCYTES IN PRIMARY CULTURE.
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Zinc is known to be essential for growth of cells in vivo and in vitro. We have previously reported the relationship between Zn^{2+} and DNA synthesis in rat livers. To understand the role of Zn^{2+} in DNA synthesis, we used primary cultures of adult rat hepatocytes. In our serum-free hepatocyte culture system, DNA synthesis was induced remarkably, and the cell number increased 2-fold during the 3 days of culture. The addition of EDTA to the culture medium inhibited hepatocyte DNA synthesis by 95%. Since this inhibition seemed to be caused by the removal of divalent cations from the culture medium, we examined whether supplement of divalent cations could restore DNA synthesis. Among the divalent cations tested (Zn^{2+}, Ca^{2+}, Mg^{2+}, Fe^{2+}, Mn^{2+}, Cu^{2+}), only Zn^{2+} could cancel the inhibitory effect of EDTA. Nextly, we investigated the effect of EDTA on the activities of thymidine kinase (TK), an enzyme related to nucleotide synthesis, and DNA polymerases (DNA pol). Addition of EDTA to the culture medium drastically suppressed the increase in TK and DNA pol activities. These results suggest that Zn^{2+} is required for the regulation of DNA synthesis through the synthesis or activity of these enzymes.

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ROLE OF PLASMIN INHIBITORS IN MAINTENANCE OF PRIMARY CULTURES OF ADULT RAT HEPATOCYTES.
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We have previously reported the induction of DNA synthesis and mitosis in primary cultures of adult rat hepatocytes in a serum-free medium. In our culture system, addition of a protease inhibitor, aprotinin (10-50 nM) was necessary to prevent hepatocytes from degeneration and to promote spreading. EACA (5 mM), tAMCHA (1 mM) and SBTI (20 μM) showed the same effect as aprotinin. But leupeptin was less effective. Other serine-protease inhibitors, e. g. DFP, PMSF, TLCK (1-100 μM) showed little effect. Dexamethasone was also effective, but other hormones, e. g. insulin, glucagon and EGF, were not. Proteolytic enzyme activities were detectable in conditioned medium of hepatocytes. Amidase activity that hydrolyzes S-2251 was correlated with degrees of hepatocyte degeneration. Hepatocyte-survival activity of protease inhibitors closely agreed with their plasmin inhibitory activity measured by using fibrin. These findings suggest that plasmin-like enzymes released from hepatocytes degraded attachment proteins, resulting in cell degeneration. When hepatocytes were cultured on dried collagen or collagen gels, addition of protease inhibitors was not necessary. Role of collagen substrate in maintenance of hepatocytes is currently under investigation.

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Effects of coenzyme Q_{10} on the olfactory cortex slice of guinea pig.
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The amplitude of field potentials evoked by electrical stimulation of lateral olfactory tract in guinea pig olfactory cortical slice (ca 400 μm thick) maintained in vitro, began to decrease when the incubation gas of 95% O_2 was replaced with 45% O_2. The amplitude diminished to about one half of the control level after 25 min of 45% O_2 gas bubbling and kept a steady state level after that time. The potential height started to increase slowly and attained a plateau at 30 min when the medium containing coenzyme Q_{10} (Co Q_{10}) was applied to the slice being kept slightly hypoxic (45% O_2). The concentration of Co Q_{10} was effective in the range of 10^{-5} to 5×10^{-6} M and the optimal concentration was 8×10^{-6} M.

On the other hand, Co Q_{10} was also effective on the slice incubated with the medium containing antimycim A (0.2 μg/ml), which brings about the depression of the function of respiratory chain, the suppression of ATP synthesis and the decrease in potential height. This would indicate that exogenous Co Q_{10} was utilized by the respiratory chain in mitochondria.

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CONTENT AND LOCALIZATION OF NERVOUS SYSTEM SPECIFC PROTEINS IN RAT SUPERIOR CERVICAL GANGLIA FOLLOWING ADRENERGIC STIMULATION. MIWA, M., ANDO, M., KATO, K.* and NAGATA, Y. Dept. Physiol., Sch. Med., Fujita-Gakuen Univ., Toyoake, Aichi 470-11, and *Dept. Biochem., Inst. Develop. Res., Aichi Pref. Colony, Kasugai, Aichi 480-03

In a search for the functional role of nervous system specific proteins in neural activity, we applied various forms of stimulation to superior cervical ganglia (SCG) excised from rats. Neither cholinergic nor depolarizing stimuli produced any significant increase in ganglionic enolase isozyme or S-100 protein contents. Addition of norepinephrine (NE), as well as isoproterenol, to the medium caused a distinct increase both in $\alpha\alpha$ and $\alpha\gamma$ enolase isozymes in the SCG after 3 hrs' incubation, and this effect was further enhanced in axotomized SCG, in which most neurons had degenerated and reactive glial proliferation was in progress. The NE-induced increase in the α isozymes was not found until 3 hrs' incubation and was inhibited by addition of cycloheximide. By contrast, NE-evoked increase in ganglionic S-100 protein was observed within 30 min, and was found to be the result of solubilization, i.e., transformation of bound protein into soluble form. Addition of propranolol to the medium caused rapid leakage of the solubilized S-100 protein from the SCG into the surrounding medium. Immunohistochemical examination revealed that α enolase and S-100 protein were localized in non-neuronal satellite elements, while γ enolase was in neurons in the SCG.

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COOPERATIVE PHENOMENA IN THE MEMBRANE POTENTIAL CHANGE OF PARATHYROID CELLS INDUCED BY DIVALENT IONS. HIROSE, T., KIKUCHI, H. AND SHINAGAWA, Y. Dept. of Physiology, Nippon Medical School, Bunkyo-ku, Tokyo 113

The membrane potentials of mouse parathyroid cells were measured by means of the intracellular microelectrode. The mean value of membrane potential in 2.5 mM of Ca^{++} was -20.5±0.4 mV(mean ± standard deviation). The low concentration of Ca^{++} (1.0 mM) caused hyperpolarization of the membrane potential to -61.7±0.8 mV (K^+ 5 mM). The membrane potential was proportional to the logarithm of the concentration of K^+ in the solution of low Ca^{++}. The membrane potentials in 2.5 mM of Ca^{++} solution were not affected by the K^+ concentration. The sigmoidal transition of membrane potentials was induced by the change of Ca^{++} concentration in the range from 1.0 to 2.5 mM. The similar sigmoidal change of the membrane potentials was observed in the solution containing 3 to 4 mM of Sr^{++}. Hill constant of Sr^{++} and Ca^{++} were both 20, the relationship between Sr^{++} and Ca^{++} was competitive inhibition. Hill constant of magnesium was 1.5, the cooperativity of membrane was reduced in Mg^{++} solution.

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ELEMENTAL DISTRIBUTION AND CYTOSKELETAL SYSTEMS OF THE POSTERIOR SILK GLAND CELLS. SASAKI, S., NAKAGAKI, I., OYABU, T., IMAI, Y. AND TASHIRO, Y.* Dept. Physiol., Osaka Med. Coll., Takattsuki, Osaka 569 &*Kansai Med. Univ., Osaka 570 Japan

Measurements of intracellular electrolytes of posterior silk gland cells have been made by the electron probe X-ray microanalysis on frozen thin sections of freshly frozen glands of normal silkworms and flimsy cocoon mutants whose posterior silk glands miss the function of the fibroin secretion. In energy dispersive X-ray spectra over the cytoplasm and glandular lumen of normal secretory glands, Ca and Cl peaks were higher, and K peak was lower than those of flimsy cocoon mutant. It was suggested that Ca and Cl were secreted from the cells into the lumen and K was absorbed from the lumen into the cell by the K^+-pump which was located on luminal plasma membrane.

Observations of cytoskeletal systems of posterior silk gland cells have also been performed by the ultrahigh voltage electron microscopy on thick sections of the epon-embedded normal and mutant glands. In the cytoplasm of mutant cells, the large accumulations of the secretory granules were found and it was suggested that the function of radial microtubule systems which transport the secretory granules from basal to apical cytoplasm, were destroyed in the mutant cells.

The functional relationships between the cytoskeletal systems and the secretion of protein and electrolytes in the posterior silk gland cells, were discussed.

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IMPEDANCE MEASUREMENT OF THE CANINE SUBMANDIBULAR GLAND.

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Salivary gland is a organ which transports a massive fluid transepithelially. Therefore considerable change in the ratio (extracellular fluid volume/intracellular fluid volume: ECF/ICF) during the secretion might be expected. The volumes of the extra- and intra-cellular and luminal spaces would be changed. It was reported that the impedance values of the cell suspension were correlated with the ratio (ECF/ICF). We estimated ECF volumes by the impedance method. The impedance of the gland was measured during rest and stimulation with/without blood supply by a YHP-LF Impedance Analyzer (frequency range: 5 Hz - 13 MHz). A low frequency impedance of the gland increased during stimulation, which increased without blood supply. The ECF volumes of the salivary glands were estimated as a 46-30 % at rest, 37-24 % during stimulation and 36-23% during stimulation without blood supply. For the comparison, we estimated the ECF volumes of muscle, liver and kidney to be 9-20 %, 13-25 % and 14-22 % respectively. These results suggested that the saliva was originally supplied from the ECF.

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ACTIVATION OF SALIVARY GLAND CELLS AND THEIR INTERCELLULAR COUPLING IN *CHIRONOMUS* LARVA.

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The salivary glands of *Chironomus plumosus* larva were isolated and three, independent microelectrodes were inserted into two, neighboring acinar cells; two electrodes into one cell for recording and for current injection, and remaining one into companion cell for recording, thus the membrane potentials and input resistances, and coupling ratio between two cells were measured. The electric field stimulation with 15-20 V of strength and 0.5 ms of duration evoked the depolarizing response accompanying with reduction of input resistance, followed by a decrease of coupling ratio for five minutes. The response was abolished by application of TTX in the medium or by superfusion of Ca-free solution. The identical response was elicited by application of 5-HT (10 μM) and it was also abolished by superfusion with Ca-free solution. The response by field stimulation was suppressed by atropine (10^{-5}M) or by hydrocortisone (10 μg/ml). The maximal response obtained by field stimulation seems to be a neurally mediated activity of gland cell and 5-HT is the most probable candidate for the transmitter, which induces a decrease of membrane resistance and decoupling between two cells.

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EFFECTS OF β-ADRENERGIC AGONISTS ON POTASSIUM UPTAKE IN SALIVARY GLANDS IN VITRO.

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Mechanisms of action of β-adrenergic agonists with regard to K uptake in the salivary glands in vitro, were investigated by measurements of net K influx, membrane potential and amylase release. Amylase release evoked by isoproterenol (Iso) and salbutamol (Sal) from the dispersed rat parotid acini was markedly reduced by propranolol, atenolol or ICI 118,551. K uptake in the rat submaxillary gland slices and hyperpolarizing response in the mouse submaxillary gland acinar cells were reduced by propranolol, while they were not significantly reduced by atenolol or ICI 118,551. Sal evoked membrane hyperpolarization with a latency of over ten sec. However, a slight reduction in input resistance preceded the hyperpolarization, indicating true latency of several sec. The hyperpolarizing response was abolished by ouabain, or in a K free or low Na solution. The results may suggest presence of β-adrenoceptor subtypes in the salivary glands although their specificities have not been clear, dissociation between the stimulant-evoked K uptake and amylase secretion, and Na uptake taking part in activation of the K uptake.

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α-ADRENERGIC RECEPTOR POTENTIATES THE β-ADRENERGIC STIMULATION OF PAROTID CYCLIC AMP ACCUMULATION. YOSHIMURA, K., NEZU, E. AND YONEYAMA, T. Dept. Physiol., Hokkaido Univ. Sch. Dent. Sapporo 060

We found that the time course of parotid cyclic AMP (cAMP) response to isoproterenol(IP) was different from that to norepinephrine (NE); that is, cAMP accumulation by IP was reached maximum at about 2 min, whereas it required about 5 to 10 min for NE. α-Adrenergic antagonists inhibited the effect of NE on cAMP accumulation, but did not the effect of IP; and the peak level of cAMP by NE in the presence of α-adrenergic antagonists was attained at about 2 min. α-Adrenergic agonists did not increase the level of cAMP, but when added immediately before IP, they potentiated the effect of IP on cAMP accumulation at 5 and 10 min. Phenyl ephrine in the presence of forskolin increased the amount of cAMP, and this effect was inhibited by α-and β-adrenergic antagonists. These findings show that the parotid cAMP response to catecholamines is regulated by both α-and β-adrenergic receptors. Apparently, β-adrenergic receptor activation is an absolute requirement and an α-adrenergic receptor activation mechanism potentiates β-adrenergic activation.

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X-RAY MICROANALYSIS OF PANCREATIC ACINAR CELLS. NAKAGAKI, I., SASAKI, S., IMAI, Y. AND SHIGUMA, M.* Dept. Physiol. & * Neurosurg., Osaka Med. Coll., Takatsuki, Osaka 569 Japan

Using the technique of electron probe X-ray microanalysis, the changes of elemental concentrations in rat and dog pancreatic acinar cells with cholecystokinen-pancreozymin (CCK-PZ), cytochalasin B and colchicine treatments were investigated.

With CCK-PZ stimulation, the Na, Cl and Ca increased and K decreased in the cytoplasm in rat acinar cells. These results were much the same of that from dog pancreatic acinar cells with pilocarpine stimulation.

With cytochalasin B treatment, the luminal surface of exocrine acinar cells was extensively enlarged and also observed the small vacuoles near the baso-lateral plasma membranes on the electronmicrographs of rat pancreatic acinar cells. By the treatment of cytochalasin B, the concentrations for Na, Cl and Ca were higher while K concentration was much lower in the cytoplasm, comparing with normal state. These results suggest that cytochalasin B enchances the secretion of enzymes and/or fluid in pancreatic acinar cells.

With colchicine treatment, the concentrations for Na, Cl, K and Ca in the cytoplasm, and the ultrastructure of acinar cells showed similar results in the normal state.

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ON THE CARCINOGENICITY OF QUERCETIN. ONISHI, M., YAMAGAMI, K. Dept. of Physiol., Sch. of Med., Kagoshima Univ., Kagoshima 890.

Quercetin are very widely distributed in edible plants: i.e., vegetables, berries and fruits. Quercetin have been shown strongly mutagenic activity for Ames's tests. The metabolism of quercetin with rat liver and kidney preparations were examined by in vitro tests. Quercetin was converted more efficiently by kidney preparations than by liver preparations. Five kidney metabolites were detected, and two metabolites were identified as isorhamnetin (3'-O-methylquercetin) and tamaraxetin (4'-O-methylquercetin) by TLC, UV, NMR and HPLC with synthetic these substances. It may be formed by action on quercetin of a catechol-O-methyltransferase reported to be present in kidney (and liver) tissues. Isorhamnetin was also detected in urine of rats orally dosed with quercetin. Isorhamnetin was weakly mutagenic activity for TA 98 in the presence of liver S-9 mix, but tamaraxetin was strongly mutagenic activity for TA 98 with liver S-9 mix.

Futher studies are in progress to characterize other metabolites of quercetin and determine its carcinogenetic active form.

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CHROMOSOME ANALYSIS OF PANCREATIC ISLET CELL TUMOR IN SYRIAN HAMSTER. IMAGAWA, M., SAKAI, S., AIZAWA, K., O'HATA, S., KATO, K.* Dept. of Physiol., Tokyo Med. Coll., Shinjuku, Tokyo 160., NIH Japan*, Tokyo 141

We have previously reported the establishment of glucagon-producing tnmor cell line (ITC 1) and glucagon-nonproducing tumor cell line (ITC 5-1) by S. C. transplantation of Insulinoma cell line (In 111) to Syrian hamsters. Glucagon-producing ascites tumor cell line (GOAT) was established by injection of GOP (IT-1 cell line). Nonproducing cells (In 111 and ITC 5-1) had small shape and rough surface. Producing cells (GOP and GOAT) were smooth surface and lager than non-producing cells. The normal hamster complement contains 44 chromosomes distributed into 21 pairs of autosomes and two sex chromosomes. In 111 and ITC 5-1 were found the former modal chromosome number was 38 and the latter was 37. GOP and GOAP were observed the modal chromosome number was 62 and 61 respectively. Banding patterns obtained with the trypsin technique were used for the identification of chromosomes from ITC 5-1 and GOP. GOP lost the typical X and Y chromosomes. Both cells had A1-like 3 long chromosomes and their length were similar to X chromosome.

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EFFECTS OF NONHOMOGENEOUS MAGNETIC FIELD ON GROWTH 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., Fac. of Engin.*, Tokushima Univ., Tokushima 770

The growth of HeLa cells exposed to a strong magnetic field was examined. For this purpose, we developed a device including a Sm-Co magnet in combination with a gilded iron needle (1 mm diameter) with a tip of 20 μm. The cells were exposed to the magnetic field of decreasing from 11 to 3 kG in its strength within 0.3 mm from the tip, as calculated by the finite element method. The magnetic field produced stronger Maxwell stress than the homogeneous field. The results were as follows. 1)Microphotographs of the cells were taken by microscope under special illuminating condition using the plastic fibers. The number of cells located within 0.3 mm from the tip was determined from the photographs. 2)The cells were stained by the Feulgen reaction and the amounts of DNA per cell were determined by the microfluorometry. The growth of cells exposed to the magnetic field for 24-96 h seemed to be slightly inhibited, however, the inhibition was insignificant. Also, the amounts of DNA were not significantly affected by the magnetic force.

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EFFECTS OF AN IONOPHORE ON THE INTRACELLULAR LEVELS OF MONOVALENT CATIONS IN HELA CELLS. IKEHARA, T., YAMAGUCHI, H., HOSOKAWA, K. AND MIYAMOTO, H. Dept. of Physiol. Sch. of Med., Univ. of Tokushima, Tokushima 770)

By treatment with nystatin, we attempted to widely regulate the intracellular levels of monovalent cations of HeLa cells incubated with hypertonic media. The total concentration of the cations in the media was adjusted to be 300 mM by addition of choline chloride. Nystatin (50 μg/ml) seemed to strongly stimulate fluxes of both Na^+ and K^+. The intracellular levels of Na^+ and K^+ continued to increase, being unsaturated during a 5 h-incubation. However, on concomitant addition of 100 mM sucrose with nystatin, the intracellular cations reached saturating levels in 3 h. Also, cell swelling caused by the increased cation influxes in the presence of nystatin was protected by addition of sucrose (>30 mM). After 5 h-incubation with the hypertonic media containing nystatin and sucrose the intracellular level of Na^+ or K^+ was shown to change in proportion to its extracellular concentration, provided the extracellular concentration of the other cation was kept constant.

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GLUCAGON SYNTHESIS IN GLUCAGON-PRODUCING TUMOR CELLS (ITC-1). SAKAI, S., IMAGAWA, M., SAITO, H., AIZAWA, K., O'HATA, S., AKIYAMA, S.*[1] AND KANAZAWA, M.[1] Dept. of Physiol., [1]Int. Med., Tokyo Med. Coll., Shinjuku-ku, Tokyo 160

A carboxy-terminal specific antiserum against pancreatic glucagon was obtained after repeated immunization of rabbits, by the method of Schenck ('77). ITC-1 cells were grown as monolayers in RPMI 1640 medium supplemented with 10% new born calf serum. The cells were pulse-labeled with ^{3}H-leucine for 15 min and chased for 0, 15, 120, 180 min. Immunoreactive glucagon (IRG) in the cells was extracted by Kenny's method and gel-filtered on Bio Gel P-30 column. Each fraction was immunoprecipitated with 30K (Unger) and counted radioactivity of ^{3}H-leucine in the immunoprecipitates by liquid scintillation counter.The results indicated that big IRG (>40 Kd) was rapidly synthesized and converted to 12 Kd IRG within 15-30 min. It took two or three hours for the synthesis of true glucagon (3.5 Kd). The big IRG and 12 Kd IRG may represent preproglucagon or intermediates in the conversion process of glucagon.

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CONDITIONS FOR GROWTH AND MORPHOGENESIS OF NORMAL AND NEOPLASTIC MAMMARY EPITHELIAL CELLS EMBEDDED IN COLLAGEN GEL MATRIX. ENAMI, J. AND KOGA, M. Dept. Physiol., Dokkyo Univ. Sch. Med., Mibu, Tochigi 321-02

We have examined the conditions required for growth and morphogenesis of normal and neoplastic mammary epithelial cells embedded inside the collagen gel matrix. Collagen concentration (0.1-0.3%) greatly influenced the morphology of colonies. In an established line of mammary tumor cells (MTD cells) the formation of branched outgrowth was facilitated by lower concentrations of collagen, although the growth rate was only slightly affected by different concentrations of collagen. In freshly dissociated normal mammary epithelial cells of the mouse, branched morphogenesis was observed in all concentrations of collagen. In freshly dissociated mammary tumor cells, more compact aggregates of cells appeared in higher concentrations of collagen in addition to the branched structures. We have further examined the effect of hormones on growth of normal mammary epithelial cells and pregnancy-dependent mammary tumor cells. Under serum-free conditions, both types of cells responded to mammogenic hormones only after cultivation in collagen gels. Tumor-like compact aggregates of cells were formed in tumor cells cultured in higher concentrations of collagen. From these results, it is suggested that collagen may play an important role in growth and morphogenesis of mammary epithelial cells.

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REGULATION OF FORMATION AND MAINTENANCE OF INTERCELLULAR COMMUNICATION. 2. EFFECT OF MONOCLONAL ANTIBODY ECCD-1. SASAKI, Y., SHIBA, Y., TAKEICHI, M*. AND KANNO, Y. Dept. of Physiol., Hiroshima Univ. Sch. of Dent., Hiroshima, 734 and Dept. of Biophysics, Fac. of Sci., Kyoto Univ., Kyoto 606

The monoclonal antibody ECCD-1 inhibits the Ca^{2+}-dependent cell-to-cell adhesion in teratocarcinoma stem cells. In this study, we investigated the effect of ECCD-1 on cell-to-cell communication in PCC3 cells by measuring the transfer of Lucifer Yellow CH between cells. PCC3 cells were grown for a few days until colonies were formed. Out of them, the compactly packed colonies were marked and used for the following experiments. The dye was injected into a cell located in the center of colony. The number of cells to which the dye was transferred was counted 2 min after injection. Antibody began to change cell shape of PCC3 cells after 20-30 min exposure and also inhibited the dye transfer between cells. Antibody treatment decreased the number of dye-recipient cells from 6.9 to 0.9. These results suggest that Ca^{2+}-dependent cell-to-cell adhesion system is required for the maintenance of cell-to-cell communication. This work was supported by a Grant-in-Aid for Scientific Research (56440024) and by a Grant-in-Aid for Cancer Research (58010034, 58015077) from the Ministry of Education, Science and Culture, Japan.

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TUMOR PROMOTERS INHIBIT CELL-TO-CELL COMMUNICATION BETWEEN BALB/c 3T3 CELLS AND cAMP PREVENTS TPA-INDUCED INHIBITION. KANNO, Y., ENOMOTO, T. AND YAMASAKI, H.* Dept. of Physiology, Hiroshima University School of Dentistry, Hiroshima 734 and I.A.R.C., Lyon, Cedex 02, France

The effect of tumor promoters and cAMP on the cell-to-cell communication between 3T3 cells was studied using dye (Lucifer Yellow CH) transfer method. The number of dye coupled cells was 31.0±3.1 in control at 5 min after dye injection, while in the cells treated with TPA (100ng/ml) for 4 hr, the number of dye coupled cells decreased to 2.5±1.6. Although other tumor promoters such as PDD, PDBu and mezerein showed the similar effect, non-promoting derivatives phorbol, 4α-PDD and 4-O-MeTPA had no effect. The addition of 1.0 mM dbcAMP and 1.0 mM caffein to the cultures increased the number of dye coupled cells to 84.5±5.5, 6 hr later. When the cells were cultured in TPA containing medium together with cAMP plus caffein, the inhibitory effect of TPA on cell communication was prevented and the number of dye coupled cells was transiently decreased to 18.1±5.0, 2 hr later, however, increased to 83.8±8.2, 6 hr later. A study with cycloheximide suggests that inhibition of cell communication may not need protein synthesis but upregulation by cAMP requires protein synthesis. This work was supported by a Grant-in-Aid for Cancer research (58010034, 58015077) and by a Grant-in-Aid for Scientific Research (56440024) from the Ministry of Education, Science and Culture, Japan.

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SYNTHESIS OF NETWORK MODEL ON THE ACTIVE TRANSPORT SYSTEM

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Bio-power system was studied theoretically using the power balance equation of all powers included in it. It was demonstrated that the network model of power system could be synthesized with representations on the free energy changes of each power, on the power dissipations and on the power couplings between them. The active transport system in the membrane transport could be treated as a power system in which a chemical reaction and an ionic transport progressed and coupled together. In principle, such a two-powers system could be represented as an elementary circuit which included two elements of the free energy changes (two capacitors),two elements of the power dissipations (two resistors) and an element of the power transmission (one transducer). The model of the epithelial transport system,including the active Na-K pump and the Na-Cl co-transport ,was synthesized by combinations of several elementary circuits.The model behaviore was discussed with the real epithelial transport using computor simulations.

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ION TRANSPORT IN CHOROID PLEXUS EPITHELIUM: INTRACELLULAR Cl ACTIVITY MEASUREMENTS.

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Choroid plexus transports Na, Cl and HCO_3 from the blood to ventricles. We have shown that an elevation of the cellular cAMP (but not cGMP) concentration enhances HCO_3 secretion by increasing the HCO_3 conductance across the apical membrane of the epithelium. Present paper deals with the effects of cAMP on Cl movements across the membranes. Intracellular Cl activities determined with Cl-selective microelectrodes was 22 mEq/l, a value two times higher than that predicted for passive distribution. The activity in the subepithelial compartment was 10 mEq/l lower than the equilibrium activity. Cellular Cl accumulation was observed also under HCO_3-free conditions. Addition of phosphodiesterase inhibitor depolarized the potential and increased the conductance across the apical membrane. Cell Cl activity either increased or decreased depending on the magnitude of membrane depolarization and approached the equilibrium activity. It was concluded that Cl entered the epithelial cells by an active process across the basolateral membrane and left the cells down the electrochemical potential gradient across the apical membrane. Cyclic AMP increased the Cl conductance of the apical membrane but the Cl movement was not the primary cause of the cAMP induced apical membrane depolarization and the conductance increase.

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REGULATION OF TRANSEPITHELIAL ACTIVE SODIUM TRANSPORT BY CELL CALCIUM IN FROG SKIN.

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Reduction of sodium, increase of calcium, or addition of calcium ionophore A23187 of corium side solution all caused decrease in short circuit current(I_{SC}) and transepithelial conductance(g_t) in abdominal skins of the frog species, Rana japonica(red frog) and Rana catesbeiana(bull frog). The reduction of I_{SC}, however, occurred about three times faster in skins of red fog than bull frog. To explore the responces of epithelial cells against these perturbations, intracellular potential(PD_{SC}) and fractional resistance(fR_O) were measured using microelectrode under short circuited condition. Cells were impaled from epithelial side and had values PD_{SC}=-46±9(-16 -75)mV, fR_O=0.7 0.95 respectively. In 15 experiments of bull frog skins, 7 cells were successfully impaled over 40 minutes during sodium withdrawal experiment. Each cell began to depolarize 10-20 mV within 5 minutes and maintained the value, despite I_{SC} decreased after small increase and fR_O decreased significantly. These results suggest that the increase in cell calcium may decrease the basolateral membrane conductance by inhibiting pump activity directly or by affecting cell energy metabolism indirectly.

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DISTRIBUTION AND FUNCTION OF (NA^+, K^+)ATPASE ON LIVER CELL MEMBRANE.
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Distribution of (Na^+, K^+)ATPase on the cell surface of canine hepatocytes was investigated quantitatively by incubating prefixed liver cells with ferritin antibody conjugates against canine kidney holo(Na^+, K^+)ATPase. It was revealed that (Na^+, K^+)ATPase exists bilaterally both on the bile canalicular and sinusoid-lateral regions. The particle density on the bile canalicular region was much higher (∿3 times) than on the sinusoid-lateral region. In the latter region, the enzyme exist almost equally both on the sinusoidal and lateral region. We suggest that the (Na^+, K^+)ATPase on the bile canalicular region is responsible for the bile acid-independent bile flow.

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ACCELERATIVE EFFECT OF NERVE GROWTH FACTOR ON AMINO ACID UPTAKE IN EXCISED SUPERIOR CERVICAL SYMPATHETIC GANGLIA OF RATS DURING IN VITRO INCUBATION.
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Active uptake of a labelled non-metabolizable amino acid (aminoisobutyrate, α-[methyl-^{3}H], AIB) into excised superior cervical sympathetic ganglia (SCG) of rats was significantly enhanced by addition of nerve growth factor (NGF) to the medium during 1 to 5 hours' incubation at 37°. Although the Km value of the AIB uptake by the SCG did not change with addition of NGF, Vmax was nearly doubled. The NGF-evoked increase in AIB uptake was antagonized by addition of specific antisera and was greatly suppressed in Na^+-free, K^+-free, or ouabain containing media. In SCG axotomized one week prior examination, in which most of the ganglionic neurones had degenerated completely and reactive proliferation of satellite glial components was in progress, the NGF-induced increase of AIB uptake was completely abolished. Therefore, active accumulation of the amino acid is considered to occur not in the glial but only in the neuronal elements of the SCG.

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THE ROLE OF Na-K PUMP ON SALIVARY SECRETION IN PERFUSED DOG SUBMANDIBULAR GLAND.
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The contribution of Na-K pump activity on salivary secretion was studied in saline-perfused dog salivary gland by replacing perfusate to K free, oxygen eliminated and ouabain contained solutions. Then, salivary secretion rate, oxygen uptake and K flux across the basolateral membrane were simulteneously measured during persistent or temporary stimulation with 10^{-6} M ACh. Persistent stimulation evoked two distinctive type of secretions, initial phase and steady phase. Initial phase appeared in very early stage after onset of stimulation and showed a high salivary secretion rate. This phase was not significantly altered by K free , anoxic conditions and the presence of ouabain which are all considered to suppress the Na-K pump activity . Furthermore, initial secretion phase did expend oxygen only a little. By contrast,the steady secretion phase decreased correponding to the suppression of Na-K pump activity which was also accompanied with a decrese in oxygen uptake. Ouabain abolished the steady phase as well as extra-oxygen uptake enhanced by stimulation.These results suggested that secretion would be provoked by the combination of Na-K pump , utilization of some stored energy,probably Na electrochemical potential and an amount of Na influx.

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EFFECTS OF LOW NA^+ PERFUSION ON SALIVARY SECRETION: DIFFERENCE BETWEEN LI^+ AND CHOLINE REPLACEMENTS. *MURAKAMI,M., NOVAK,I.*, and YOUNG,J.A.* Physiol.Dept.Osaka Med. Col. Takatsuki 569 Japan. *Dept.Physiol. University of Sydney, N.S.W. 2006, Australia.*

Na^+ dependency of fluid secretion of the isolated perfused mandibular gland of rat was studied by replacing Na^+ in the perfusate with Li^+. The gland was stimulated continuously by acetylcholine (1 μmol/l). In general, the stimulated fluid secretion is decreased with time during stimulation by acetylcholine (tachyphylaxis). The Na^+ dependency curve of 1 hour fluid secretion was sigmoid (Hill number was 6), comparing with those during Na^+ replacement with choline which also showed sigmoidal shape (Hill number was 3) shifted to the left of Li^+ curve. Curves of Na^+ dependency measured at different measuring points along the tachyphylaxis were plotted on a same curve. Li^+ concentration in the saliva depended positively on the rate of fluid secretion, suggesting that Li^+ is reabsorbed by ductal system assuming the same concentration of Li^+ in the primary saliva as in the perfusate. The sum of Li^+ and Na^+ concentration in the saliva was less than Na^+ concentration in control experiment, suggesting that the affinity of Li^+ in the ductal reabsorbing system is lower than those for Na^+.

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STUDIES OF INTRA- AND EXTRACELLULAR SODIUM BY MEANS OF SODIUM-23 NMR USING AN AQUEOUS SHIFT REAGENT. NISHIKAWA, H. AND SEO, Y. Dept. Physiol., Kyoto Pref. Univ. Med., Kyoto 602

The aqueous shift reagent, $Dy(PPPi)_2^{7-}$ had been applied to ^{23}Na-NMR in frog sciatic nerves and sartorius. Two resonance lines were observed, reflecting the intracellular Na^+ and the extracellular Na^+ interacting with the shift reagent. The concentrations of intracellular Na^+ and the extracellular spaces in the nerve and the muscle were calculated from the following equations: [Na]in x (1 - Sex) / [Na]out x Sex = Ain / Aout, [Na]in x (1 - Sex) + [Na]out x Sex = [Na]total. [Na]in, [Na]out, [Na]total, Sex, Ain and Aout were the concentrations of intracellular, extracellular and whole Na^+, the extracellular space and the signal intensities of intra- and extracellular Na^+ in the nerve or the muscle respectively. The concentrations of intracellular Na^+ in the nerve and muscle were 63.8 mmol/l of tissue water and 7.1 mmol/l of tissue water respectively. The extracellular spaces in the nerve and muscle were 41 percent and 30 percent respectively. Further ^{23}Na-NMR using the shift reagent had been tried in the experiment of the sodium transport across the nerve membranes. The nerves were equilibrated in the Ringer's solution containing 10 mM K^+, and then stimulated electrically. The signal intensity of intracellular Na^+ increased by 40 percent reversively as comparison with the nerve equilibrated in the normal Ringer's solution.

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CHARACTERISTICS OF THE 3-O-METHYL-D-GLUCOSE TRANSPORT SYSTEM IN FROG SKELETAL MUSCLE. MARUNAKA, Y., KITASATO, H., MURAYAMA, K. AND KANBE, M.
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We investigated characteristics of the 3-O-methyl-D-glucose (3-O-MG) transport system in sartorius muscle of *Rana catesbeiana*. 3-O-MG influx was consisted with the Na-dependent and the Na-independent components; the former was insulin-sensitive and the latter was insulin-insensitive. The Na-dependent 3-O-MG influx was inhibited by both phlorizin and cytochalasin B. The Na-independent 3-O-MG influx was inhibited by neither phlorizin nor cytochalasin B. Neither the Na-dependent nor the Na-independent 3-O-MG influx was inhibited by phloretin. D-glucose had an inhibitory effect only on the Na-dependent 3-O-MG influx. In a solution containing 2mM 3-O-MG 5mM D-glucose decreased the maximal rate of Na-dependent 3-O-MG influx by 40% of that observed in the absence of D-glucose, while the half saturation concentration was not significantly affected by D-glucose. Further, in a solution containing 2mM 3-O-MG 5mM D-glucose increased the Hill's coefficient of Na-dependent 3-O-MG influx from 1.5 to 1.8. Valinomycin stimulated the Na-dependent 3-O-MG influx, but had no significant effect on the Na-independent 3-O-MG influx. On the other hand, in the presence of insulin Valinomycin inhibited the Na-dependent 3-O-MG influx.

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EFFECT OF INSULIN ON THE Na,K-ATPase ACTIVITY OF PLASMA MEMBRANE VESICLES PREPARED FROM FROG MUSCLES.
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Membrane fractions were prepared from skeletal muscles of *Rana catesbeiana* by a sucrose density gradient centrifugation. The low density fractions, which had low protein concentration, showed high Na,K-ATPase and low NADH-oxidase activities, whereas the high density fractions, which had high protein concentration, showed high activities of Na-independent ATPase and NADH-oxidase. The activity of Na,K-ATPase was inhibited by 2×10^{-5}M digitoxin to the level in the Na-free solution. Either 10^{-5}M ouabain or 3mM EGTA did not inhibit the Na,K-ATPase activity. A very low activity of galactosyl transferase was found in the low density fractions. High activities of 5'-nucleotidase were found in both fractions of low and high densities. Therefore, the major component of the low density fractions was thought to be plasma membrane vesicles and that of the high density was mitochondrial. Na,K-ATPase specific activity of plasma membrane vesicles was increased 1.9-fold by pretreating muscles with insulin, whereas the same pretreatment reduced Na-independent ATPase activity by 10%. These observations support the idea that the externally applied insulin stimulates the activity of Na/K transport possibly by increasing the number of active pumping units on the plasma membrane of skeletal muscles.

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NACL-KCL COTRANSPORT ACROSS THE SEAWATER EEL INTESTINE.
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Simultaneous measurements of net Na^+, K^+ and Cl^- fluxes were made in the stripped intestine of the seawater eel, and the effects of furosemide and ethacrynic acid (EA) on these ion fluxes were examined under the standard condition (mucosal KCl and seroal NaCl Ringer's solutions). When furosemide was added to the mucosal KCl Ringer's solution, the net Na^+, K^+ and Cl^- fluxes from mucosa to serosa were all reduced. On the other hand, EA inhibited only Na^+ and Cl^- fluxes, but not K^+ flux, implying existence of a K^+ transport system independent of Na^+ and Cl^- transport. However, this possibility was denied, because simultaneous treatment with furosemide and EA did not inhibit the net K^+ flux. Since it would be also feasible that EA might increase K^+ permeability and thus the increased K^+ flux might compensate the inhibition of the K^+ flux induced by furosemide, effects of EA on K^+ permeability was examined in the next experiment. However, EA did not enhance the passive K^+ flux after blocking active Na^+, K^+ and Cl^- fluxes completely by treatment with ouabain for more than 1 hr. Moreover, pretreatment with EA diminished the effect of Na^+ removal on the net K^+ flux, whereas it was inhibited after removal of serosal Na^+ in the absence of EA. Therefore, it is likely that EA separates KCl cotransport system from NaCl cotransport system, whereas they usually depend on each other.

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INTRACELLULAR Ca^{2+} MOBILIZATION UPON SECRETAGOGUE STIMULATION OF CULTURED INTESTINAL EPITHELIAL CELLS: THE STUDIES WITH Ca^{2+}-SELECTIVE MICROELECTRODES.
*OIKI, S., ***YADA, T., **UEDA, S. AND OKADA, Y. Dept. of Physiol., *Dept. of Anesthesiol., and **Dept. of Internal Med., Fac. of Med., Kyoto Univ., Kyoto 606, and ***Dept. of Physiol., Tokyo Med. Dent. Univ., Tokyo 113

Cultured Intestinal 407 cells, derived from human fetal intestinal epithelium, exhibit hyperpolarizing responses upon receptor stimulation by various intestinal secretagogues. A number of observations have suggested that significant increases in the free Ca^{2+} concentration within the cells are associated with the hyperpolarizing responses. To verify this inference, the cytosolic free Ca^{2+} concentration was directly measured using Ca^{2+}-selective microelectrodes. It was observed that the hyperpolarizing responses induced by acetylcholine, histamine and serotonin, whose second messenger is known to be Ca^{2+}, coincided with significant increases in the cytosolic free Ca^{2+} from submicromolar to micromolar concentrations. Furthermore, secretin and vasoactive intestinal polypeptide, which are putative cyclic AMP-mediated secretagogues in the intestinal epithelium, as well as cyclic AMP itself were found to induce increases in intracellular Ca^{2+} and hyperpolarizing responses. These observations suggest that an increase in cytosolic cyclic AMP may induce Ca^{2+} mobilization within the cells and Ca^{2+} may be a final mediator in the cyclic AMP-mediated intestinal secretion.

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LIPID/PROTEIN MONOLAYER FORMATION FROM INTESTINAL BRUSH BORDER MEMBRANE VESICLES: EXISTENCE OF Na^+-D-GLUCOSE COTRANSPORTERS IN THE MONOLAYERS. FUJITA,Y., and HOSHI,T. Dept. of Physiol., Faculty of Med., Univ. of Tokyo, Bunkyo-ku, Tokyo 113

We have previously demonstrated that a lipid/protein monolayer could be formed when intestinal brush border membrane vesicles (BBMV) were dispersed at the air/water interface. Modification of thiol or amino groups of BBMV in the presence or absence of D-glucose, and affinity chromatography with phlorizin polymers were employed to identify the protein components contributing to D-glucose cotransport. Molecular weights of the components commonly found by three independent methods were 30-40k, 45k, 70-90k and 100-150kdalton on SDS gel. Electron microscopic examination of monolayer adsorbed on alkylated mica revealed a rough surface which is indicative of the presence of protein molecules in the monolayer. Rinsed and collected materials from BBMV monolayer were applied on phlorizin polymer to analyze for the presence of cotransporters. The fraction eluted specifically with D-glucose contained the components identical in molecular weight to those found to be influenced by the presence of D-glucose during modification experiments. This suggests the presence of Na^+/D-glucose cotransporter in the monolayer formed from BBMV.

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L-ALANINE TRANSPORT ACROSS INTRACELLULAR MEMBRANE BY RAT JEJUNUM. HANAI, H., FUJITA, M.* AND HONDA, N. Dept.Medicine. Dept.Biochemistry.* Hamamatsu University School of Medicine Hamamatsu, 431-31

Amino acids are actively transported across the small intestinal mucosa. Further confirmation of the mechanisms has come from studies with isolated brush border membrane and basolateral membrane vesicles. The present work was undertaken to examine the amino acids transporting property of the endoplasmic reticulum membrane(ER)-enriched fraction obtained from rat jejunal absorptive cells. The membrane vesicles were osmotically active. They were permeable to L-alanine but less permeable to the D-isomer. The transport of L-alanine across the ER-enriched membrane was found to be enhanced by chloride gradient. There seemed to be enhanced by chloride gradient. There seemed to be no cation-specificity. However, K^+ ions seemed to be peculiar in that KCl stimulated the uptake of L-alanine even after the preincubation of membrane vesicles with salt. The addition of valinomycin did not affect the KCl-enhancement regardless of whether or not the vesicles were preincubated the KCl. Valinomycin was also without effect for the uptake of L-alanine in the presence of KSCN. A proton specific ionophore , FCCP(carbonylcyanide P-nitrofluoromethoxy phenyl-hydrazone), did not affect the amino acid uptake in the presence of choline chloride. An anion transport inhibitor, SITS abolished the overshoot phenomenon and inhibited the uptake.

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EFFECT OF NORADRENALIN ON POTASSIUM TRANSPORT IN GUINEA PIG COLON. ISHIDA, H. AND SUZUKI, Y. Dept. of Physiol., Yamagata Univ. Sch. Med., Yamagata 990-23

We have examined the effects of noradrenalin(Nad) on short-circuit-current(Isc), transepithelial conductance(Gt) or K flux from serosa to mucosa in isolated guinea pig colon. Nad(10^{-5}M) added to the serosal solution caused a decrease in Isc with an increase in Gt, consistent with either stimulation of electrogenic cation secretion or anion absorption.

Not only replacement of either Na with choline or Cl with sulfate in the serosal solution, but also addition of bumetanide(10^{-4}M) to the serosal solution greatly diminished these electrical changes indused by Nad, suggesting the involvement of Na-(K)-Cl cotransport in the serosal membrane process of these effects. Bumetanide alone elicited an increase in Isc with a decrease in Gt, and these changes were shown to be due to reduction of K secretion, when the K flux from serosa to mucosa was determined by flame photometry. From the above data, we speculate that Nad stimulates K secretion in this preparation. Bumetanide inhibitable Na-Cl or Na-K-Cl cotransport might be responsible for the serosal membrane process of this secretion.

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H^+/K^+ ANTIPORT IN THE COLONIC EPITHELIUM. SUZUKI, Y. AND KANEKO, K. Dept. of Physiol., Yamagata Univ. Sch. of Med., Yamagata 990-23

H^+ secretion by guinea pig colonic mucosa was inhibited completely when K^+ was removed from the mucosal solution, indicating a crucial role of mucosal K^+ in the mucosal membrane process of H^+ secretion. In order to elucidate the mechanism by which mucosal K^+ activate the H^+ secretion across the mucosal membrane, ^{86}Rb influxes were determined under a variety of experimental conditions using everted sac preparations. Addition of ouabain (10^{-4}M) into the mucosal solution, a condition where H^+ secretion was specifically and completely inhibited, reduced the ^{86}Rb influx across the mucosal border, demonstrating that part of ^{86}Rb influx was closely associated with H^+ secretion. This ouabain-sensitive ^{86}Rb influx showed a saturable property when Rb^+ concentration in the mucosal solution was elevated from 1mM to 2mM or 5.4mM. Incubation of tissues for 15 min in the presence of acetazolamide (10^{-4}M) or DNP (2×10^{-4}M) decreased the ouabain-sensitive ^{86}Rb influxes. Under all these conditions, the ratios of ouabain-sensitive ^{86}Rb influx to H^+ secretion rate remained to be constant and were not different from unity. These results suggest that H^+/Rb^+ (K^+) antiporter, operating in a one for one fashion, might be responsible for the mucosal process of H^+ secretion in the colon.

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BARIUM EFFECTS ON POTASSIUM RECYCLING AND ACTIVE CHLORIDE TRANSPORT ACROSS THE DILUTING SEGMENT OF TRITURUS NEPHRON. SAKAMOTO, H., KURAMOCHI, G. and HOSHI, T. Dept. of Physiology, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113

The rate-limiting step of active Cl^- transport across the diluting segment is known to be $2Cl^-/Na^+/K^+$ cotransport across the luminal membrane. For continuous operation of this cotransport, a continuous supply of K^+ into the lumen is needed since luminal concentration of K^+ is much lower than those of Na^+ and Cl^-. To determine the true pathway of K^+ supply, effects of Ba^{++}, triaminopyrimidine(TAP) and Kinetin on transtubular PD(Vt), resistance(Rt) and equivalent short-circuit current(Isc) were studied in Triturus early distal tubule. Intraluminal Ba^{++}, as well as TAP and Kinetin which block paracellular cation permeability in epithelia, completely suppressed generation of Vt and Isc during stopped-perfusion of the lumen with a K^+-free solution. Ba^{++} increased the Rt from 31 to 58 Ωcm^2, the increase being unexplainable without assuming the paracellular resistance increase. In the absence of the tripple cotransport, step changes in luminal K^+ or Na^+ concentration generated a large transient diffusion potential, which was also greatly suppressed by Ba^{++}. The results indicate that the paracellular pathway is principally important for K^+ recycling and Ba^{++} blocks this recycling pathway.

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PASSIVE POTASSIUM TRANSPORT PATHWAY ACROSS THE APICAL MEMBRANE OF THE NEWT PROXIMAL TUBULAR CELLS. KAWAHARA, K. Department of Physiology, Faculty of Medicine, University of Tokyo, Bunkyo-ku, Tokyo 113

Potassium transport pathway across the apical membrane of the newt proximal tubular cells was examined electrophysiologically. Intracellular potassium activity as measured by a K^+-selective electrode was 82.4 $\pm$ 3.6 meq/l (N = 16), the value being significantly higher than that predicted from the Nernst equation. During intraluminal perfusion with a solution of a low sodium concentration, the slope of apical membrane potential changes per 10-fold change in the luminal potassium concentration was 50 mV in the range of potassium concentration above 25 mM. The slope was reduced to 24 mV by addition of 10^{-3} M Ba^{2+} to the lumen. The apical membrane resistance per unit length decreased by 58% when a high potassium Ringer solution(25 mM K^+) was infused and increased by 70% when Ba^{2+}(10^{-3} M) was added to the luminal fluid. Ouabain(10^{-3} M in the lumen) had no effects on both cell membrane potential and intracellular potassium activity. The results indicate that cell potassium can passively diffuse out of the cells into the lumen through this potassium conductive pathway of the apical membrane.

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Inward-going rectification properties of K conductance channels across the basolateral membrane of amphibian kidney proximal tubule.

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To evaluate the movement of ions, especially of K, across the proximal tubule basolateral membrane, we used the doubly-perfused amphibian kidneys with high resistance mineral oil placed intraluminally. This procedure permitted us to measure the basolateral membrane resistance (R_{bl}) from the cable equations, in which $R_{z\text{-oil-filled}}$ was substituted for R_{bl}. Replacement of HEPES for HCO_3, administration of $5x10^{-4}$ M SITS and increase of external K concentration up to 30 mM made the membrane highly selective to K ion,the apparent transference number being $t'_{K\ 10-30}$ of 0.84 and $t'_{K\ 30-50}$ of 0.99 on the average. Under these conditions, a cable analysis with alternatively hyperpolarizing or depolarizing currents of $0.78x10^{-8}$ A to $12.48x10^{-8}$ A in 16 steps shows an inward-going rectification, i.e., the resistance for outward K movement is by 1.5 to 2.2 times higher than that for inward K movement.

These facts suggest that there is an inward-going K channel across the basolateral membrane of the proximal tubule.

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THE RELATIONSHIP OF ELECTROGENIC URINARY ACIDIFICATION PROCESS AND CELL MEMBRANE POTENTIAL PROFILE OF THE URINARY BLADDER OF FRESH WATER TURTLE.

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The mechanism of the urinary acidification across the turtle bladder was examined, by measuring the mucosal and serosal membrane potential (mEm,sEm) and individual membrane fraction of resistance (fm,fs) with simultaneous recordings of transepitherial potential difference (PD), short-circuit current (Isc) and resistance (R) under constant current pulse. After amiloride (AML) administration, R increased about 4 times from 1.7 kΩ to 6.6 kΩ (n=8) with an increase of mucosal membrane fraction ($fm=\Delta Vm/\Delta Vt$) from 0.81 to 0.97 (n=6), whereas, serosal membrane fraction (fs) decreased from 0.13 to negligible value. The most significant change after AML was the increase of mEm from -6.1 mV (in control) to 92 mV (n=6), with a reverse of PD from 47 to -27 mV (n=8) and of Isc from 35 to -8 μA, simultaneously. The change of sEm was a slight depolarization followed by hyperpolarization by 7 mV above control level (53 mV). With serosal administration of Acetazolamide, the reversed PD and Isc after AML decreased by 76 % (n=5) and 74 % (n=7), respectively. This decrease of PD associated with the decrease of mEm. The above results indicate that the occurence of reversed PD after AML treatment is a reflection of urinary acidification process of an electrogenic nature.

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H^+ TRANSPORT MECHANISM ACROSS THE PERITUBULAR MEMBRANES OF BULLFROG KIDNEY.

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To investigate the mechanism of renal tubular acid-base handling, the intracellular pH (pH_i), as well as the peritubular membrane potential (E_M), was determined in the bullfrog proximal tubule perfused *in vitro*, using double-barreled liquid ion-exchanger pH microelectrodes. In control conditions, in which Ringer solution (pH = 7.6 PCO_2 = 11 mmHg, $[HCO_3^-]$ = 15 mM) was perfused in both luminal and peritubular sides. In the 1st experiment, in which the peritubular PCO_2 and $[HCO_3^-]$ were raised about 3 times the control at constant pH, the pH_i initially decreased and then rapidly increased from 7.2 to 7.5 within 2 min with a marked hyperpolarization by 15 mV. In the 2nd experiment, in which the peritubular PCO_2 was raised to 37 mmHg and pH shifted to 7.1 at constant $[HCO_3^-]$, the pH_i gradually decreased from 7.3 to 7.2 with a depolarization by 5 mV in a few min. In the 3rd experiment, in which the peritubular $[HCO_3^-]$ was raised to 45 mM at constant PCO_2 (pH shifted to 8.1), the pH_i was slowly increased from 7.2 to 7.7 for 10 min with a hyperpolarization of E_M by about 15 mV. These data indicate that the peritubular membrane is partly permeable to H^+ or HCO_3^-, besides CO_2, and the E_M can be affected by chemical potential gradients of pH and HCO_3^- across the peritubular membrane.

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THE RESISTANT AND CAPACITANCE OF THE SQUID GIANT AXON BY THE FINITE ALTERNATING POTENTIAL SAITO, T. Depertment of Physiology, Nippon Dental University, Tokyo, Japan.

As there are immidiate relations between the impedance and excitation of the living menbrane, the messurment of impedance is useful methode to research mechanism of the excitation. The auther invents the theory and methode to messure the impedance by using the finite alternating potential and made the apparatus.

The finite potential is E sin t and the electric model of the menbrane is the sereis circuit of the resistant r and capacitance c. The passing potential is v and the imput resistance of the messuring apparatus is R.

$$v = \frac{RE_m}{R+r}\sin\phi\cos\phi\left\{1-\exp(-n\pi\tan\phi)\right\},\quad \phi=\omega\tau,\quad \tan\phi=\frac{1}{\omega(R+r)c}$$

$$v = V_m\left\{\sin(\omega t+\phi)-\sin\phi\,\varepsilon^{-\frac{t}{(R+r)c}}\right\},\quad V_m=\frac{RE_m}{\sqrt{(R+r)^2+(1/\omega c)^2}},\quad \tan\phi=\frac{1}{\omega(R+r)c}$$

$$\frac{dv}{dt}=\omega V_m\left\{\cos(\omega t+\phi)+\cos\phi\cdot\tan^2\phi\,\varepsilon^{-\frac{t}{(R+r)c}}\right\}$$

We can get those values when the menbran excites or not by those formula and the auther manages the squid giant axon by this methode.

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SALTATORY CONDUCTION ANALYSED BY UNIDIMENSIONAL LATENCY-TOPOGRAPHY. HOMMA, S., NAKAJIMA, Y. AND MURAKAMI, M*. Dept. Physiol., Sch. of Med., Chiba Univ., Chiba 280

A conducting action potential was simultaneously recorded from twelve electrodes with short inter-electrode distance. The action potentials thus recorded were interpolated according to the sampling theorem, latency and electrode distance being scaled as ordinate and abscissa, respectively. The amplitude gradient between these scales was divided into eleven subdivisions, positive ones being displayed with reddish tints, while negative with bluish tints on a color CRT. Thus unidimensional latency-topography was obtained. Unidimensional latency-topography applied to an impulse conducted along a muscle fiber revealed a linear equi-potential line, of which slope corresponds to the conduction velocity of the impulse. Unidimensional latency-topography applied to an impulse conducted along a nerve fiber revealed a step-like equi-potential curve, indicating a saltatory conduction of the impulse. In this study, we applied this topographic analysis to nerve fibers of identified function, and to demyelinated fibers caused by intraperitoneal injection of clioquinol. From these observations, we discuss on a relation between internodal length and conduction velocity, one between functional myelin length and conduction velocity, and on delayed conduction along demyelinated fibers.

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NEURON MODEL WITH NONUNIFORM PASSIVE MEMBRANE PROPERTIES. KAWATO, M. AND TSUKAHARA, N. Dept. Biophys. Eng., Osaka Univ., Toyonaka, Osaka 560 and National Institute for Physiological Sciences, Myodaiji, Okazaki, Aichi 444

We propose an exact, self-closed and simple method for simultaneous estimation of the electrotonic length of the equivalent dendritic cylinder, and the ratio of dendritic to somatic input conductance in the Rall's motoneuron model (1969), from a voltage transient at the soma in response to a current step applied to the soma. We prove the theoretical constraint in the Rall's motoneuron model that one half of the ratio of amplitudes of the first two peeled exponentials must be smaller than the ratio of the corresponding first two time constants. This theoretical prediction is not satisfied for several types of neurons, and our method to estimate cable parameters is not applicable to these neurons. By extending the Rall's neuron model, we develop a neuron model, which contains two membrane resistance per unit area; one for somatic membrane and one for dendritic membrane. In this model we obtain the transient solution of membrane potential in response to a current step. It is shown that the amplitude ratio can be larger than double of the time constant ratio when the somatic resistance is lower than the dendritic resistance. Moreover, we give a purely electrophysiological method to estimate cable parameters of the extended model from soma transient in response to a current step applied to the soma.

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RECTIFYING PROPERTIES OF RABBIT NODOSE GANGLION CELL MEMBRANE AND THEIR MODIFICATION BY PHARMACOLOGICAL AGENTS. MIYAZAKI, T., HASHIGUCHI, T. AND KOBAYASHI, H. Department of Physiology, Tokyo Medical College, Shinjuku-ku, Tokyo 160.

A remarkable outward rectification was detected at the levels of membrane potentials (MPs) more positive than about -55 mV in C-type cells of the rabbit nodose ganglion (NG) through the analysis of steady state current-voltage relationship. In addition to the nicotinic type depolarization (DP) with increase in membrane conductance (Gm), acetylcholine induced the second type of DP associated with no change in Gm which was apparently muscarinic. Muscarine itself evoked only the latter type DP in some cells. Unlike its effect on the sympathetic ganglion (SG) cells, muscarine did not antagonize the outward rectification in NG cells. Barium and tetraethylammonium evidently antagonized the outward rectification, but they also reduced the slope conductance at the MPs more negative than -55 mV, indicating their effects were not selective to the outward rectification. Histamine induced only DP with no change in Gm. Dibutyryl cyclic GMP (100 μM) induced a small DP with no change in Gm at the levels of MPs more depolarized than -55 mV, while it brought about a marked increase in Gm at much hyperpolarized levels. In summary, the outward rectification found in NG cells appeared different from that found in SG cells which was known due to the "M-current". An intracellular mediation of muscarine- or histamine-induced changes by cyclic GMP was inconclusive.

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THE OPTICAL ACTIVITY SIGNAL RECORDED FROM A LOBSTER NERVE USING A PHOTOELASTIC MODULATOR. WATANABE, A. Dept. of Cell Physiology, Nat. Inst. Physiol. Sci., Okazaki, Aichi 444

The change in optical activity of the lobster nerve was recorded using a photo-elastic modulator. The light source was a 300 W tungsten incandescent lamp. Using interference filters a quasi-monochromatic light at a wavelength between 450 and 570 nm was obtained. The light was circularly polarized with a Polaroid Film HNCP 37, and passed through a photoelastie modulator, which changed the state of polarization of the beam alternatively from a linearly plarized light with 45° azimuth to another with -45° azimuth. The frequency of the change was 50 KHz. The nerve was placed usually at 0° azimuth and the analyzer (a linear polarizer) was placed also at 0° azimuth. The light was received by a photodiode. The 50 KHz ripple of the photocurrent was rectified with a lock-in amplifier. The change in output of the lock-in amplifier was further amplified and fed to an averager. When the nerve was stimulated the change in optical activity could be detected. The duration of the change was usually less than 10 ms. The time course was different from other signals which came from birefringence change, absorbance change or from linear dichroism change. The optical properties of the signal were examined by changing the azimuth angle of the nerve or the analyzer.

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MULTIPLE-SITE OPTICAL RECORDING OF ELECTRICAL ACTIVITY IN EARLY EMBRYONIC SEMILUNAR GANGLION. SAKAI, T., KOMURO, H., YADA, T., HIROTA, A. and KAMINO, K. Dept. of Physiol. Tokyo Medical and Dental University School of Medicine, Bunkyo-ku, Tokyo 113.

Changes in absorbance of voltage-sensitive merocyanine-rhodanine dye were used to monitor electrical responses in the semilunar ganglion of 4-10-day-old developing chick embryos. The electrical responses were simultaneously recorded from several positions in the ganglion. Stimulation of the afferent nerve fibers (the ophthalmic division of cranial nerve V) with a suction electrode led to changes in light absorption of the dyed ganglia. In the 4-10-day-old embryonic ganglia, two types of the optical membrane potential responses, "non-conducted" and "conducted" responses were indentified. The non-conducted response exhibited a local response-like "initial upstroke-response" followed by the steady-state plateau evoked by larger depolarizing pulses. The conducted response was blocked by tetrodotoxin(TTX). The incidence of the conducted responses increased as development proceeded from 5-day to 10-day-old. Thus, the TTX-sensitive action potential activity is probably initially generated in the semilunar ganglion during the 5-10-day-stage of development. These data represent the first demonstration of membrane potential responses in early embryonic chick neuronal cells in situ. And these studies demonstrate the usefulness of voltage-sensitive dyes in the analysis of the organizing process of the neuronal functions in the early developing embryos.

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MECHANICAL SUPPORT OF THE EXCITABLE MEMBRANE BY CYTOSKELETAL PROTEINS

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Effects of the intracellular pressure on the excitability of the squid axon were examined by the voltage clamp method. When the axon was perfused intracellularly with a solution mainly containing KF, the intracellular pressure applied in the range of 0 - 20 cmH_2O had a small effect on the inward current. However, when the axon was perfused with a KCl- or KBr-solution, raising the pressure suppressed the inward current in 10 -20 min, whereas maintaining the pressure at zero level did not. Electron microscopic observations revealed that the cytoskeletal structure was preserved by the KF-solution but dissolved completely by the KCl- or KBr-solution. There was a clear correlation between the loss of cytoskeleton and the increase in sensitivity of the membrane to the mechanical stress. Intracellular application of 10 mM colchicine reduced the inward current to half of the control value when the intracellular pressure was kept high. In contrast, if the intracellular pressure was reduced to zero, colchicine did not exert its effect. These observations suggested that submembranous cytoskeletal fibers actually support the excitable membrane mechanically, although cytoskeletal fibers are not directly involved in the molecular machinery of excitation.

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MEMBRANE POTENTIAL OF CYNOPS BLASTOMERE.

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Membrane potential (Em) of the egg of Japanese newt, Cynops pyrrhogaster, at the first cleavage stage was measured in a modified Holtfreter's solution (NaCl, 60 mM; KCl, 0.7 mM; $CaCl_2$, 1.4 mM; HEPES, 2.0 mM). The egg which had been removed of its jelly coat and vitelline membrane before the beginning of the first cleavage was impaled with two glass microelectrodes. 1) Before cleavage, Em was -12.1±2.1 mV and remained almost constant when the ionic concentrations and pH of the solution were altered. 2) During the first cleavage, when new membrane of the inner surface of blastomere was formed, Em gradually increased up to the value of over -80 mV (at pH 7-8), and membrane resistance decreased (6.8±1.5 MΩ before cleavage, 0.5±0.3 MΩ after cleavage). 3) After the beginning of the first cleavage, Em was changed mainly by the alteration of pH of the solution (-91.9±7.0, -75.1±4.7, -41.8±12.3, -2.9±8.7 and +43.1±10.8 mV at pH 8.0, 7.0, 6.0, 5.0 and 4.0, respectively). The 10 times increase of K^+ concentration in the solution depolarized the egg by about 30 mV, 20 mV and several mV at pH 8.0, 7.0 and 6.0, respectively. The decrease of Na^+ (to 1/10 concentration) depolarized the egg at pH 8.0 and hyperpolarized at pH 4.0 by 10-20 mV. The exchange of Ca^{2+} for Mg^{2+} did not affect Em at pH 8.0 and 4.0. The decrease of Cl^- (to 1/10 concentration) hyperpolarized the egg by several mV at pH 8.0.

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RECTIFICATION OF K-CHANNEL IN A SQUID GIANT AXON INDUCED BY WATER FLOW.

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An osmotic water flow was applied to modify the properties of K-channel in a squid giant axon. The outward water flow made an undershoot of the action potential and an E_K more negative. The potassium conductance obtained from the instantaneous I-V relation for the tail currents had a marked rectification. The conductance in the direction opposite to the water flow applied decreased extremly. The undershoot and the E_K became more sensitive to potassium ions in the external solution than those in the axon under the normal isotonic condition. However, potassium ions applied externally gave no effect on the tail current. These changes were restored immediately when the water flow was stopped by matching the osmolalities on both sides at a high osmolality, but the recovery took much longer time when the osmolality change was restored to the initial isotonic value without matching osmolalities first. This rectification cannot be explained by the depletion of potassium ions outside but some sructural change in the potassium channel induced by the water flow might be the cause of this rectification.

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PHARMACOLOGICAL PROPERTIES OF K-CHANNELS ACTIVATED UNDER EXTERNAL K-RICH CONDITIONS IN MOUSE NEUROBLASTOMA (II)

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Our previous experiments using various K-channel blockers indicated that mouse neuroblastoma cells (clone N-18) carry a high (above 10-15mM) $[K^+]_o$-activated K-channel which is distinct from the voltage dependent and the Ca-dependent ones. In the present study, we further examined the pharmacological and physiological properties of this K-channel. The results obtained are as follows. 1) The replacement of external Ca^{2+} (1.8 mM) with Ba^{2+} (1.8 mM) had no effect on the high $[K^+]_o$-induced increase in Rb efflux (used as an index of the membrane K-permeability). 2) The increase in Rb efflux was observed even in the absence of extracellular Ca^{2+}. 3) The elevation of $[Ca^{2+}]_o$ (e.g. from 1.8 to 10 mM) inhibited the increase of the Rb efflux in seemingly competitive manner to $[K^+]$. The other divalent cations, Co^{2+} and Cd^{2+}, exhibited a similar blocking effect. 4) This type of K-channels was also activated more or less by the extracellular Rb^+ and Cs^+. 5) These results confirm the idea that the K-channel under consideration is a novel one that was opened by the binding of K^+ (Rb^+ or Cs^+) to the channel. 6) The activation of this novel K-channel may be responsible, at least in part, for the suppression of action potentials at high $[K^+]_o$ above 10-15 mM.

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POTASSIUM CURRENT NOISE IN SINGLE SALIVARY GLAND CELLS.

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Voltage dependency of K channel in the salivary gland acinar cells was investigated from fluctuation analysis. Single cells from rat parotid gland were voltage clamped using giga-seal whole cell clamp mode. Sets of 20 to 40 identical, 60 (ms) depolarizations and hyperpolarizations were applied. The resulting current records were processed by MINC laboratory computer to obtain the mean current and the variance. The time course of the mean current was fitted by two time constants. It means that 3-state model is more realistic. The simplest plausible model could be 2-close 1 open model. Assuming this model, the relationship between the variance and the mean current could be fitted by the function $\delta^2/I = i - I/N$. The size of the population of channels per cell ranged between 50 - 100 and the single channel current was about 5 to 6 (pA) at 0 (mV). Those values should still be the approximated values but not far from the true values. It was tested by the simulation in the time course of the variance.

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EFFECTS OF TMA^+ ANALOGUE $[TRIMETHYL\text{-}N\text{-}ALKYLAMMONIUM]^+$ ON IONIC CHANNELS OF THE SQUID GIANT AXON.

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$[Trimethyl\text{-}n\text{-}alkylammonium]^+$ has a hydrocarbon chain with n carbon atoms and the agents(n=8,10,12,14) were applied intracellularly to the squid giant axons. The agents inhibited both peak inward-currents and late outward-currents and the half inhibition doses were 600μM(n=8), 150μM(n=10), 40μM(n=12) and 3μM(n=14). These inhibition effects of the agents increased as the length of a hydrocarbon chain increased and were reversible. When the agent(n=10) at concentration,5mM,was externally applied to the axon perfused intracellularly, both values of the peak inward-current and late outward-current decreased. To stop the intracellular perfusion specifically reduced the value of late outward-current and the value recovered after starting the intracellular perfusion. This suggests that the agent might penetrate the membrane from outside to inside of the axon, attach a TMA^+ site of the agent to a potassium channel and inhibit the late outward-current.

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INFLUENCES OF MEMBRANE POTENTIAL AND EXTERNAL PH ON BINDING PROPERTIES OF SEA ANEMONE TOXINS TO NA CHANNELS IN THE CRAYFISH GIANT AXON. OGURA, T.* AND WARASHINA, A.† Dept. Biol., Fac. Sci., Niigata Univ. and †Dept. Physiol., Niigata Univ. Sch. Med., Niigata.

Four different sea anemone toxins from Anthopleura (AP-A and -C; gifted by Dr. T.R. Norton), Anemonia (ATX II) and Parasicyonis (PaTX) were applied to crayfish giant axons. These toxins suppressed the Na inactivation process similarly to produce maintained currents, which could be used to determine the fraction of saturable toxin binding to Na channels. When washing AP-A-affected axons with a toxin-free ringer, AP-A detached at pH 8.5 faster than at pH 7.5. PaTX and ATX II did not dissociate appreciably at either pH. AP-C dissociation was observed at pH 8.5, but not at pH 7.5. These natures may be related to localization of positive charges on the toxin molecules, referring to their amino acid sequences. By depolarizing the membrane, the binding of each kind of toxin was reduced in a characteristic manner. The depression of binding in case of AP-A under depolarization mainly caused by an increase in dissociation rate, whereas that with PaTX was ascribed solely to an decrease in association rate. These potential-dependent associations and dissociations are not suitably accounted by a simple interaction between net charges on the toxins and the membrane potential gradient, suggesting that additional factors, which might be related to gating mechanisms of Na channel, are possibly involved in the phenomena.

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CHARACTERIZATION OF VOLTAGE DEPENDENT CHLORIDE CHANNEL OF THE SQUID AXON MEMBRANE. INOUE, I., Inst. Marine Biol., Sch. Medicine, Tokushima Univ., Seto-cho, Naruto City, Tokushima, 771-03

Giant axons of squid, Sepioteuthis, were internally perfused with a solution of 100 mM TEA-salt or of 100 mM TMA-salt, and were bathed in a 100 mM Ca-salt solution containing TTX. The anion composition of both internal and external solutions was changed. Under these conditions the membrane behaved like an anion selective electrode especially sensitive to Cl^-. Membrane currents recorded from those axons showed practically no time-dependent property, but they had a strong voltage dependent characteristic, i.e. outward rectification. Cl^- had a strong effect upon the voltage dependency; externally applied Cl^- enhanced the outward rectification, and internally applied Cl^- increased the inward currents. This non-linear characteristic of the current was almost completely suppressed by some disulfonic stilbene derivatives, such as SITS and as DIDS which are known as anion-channel blockers. These results have demonstrated that a voltage dependent chloride channel exists in the squid axon membrane. The chloride permeability of the resting membrane (at -60 mV) is calculated to be 3.0×10^{-7} cm/s.

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EFFECT OF ALIPHATIC SULFONIC ACIDS ON THE EXCITABLE MEMBRANE IN THE SQUID GIANT AXON. TAKENAKA,T.,HORI,N. AND HORIE,H. Dept. of Physiol., Sch. of Med.,Yokohama City Univ.,Minamiku,Yokohama,232.

The effects of aliphatic sulfonic acids on the excitability of the squid giant axon were studied by using a voltage clamp. The aliphatic sulfonic acids which have carbon atoms from 9 (nonane-) to 13 (tridecane -) were applied externally and internally in an internal perfused axon. These drugs inhibit only sodium current. The half inhibition dose was 1.3 mM at nonanesulfonate and 11 μM at tridecanesulfonate by internal application. The inhibitory effect increased three times when the carbon atoms increased one. These effects were completely reversible by washing with artificial internal solution. These drugs were also effective by external application but its effect was one sixth compared with the case of internal application. These data show that the inhibitory effects of sodium-current depends on the number of carbon atoms in the compounds and the effective site of these drugs located in the internal surface of the membrane.

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INTRACELLULARLY APPLIED TRYPARGINE SELECTIVELY BLOCKS THE SODIUM CHANNEL OF SQUID GIANT AXON. SEYAMA, I. AND YAKEHIRO, M. Dept. Physiol., Med. Sch., Hiroshima Univ. Hiroshima 734

The pharmacological action of trypargine(TRG), 1,2,3,4-tetrahydro-β-carboline, was examined on squid axons isolated from Doryteuthis bleekeli or Sepioteuthis lessoniana. A combined method for internal perfusion and voltage clamp was used in all experiments. TRG specifically suppresses the Na current when applied to the internal surface of the membrane without affecting the K current. The block of the Na channel in the intact axon occurred in a frequency and voltage dependent manner. However, in the pronase-treated axon, the frequency dependent block of the Na channel disappeared, but the voltage dependency remained unchanged, when the duration of depolarizing pulses was long enough to saturate TRG with receptor. Decay of Na current in pronase-treated axon in response to various cathodal pulses followed a time course of single exponential function where time constants are voltage dependent. Therefore the mechanism of blocking action of TRG can be explained by the two state model, open $\rightleftharpoons$ occluded. The binding of TRG to receptor occurs at a site about halfway through the membrane electric field from the outside; the dissociation constant is 11 uM at 0 mV.

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EFFECTS OF ALKALOID TOXINS ON THE SODIUM CONDUCTANCE IN THE CRAYFISH GIANT AXON. WARASHINA, A. Dept. of Physiol., Niigata Univ. Sch. of Med., Niigata 951

Two kinds of alkaloid toxins, veratridine and aconitine, were applied to voltage-clamped crayfish giant axons to analyze detailed modes of toxin actions. Veratridine at a concentration of 50 μM induced three major effects on the membrane Na current: 1) increase in steady current at the holding potential (-100 mV), 2) reduction of the maximum peak inward current, 3) shift in the reversal potential to the negative direction. These toxin actions developed effectively only when the axon was stimulated repetitively with depolarizing pulses to voltages above the activation threshold, suggesting that veratridine interacted with Na channels being in an open state. It was found, however, that the steady-current-inducing effect described in item 1) was quickly removed by stopping the repetitive stimulations in the presence of veratridine in the bath or washing the axon with a toxin-free ringer, whereas the other two effects remained persistently under those conditions. Aconitine also acted to depress the transient Na current in a frequency-dependent manner but neither induced a steady current or shifted the reversal potential. These results seem to suggest that the alkaloids may cause different types of toxin actions on the crayfish giant axon through different modes of interactions between Na channels and toxin molecules.

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MECHANISMS OF ACTION OF PYRETHROIDS ON THE GATING OF Na CHANNELS IN THE CRAYFISH GIANT AXON. MURAYAMA, K., OMATSU, M.*, KITASATO, H., MARUNAKA, Y. and KANBE, M. Dept. of Physiol., Shiga Univ. of Med. Sci., Ohtsu 520-21 and Dept. of Agric. Chem., Kyoto Univ., Kyoto 606.

To search for suitable chemical modifiers to Na channels, the mode of action of a series of substituted benzyl chrysanthemates was characterized using crayfish giant axons. The pharmacological features observed, 1) depolarization of the resting membrane, 2) depression of peak g_{Na}, 3) slowing Na inactivation process and 4) an appearance of the residual I_{Na} followed by a Na tail current, were found to be correlated with the steric volume and position of substituents. Next, of typical pyrethroids, p-SO_2Me and o-SO_2Me, mechanisms of the action were studied on asymmetrical capacitance currents (I_g). Both compounds immobilized gating charges in I_g-on by 20-30% of the control, which corresponds to the suppression of peak g_{Na}. Integrated I_g-on (Q-on) vs time and kinetic analysis of I_g-on relaxation decay showed that a fraction of movements of gating charges on depolarization was slowed by both compounds, the rate being slower in p-SO_2Me than in o-SO_2Me. These results suggest that slowing Na inactivation or an appearance of residual Na currents may be ascribed to the slowly opening fraction of Na channels. The Na tail current with p-SO_2Me was clearly shown to be due to very slow return of gating charges to the closed position upon repolarization.

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SPECIFIC REDUCTION IN SODIUM CHANNELS DURING PROLIFERATION OF HERPES SIMPLEX VIRUS (HSV) IN THE TISSUE-CULTURED MAMMALIAN NERVE CELLS. FUKUDA*, J., KURATA, T. and YAMAGUCHI, K., Department of Physiology, Faculty of Medicine and *Department of Pathology, Institute of Medical Sciences, University of Tokyo, Tokyo 113.

This paper reports that HSV proliferation blocks Na spikes but not Ca spikes in a tissue-cultured mammalian nerve cell. Nerve cells were isolated from dorsal root ganglia of adult guinea pigs and were grown on a plastic dish (see Fukuda et al., Nature 279, 546, 1979). On day 2, nerve cells were infected with type 2 HSV (Ogiwara strain; 2×10^4 p.f.u./ml) and then were grown for another 4-6 days in vitro. No change in nerve cell morphology was detected during the early stage of the HSV-infection. Staining the nerve cells with FITC-labelled anti-HSV-antibody showed presence of HSV-antigen in approximately 10-15 % of the nerve cells. Intracellular recording with a glass microelectrode demonstrated a reduced value of the resting membrane potential by 5-10 mV. Intracellular passage of depolarizing pulse currents failed to generate a full-sized Na spike in the HSV-infected nerve cells even after holding the membrane potential at -100 mV which was provided to remove inactivation of Na channels in the membrane. By contrast, these HSV-infected nerve cells generated full-sized Ca spikes when bathed in a Na-free, TEA-containing solution. These suggest that the HSV (a DNA virus) inhibits production or maintenance of the Na channels in the plasma membrane of the nerve cells.

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KINETICS OF 9-AMINOACRIDINE BLOCK OF SINGLE Na CHANNELS. YAMAMOTO, D. Lab. Neurophysiol., Mitsubishi-Kasei Inst. Life Sci., Machida, Tokyo 194.

Kinetics of 9-aminoacridine (9-AA) block of single sodium channels in neuroblastoma N1E-115 cells was studied using the gigaohm seal patch clamp technique, under the condition in which the inactivation gate of Na channels had been removed by treatment with N-bromoacetamide. When 9-AA was applied to the cytoplasmic phase of Na channels, it caused flickering within single openings of sodium channels. In the presence of 30 μM 9-AA, the mean open time (τ_o) was 1.6 msec and the mean blocked time (τ_b) was 1.3 msec at -40 mV. The dissociation rate constant (1) calculated from the τ_b was strongly voltage dependent, the mean rate constant being 214 sec^{-1} at 0 mV. The association rate constant (k), calculated from the slope of the curve relating the reciprocal τ_o to 9-AA concentration showed little voltage dependence, the rate constant being on the order of 6×10^6 $M^{-1}sec^{-1}$. The equilibrium dissociation constant of 9-AA block of sodium channel, defined by the relation of 1/k, was calculated to be 36 μM at 0 mV. 9-AA also prolonged the duration of individual openings of single Na channels. All of the features of 9-AA block of single sodium channels in the absence of inactivation gate were compatible with the sequential model in which 9-AA molecules blocked open sodium channels, and the blocked channels could not close until 9-AA molecules had left the blocking site in the channels.

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DEVELOPMENTS OF Ca AND Na CHANNELS IN L6 MYOTUBES IN SERUM-FREE CULTURE. AMAGAI, Y., IIJIMA, M. AND KASAI, S. Dept. Physiol., Tohoku Dent. Univ., Koriyama, Fukushima 963

Developments of Ca and Na action potential mechanisms in L6 myotubes cultured in serum-free medium were studied. L6 cells were plated at 5×10^4 cells / 35 mm gelatin-coated dish in Ham's F-12 supplemented with 10% FCS and incubated for 24 hr. Then they were transferred to serum-free condition by replacing the medium with MM-1, which consisted of F-12, 10^{-5} M fetuin, 10^{-6} M insulin, and 10^{-7} M dexamethasone. Cells in MM-1 proliferated with doubling time of about 20 hr to achieve confluent state and differentiated into myotubes as those grown in serum-supplemented medium. These myotubes had membrane potential of -62.0±3.6 mV similar to the control (-64.8±4.7 mV) and generated action potentials due to both Ca and Na current components. The development of Ca current system was equivalent with control as judged from the amplitude of overshoot of Ca spike in 10 mM-Ca solution. However, that of Na spike mechanism was reduced so that the maximum rate of rise became about 40% of the control. These results indicate that MM-1 cannot sufficiently support the differentiation of L6 myogenic cell line with respect to the development of Na spike system.

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MAINTENANCE OF HIGH DETECTION SENSITIVITY IN THE AMPULLARY ELECTRORECEPTORS: IONIC CONTROL IN THE STEADY STATE. SUGAWARA, Y. and OBARA, S. Dept. of Physiol., Teikyo Univ., Sch. of Med., Tokyo 173

The sensory epithelium of Plotosus electroreceptors in situ is optimally biased for high sensitivity, with a steady epithelial current which consists of a passive bias current and active currents, with a continual Ca influx. Stable operation of such a system requires some regulating mechanisms. The reversal potential for the inward current under V-clamp was much lower than a suppression potential for PSP in the afferent, which suggests a non-specific outward current flowing in the open Ca channels. The inward current was partially suppressed by preceding positive command pulses, which suggests a Ca-dependent inactivation of Ca channels. Under simulated in situ conditions, 4-AP abolished the oscillation and shifted the DC level in the positive direction, i.e. $I_{K(Ca)}$ is contributing to repolarize the resting DC level. As for the leakage conductance (G_L), changes in external K or Na had little effect. High Ca increased G_L, while low Ca decreased it. KCN increased G_L but reduced peak I_{Ca}, whereas ouabain only reduced I_{Ca}. Thus, Ca_i due to the continual influx appears to be regulated in two ways, by inducing a set of ionic conductances which mostly tend to reset the resting DC level and by activating Ca pump mechanism.

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EFFECTS OF INTRACELLULAR AND EXTRACELLULAR Ca AND OTHER DIVALENT CATIONS ON MEMBRANE EXCITABILITY OF SQUID GIANT AXONS. YAMAGISHI, S. and FURUYA, K. Dept. of Cell Physiology, National Inst. for Physiol. Sci., Myodaiji, OKAZAKI 444.

Effects of 10 species of divalent cations on membrane excitability were examined by extracellular or intracellular addition to the standard solution. Among externally added divalent cations at the concentration of 80 mM, only Cd and Be ions suppressed the action potential by 30 percent. While all species of divalent cations at the concentration of 1 mM added to the internal solution (100 mM K^+) suppressed the action potential. The inward current (I_{Na}) and the outward current (I_K) were suppressed more or less by internal addition of every species of divalent cations. The modes of suppression, however, showed different manners. According to the degree of I_{Na} and I_K suppression, divalent cations are classified into three groups, namely, (1) slightly suppressed group; Mg, Mn, Ca, Sr, and Co ions, (2) strongly suppressed I_K group: Ni, Ba and Zn ions, (3) strongly suppressed I_{Na} and I_K group; Be and Cd ions. In addition to that, the prolongation of Na activation was observed by internal application of Ni, Zn, Be, and Cd ions.

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INTRACELLULAR FREE CALCIUM DURING PENTYLENETETRAZOLE-INDUCED BURSTING ACTIVITY IN SNAIL NEURONS. FURUICHI, H., IMAI, S., ONOZUKA, M. AND SUGAYA, E. Dept. of Physiol., Kanagawa Dental College, Inaoka-cho, Yokosuka

To clarify the role of intracellular Ca^{2+} during pentylenetetrazole (PTZ)-induced bursting activity (BA) in sanil neurons, $[Ca^{2+}]i$ was directly measured in the PTZ-sensitive neurons of the suboesophageal ganglion of Euhadra peliomphala with a Ca^{2+} selective microelectrode containing liquid neutral carrier ion exchanger ETH 1001 and the following results were obtained. (1) During PTZ-induced BA, $[Ca^{2+}]i$ increased compared with that before PTZ application. (2) By the application of calcium ionophore A23187 (50-100 μM), succession of BA followed by a long-lasting hyperpolarization was observed. (3) $[Ca^{2+}]i$ during A23187-induced BA was almost the same level as that induced by PTZ; $[Ca^{2+}]i$ during A23187-induced hyperpolarization was higher than that during PTZ-induced BA. (4) Co^{2+} in the bathing medium did not suppress the above mentioned PTZ-induced $[Ca^{2+}]i$ change.

These findings indicate that the generation of PTZ-induced BA requires an optimum increase in $[Ca^{2+}]i$ which is less than Ca^{2+}-dependent K^+ conductance increase generation and also requires calcium release from intracellular stored site.

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ENHANCEMENT OF Ca-CURRENTS BY SERUM FACTORS IN CULTURED NERVE CELLS.
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This study aimed to examine chemical properties of serum-factors which enhanced Ca-spikes in nerve cells. Neurons of dorsal root ganglion (DRG) were grown in media which contained various concentrations of serum-factors, and development of Ca-currents was examined by means of voltage clamp technique.

Neurons of DRG from adult guinea pig were cultured in a serum-free N1 medium. Addition of fetal calf serum (more than 0.15 %, V/V) to the N1 medium enhanced the maximum rate of rise of Ca-spikes in 5 days. Voltage clamp study of the nerve cell with a patch electrode demonstrated that the serum-factors enhanced amplitude of the Ca-currents, but did not change voltage-dependency of the Ca-currents. No change in K-currents by serum factors was exhibited in the presence of Ca-channel inhibitor. Heat-treatment of serum factors caused no reduction in capability of enhancing Ca-currents. While dialysis of the serum through a cellophane membrane induced a complete loss of capabilities of enhancing Ca-currents. It is thus concluded that effective components of the serum were suggested to be small, heat-stable molecules.

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ACTIVATION KINETICS AND ITS MODULATION BY COBALT IONS IN TWO TYPES OF CALCIUM CHANNELS FOUND IN ASCIDIAN EGG AND EMBRYO.
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Activation kinetics of Ca channels was studied by voltage-clamp technique and compared between the egg type and the differentiated type whose identification was reported previously. Activation kinetics of the egg Ca channel was described with m^1 form, while that of the differentiated Ca channel with m^2 form. Co^{2+} slowed the rising phase of egg-Ca-channel current by adding a slow component. The slowing can be explained by a model that the rate constant of the transition of the activation gate is slowed in a modified channel to which Co^{2+} is bound. Co^{2+} also revealed a slow component in the rising phase of differentiated-Ca-channel current, but a similar model to that of the egg Ca channel can not explain the slowing, because there was a clear discrepancy between slow components estimated in the rising phase of the inward current and in the falling phase of the tail current. The model which can explain the result is that Co^{2+} modifies each of two activation gates independently in a differentiated Ca channel, indicating that the channel has two independent binding sites to Co^{2+} for the kinetic modulation.

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MEASUREMENT OF INTRACELLULAR Ca IONS WITH Ca-SENSITIVE MICROELECTRODE DURING HYPERPOLARIZING EXCITATION IN HAMSTER EGGS.
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We have shown that, in golden hamster eggs at fertilization, a series of periodic hyperpolarizing responses (HRs) appears in an all-or-none fashion and that Ca injection into unfertilized eggs induces a regenerative HR with an apparent threshold. We measured directly the intracellular Ca ion concentration using a Ca-sensitive microelectrode during the HR which is based on the Ca-activated K conductance. A neutral Ca-ionophore ETH1001 was used as a Ca-sensor. A glass micropipette was broken or bevelled to have the tip of 1 to 1.5 μm and then filled with the sensor in 100 to 200 μm of the tip. After calibration of the sensitivity of Ca-electrodes, an unfertilized egg was impaled with a Ca-electrode and two KCl electrodes, one for a reference electrode and another for voltage clamp. The basal Ca concentration was 0.1 to 0.3 μM. After insemination the Ca concentration increased beyond 1 μM in an all-or-none fashion, corresponding to periodic outward K currents. Iontophoretic injection of Ca ions from a $CaCl_2$-filled electrode with 2 sec pulse demonstrated a regenerative increase in intracellular Ca ions. These findings strongly suggest that the mechanism of Ca-induced Ca release from intracellular stores exists in the hamster egg and it operates periodically during fertilization.

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IONIC MECHANISMS OF THE EXCITABILITY IN PREIMPLANTATIONAL EMBRYOS OF THE MOUSE.
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Ionic mechanisms of action potentials were studied in mouse oocytes and embryos up to the 16-cell stage of development using intracellular recording techniques. At all stages examined, action potentials dependent on divalent cations (Ca^{2+},Sr^{2+},Ba^{2+}) were detected. Such action potentials were insensitive to tetrodotoxin (TTX), and were blocked by inorganic (Co^{2+},Mn^{2+},Cd^{2+},La^{3+}) and organic (diltiazem) Ca antagonists. In addition, action potentials dependent on monovalent cations (Na^{+},Li^{+}) were also observed at all stages. Both the Na and Li action potentials were resistant to TTX, and were inhibited by organic and inorganic Ca antagonists. Furthermore, a competition phenomenon was observed between permeant monovalent and divalent cations. The results suggest that monovalent cations pass through the Ca channels instead of the Na channels. Distinct Na channels were neither detected nor induced at any stages examined.

It is concluded that the cell membranes of the mouse have voltage-dependent Ca channels which are permeable to divalent and monovalent cations during preimplantational stages of development.

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CALCIUM AND POTASSIUM CURRENTS IN SPERMATOGENIC CELLS OF THE RAT STUDIED WITH PATCH ELECTRODE.
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Spermatogenic cells were isolated from rat testes and whole-cell currents were measured with patch electrode (Hamill et al, 1981). When the membrane potential was depolarized from holding potential (-84∼ -94 mV), transient inward current was produced which was followed by slowly developing outward current. The inward current was identified as Ca^{2+} carried current since replacement of external Ca^{2+} with Mn^{2+} reversibly diminished the current whereas Ba^{2+} or Sr^{2+} substituted for Ca^{2+}. The outward current was independent of $[Ca^{2+}]_o$ and was blocked by tetraethylammonium chloride. The reversal potential of the outward current changed from -65mV to -12mV when $[K^{+}]_o$ was raised from 5 mM to 100 mM. Thus, this outward current was identified as membrane potential dependent K current. During spermatogensis from spermatogenia to early spermatids, the density of Ca current increases while the K current density decreases significantly.

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IONIC CONDUCTANCES IN RED NUCLEUS NEURONS IN VITRO.
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Intracellular recordings were made from red nucleus (RN) neurons in guinea-pig slice preparations. The action potential was followed by a fast and a slow afterhyperpolarization (AHP). The fast AHP was inhibited by tetraethylammonium (TEA). The slow AHP was reversibly abolished by Co or Mn. Amplitudes of both the fast and the slow AHP were dependent on the extracellular K concentration. When TEA was added, a tetrodotoxin (TTX) resistant regenerative depolarization was evoked which was blocked by Mn or Co. When the membrane potential was hyperpolarized, a time-dependent inward rectification was observed which was inhibited by Cs but not by TTX, TEA, Co or Ba. These results suggest that the fast AHP is produced by the voltage-dependent K conductance and the slow AHP is produced by the Ca-activated K conductance and that RN neurons have the voltage-dependent Ca conductance and h conductance.

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ELECTROPHYSIOLOGICAL PROPERTIES OF VENTROMEDIAL HYPOTHALAMIC NEURONS IN GUINEA PIG AND THEIR RESPONSES TO GLUCOSE: A VITRO STUDY. MINAMI, T., SUGIMORI. M.*, AND OOMURA, Y. Dept. Physiol., Fac. Med., Kyushu Univ., Fukuoka 812, Dept. Physiol., Sch. Med., New York Univ. U.S.A.

Intracellular recodings were made from 45 ventromedial hypothalamic (VMH) neurons in slice preparations. The measured values for the resting potential (range 51.4-75mV), the input resistance (range 84.8-355MΩ), and the time constnt (range 6-21.4msec) varied from cell to cell. Three types of activity were recognized. The first type was characterized by small Ca components when treated with TTX solution, small after hyperpolarization (AHP), high firing rate by intracellular current injection and easy activation by local stimulation at the lateral edge of the nucleus. The second type showed big Ca components, large AHP, low frequency firing by current injection and an excitation-inhibition response on local stimulation. The third type exhibited large hump trajectories, occasional extra spikes, big Ca components, large AHP and high frequency firing by current injection. These observations suggest that the differences in the electrophysiological properties between the first and second type neurons can be attributed to the Ca component in spike and the Ca induced K current. Fourteen VMH neurons were also analysed for their glucose responsiveness. Three neurons (21%) depolarlized (8-13mV) with a significant increase in the input resistance at high glucose concentration (30mM), and mean reversal potential of -89.5 mV, as seen in the I-V plot.

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INHIBITION OF CALCIUM ION CHANNEL CURRENTS IN NEUROBLASTOMA CELLS BY ADENOSINE DERIVATIVES. Y. KURODA, M. YOSHII*, A. TSUNOO* and T. NARAHASHI*. Dept. of Neurochemistry, Tokyo Metropolitan Institute for Neurosciences, Fuchu-shi, Tokyo 183 and *Dept. of Pharmacology, Northwestern University Medical School, Chicago, Ill 60611, U.S.A.

Electrophysiological and biochemical data on guinea pig brain slices indicate that adenosine derivatives modulate synaptic transmission in mammalian CNS. Inhibition of the postsynaptic potentials by adenosine is mediated by a "presynaptic receptor"; ADO-1. ADO-1 agonists reduce the transmitter release and its molecular mechanism has been suggested to be an inhibition of Ca^{2+} influx (Y. Kuroda, in "Physiology and Pharmacology of Adenosine Derivatives", J. Daly et al. eds, Raven Press, p245-256, 1983). Using patch clamp technique, direct evidence was obtained in neuroblastoma cells. Ba^{2+} currents through calcium channels, observed under the whole cell clamp condition, were completely abolished after the bath application of adenosine and 2-chloroadenosine (100 μM), and recovered to its original level after washing. This suggests a coupling of ADO-1 receptors and voltage-dependent calcium channel molecules.

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NERVOUS CONTROLS OF FEEDING MOTONEURONS IN APLYSIA. NAGAHAMA, T. AND TAKATA, M. Department of Physiolosy, School of Dentistry, Tokushima University, Kuramoto-cho, Tokushima 770

In Aplysia buccal ganglia we identified two types of motoneurons, I^1- and I^3-Mns, which innervated two different intrinsic buccal muscles, I^1- and I^3-muscles. The repetitive firings of the I^1- and the I^3-Mns caused mouth opening-like and closing-like movement, respectively. We examined properties of postsynaptic potentials produced in I^1- and I^3-Mns by electrical stimulation of three buccal nerves, esophageal nerve and cerebral-buccal connective. The I^1-Mns innervating the mouth opening muscle predominantly received excitatory inputs from these nerves, however, stimulation of these nerves produced mainly IPSPs in the I^3-Mns innervating the mouth closing muscle. Bathing the ganglion in high-Mg^{2+} high-Ca^{2+} ASW little altered the tendency for the properties of synaptic inputs to these motoneurons. The IPSPs produced in the motoneurons were selectively blocked by d-tubocurarine, while the EPSPs were little affected by hexamethonium. In addition, an inhibitory interneuron impinging on the I^3-Mn was identified. The results suggest that the I^1- and the I^3-Mns are reciprocally controlled by peripheral and central inputs.

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LONG-LASTING HISTAMINERGIC INHIBITION AND CYCLIC NUCLEOTIDES IN AN IDENTIFIED ONCHIDIUM NEURONE. GOTOW, T. Dept. of Physiol., Sch. of Med., Kagoshima Univ., Kagoshima 890

Inhibition of long duration (ILD) was monosynaptically produced in an identified Onchidium neurone by repetitive stimulation of the cardiac nerve. Ten stimuli at 1-2 Hz to the presynaptic nerve usually gave the ILD of about 10 mV in amplitude and 0.5-1 min in duration. Of this ILD, the early and middle phases reversed, but the late phase did not reverse. Neither phase of the ILD was affected by low Cl or Na solutions, nor by high Ca solutions. By changing the external K the shift of the reversal potentials for the early and middle phases was close to that predicted for the K-electrode, though the late ILD was insensitive to the external K. Intracellular TEA attenuated the amplitude of the ILD, but it did not shorten the duration. These suggest that the ILD had another conductance-independent mechanism simultaneously with the increase in K conductance. Inhibitors of energy supply, DNP and cyanide selectively reduced the ILD, but ouabain showed no such effect. Cimetidine specifically blocked the ILD, mimicked by application of histamine. It is concluded that the ILD mediated by a histamine receptor consisted of an increase in K conductance and a hyperpolarizing ion-pump insensitive to ouabain. Intracellularly injected cAMP and cGMP enhanced and depressed only the pump component of the ILD, respectively.

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GLYCINE-INDUCED DEPOLARIZATION OF AN IDENTIFIED NEUROSECRETORY NEURON IN APLYSIA ABDOMINAL GANGLION. ICHINOSE, M., SAWADA, M. AND MAENO, T. Dept. of Physiol., Shimane Med. Univ., Izumo, Shimane 693

The ionic mechanism of membrane effect of glycine on an identified neurosecretory neuron of Aplysia was investigated with conventional intracellular recording and voltage-clamp techniques. Glycine was found to cause a marked depolarization of the neuron. The depolarizing response can be obtained either by localized application of glycine (ejected by pressure and electrophoresis) or by bath-application. Bath-applied glycine induced a voltage-sensitive inward current which gradually reached a maximum and remained at that level until glycine was washed out. Displacement of holding potential from -46 to -106 mV increased the inward current. The extrapolated reversal potential was +38.6 ± 5.2 mV (mean ± S.E., N=5). Reduction of $[Na^+]_o$ caused a reversible decrease of the inward current at given holding potential. Alterations of $[K^+]_o$ and $[Ca^{++}]_o$, as well as bath-applied ouabain (10 μM) and sodium cyanide (2 mM) did not affect the inward current. From structure-activity studies with acidic, neutral and basic amino acids, neutral amino acids were found to be particularly potent. These results suggest that neutral amino acids can induce a voltage-sensitive Na^+-current and that the glycine-induced inward current does not reflect an active uptake by a Na^+-coupled transport system of neutral amino acids into the neuron.

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BLOCKING ACTION OF SEROTONIN ON THE TWO TYPES OF EXCITATORY DOPAMINE RECEPTORS OF APLYSIA GANGLION CELLS

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The application of dopamine(DA) on a certain ganglion cells of Aplysia elicits two types of depolarizing responses. One is a "slow" depolarization due to a decrease in K-conductance (D_K-type), whereas the other is a "fast" depolarization associated with an increase in Na-conductance (D_{Na}-type). These depolarizing responses were evaluated by the change in membrane conductance (ΔG) using the modulated resting clamp method. Pharmacological evidence indicated that the D_K-type response and D_{Na}-type response are mediated by two distinct receptors. A 2 minute exposure to 10 μM serotonin(5-HT) significantly depressed both types of responses to 10 μM DA. The statistical analysis of dose-inhibition curves obtained from a number of cells with the D_K-type receptor indicated that 5-HT competed with DA for the common binding sites at the receptor of this type. The Lineweaver-Burk type plotting obtained from a single cell with the receptor of D_{Na}-type suggested that the more of 5-HT-induced blockade for this type of DA-receptor was also competitive, although more cells are needed the examine in order to confirm it.

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EFFECTS OF LIDOCAINE AND DIAZEPAM ON THE GABA-INDUCED INHIBITORY RESPONSE OF *APLYSIA* GANGLION CELLS.

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Two types of GABA-receptors have been identified in *Aplysia* ganglion cells. One is the inhibitory type, which produces Cl^--dependent hyperpolarizing response (H_{Cl}-type) to GABA. The other is the excitatory, which produces Na^+-dependent depolarizing response to GABA. The results obtained in the H_{Cl}-type cells will be mentioned first of all; the GABA-induced responses were evaluated by the increase in membrane conductance, using either method of voltage clamp or current clamp. Then an effect of lidocaine (LIDO) on the GABA-induced response was analyzed with dose-inhibition curves. It was found that the blocking mode of LIDO to the H_{Cl}-type receptors was "noncompetitive".

The Cl^--dependent response to GABA was augmented by 10^{-6} M, but depressed by 10^{-4} M diazepam (DZ). The interaction between 10^{-3} M LIDO and 10^{-6} M DZ on the GABA-receptor was also observed. LIDO reduced 70% of the GABA-induced response, but this was restored 40% by DZ. However, in case of the pretreatment with DZ, the blocking effect of LIDO was only 10%.

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Cl ION CHANNEL AND MONOVALENT ANIONS.

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Experiments were carried on the internally-perfused and voltage-clamped cell bodies of the frog sensory neuron. The effects of foreign anions on GABA-induced currents (Cl, Br, I, NO_3, SCN, HCOO and F currents) were examined externally and internally. Permeable anions enhanced GABA-GABA receptor binding when these anions were applied externally and not internally. Therefore, when foreign anions were internally perfused with the external application of GABA in the presence of Cl ions, one can measure the currents carried through Cl channels without considering the effects of these foreign anions on GABA receptors. The relative conductance obtained in this condition was greater in the order of $I \approx NO_3 \approx Br > Cl > HCOO > F$.

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GABA ACTIVATES THREE TYPES OF THE RECEPTOR-IONOPHORE COMPLEX WITH THEIR RELATIVE DISTRIBUTION FOLLOWING ZIPF'S LAW. Report I.

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The GABA-activated chloride current (I_{Cl}) separated from other ionic currents in internally-perfused and voltage-clamped cell bodies (n=12) of the frog dorsal root ganglia was subjected to noise analysis. The estimated number of the ionophores (N) versus the calculated elementary Cl conductance (γ) was nearly exponential, thus following Zipf's law and thereby implicating an interacting differentiation during the receptor-ionophore ontogenesis. This graph also indicates the existence of three subgroupes (A, B and C) of the receptor-ionophore units; $N \approx 3.6 \times 10^5$, 2.3×10^4, 5.9×10^3, and $\gamma \approx 1.6$, 8.0, 12.9 pS for A, B and C in that order.

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GABA ACTIVATES THREE TYPES OF THE RECEPTOR-IONOPHORE COMPLEX WITH THEIR RELATIVE DISTRIBUTION FOLLOWING ZIPF'S LAW. Report II.

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Continued from Report I.

The activation appears to be phasic with A and B and tonic with C, since the given subtype emerged from noise analysis of the selective interval, i.e., either around the peak (for A and B) or the steady state (for C) of chloride responses to GABA. Single channel recordings (n=25) conformed to the presence of B and C; the failure of detecting A may be accounted for by its small γ value coupled with a rapid desensitization incurred with this subtype. Further, we established 'concentration clamp' technique in which external solution can be completely exchanged by new test solution within 8 msec. Using this technique, we could separate fast, phasic and tonic currents.

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RELEASE OF ENDOGENOUS GLUTAMATE FROM THE FROG SPINAL CORD: DORSAL ROOT STIMULATION.

TAKEUCHI, A., ONODERA, K. AND KAWAGOE, R. Dept. of Physiol., Sch. of Med., Juntendo Univ., Hongo, Tokyo 113

Hemisected spinal cord of the bullfrog was placed in a small bath and the concentrations of glutamate and aspartate in the solution during 10 min was measured by gas chromatograph-mass spectrometer (GC-MS). The stimulation was applied to 7-9th dorsal roots in a separate compartment and the ventral root potential (VRP) was recorded from 8th ventral root. The dorsal root stimulation at 10/sec or 20/sec induced a significant increase in the glutamate release, but the increase in the aspartate release was small and not significant. In low Ca solution VRP and glutamate release produced by repetitive dorsal root stimulation were suppressed. Essentially the same results were obtained with the intraarterially perfused spinal cord. Application of strong stimuli to the dorsal roots at low frequency (less than 1 Hz) produced VRP which lasted for several sec, suggesting the contribution of interneurones. With this stimulation an increase in glutamate release was found. These observations support the view that glutamate acts as the excitatory transmitter of primary afferent and of interneurones. Although the possibility of the transmitter role of aspartate is not excluded, its contribution may be relatively small.

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RELEASE OF ENDOGENOUS GLUTAMATE FROM THE FROG SPINAL CORD: CHEMICAL STIMULATION.
TAKEUCHI, A., ONODERA, K. AND KAWAGOE, R. Dept. of Physiol., Sch. of Med., Juntendo Univ., Hongo, Tokyo 113

The hemisected frog spinal cord was placed in a small bath and the concentrations of glutamate and aspartate in the solution during 10 min were measured with gas chromatograph-mass spectrometer (GC-MS). After treatment of the preparation with black widow spider venom (BWSV), irregular potential changes were recorded from the ventral root and the release of glutamate was increased significantly. It has already been shown that BWSV increases the quantal release of miniature e.p.s.p. and the release of glutamate in the crayfish neuromuscular junction. Application of substance P (SP) and bradykinin (BK)(100 μM) caused irregular potential changes in ventral root and increased the glutamate release with a small increase in the aspartate release. Application of tetrodotoxin (TTX) decreased the potential changes in ventral root but the release of glutamate by SP and BK was approximately the same as in control. Low calcium solution suppressed the release of glutamate induced by these peptides. These results suggest that BWSV and neuropeptides such as SP and BK act at the presynaptic terminals and increase the glutamate release which may be acting as the transmitter.

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KINETICS OF THE GABA-ACTIVATED CHLORIDE CHANNELS OF CAT SPINAL GANGLION CELLS.
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Fluctuation analysis of the GABA-induced membrane currents in neurones of the isolated spinal ganglion has been made to estimate the apparent dissociation constant for GABA-receptor interactions and the rate constant for the opening and closing of the GABA activated chloride channels. In the presence of nipecotic acid (an inhibitor of glial GABA uptake system), the variance of GABA current fluctuations increased with increasing GABA concentration up to 300 μM. It declined slightly at higher concentrations. The mean life time (τ) of GABA-activated channels was shortened with increasing GABA concentration; e.g., τ of 22.2 ms at a low concentration of GABA (10 μM) was decreased to 10.6 ms by raising GABA concentration to 1 mM at the room temperature (26°C). The concentration-dependent increase in the reciprocal mean life time (namely the overall rate constant) appeared to saturate at 115 s^{-1}. These measurements yielded the opening and closing rate constant of 70.5 s^{-1} and 44.5 s^{-1}, respectively, and the dissociation constant of 300 μM. The result suggests that the Katz-Miledi model which is characterized by the rate-limiting conformational change in ion channels is also applicable to describe the GABA-receptor kinetics in the cat primary afferent neurones.

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ON THE RELATION BETWEEN ACETYLCHOLINE- AND GLUTAMATE-RECEPTORS ON HELIX NEURONES.
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Acetylcholine(ACh)- and glutamate(Glu)-receptors in Helix neurones are independent each others according to the results of several chemical modifying reagents. In Glu-H and Glu-weak D type neurones, N-acetylimidazole(AI)-activated Glu-induced large and prolonged duration of depolarization due to Na^+ permeability increase. These AI-activation of Na^+ responses are due to disinhibition at the Na^+-channel gate according to their dose-response relation-shifts. AI-activated Glu-induced Na^+ responses were depressed by chemical modification of amino base with TNBS. ACh-induced Na^+ responses were also depressed with TNBS application. The results indicated that AI-activated Glu-Na^+ responses and ACh-induced Na^+ responses are produced by the same Na^+-channel activation.

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EFFECTS OF CYCLIC NUCLEOTIDES ON RHYTHMIC HYPERPOLARIZING POTENTIAL OF HAMSTER SUBMANDIBULAR GANGLION CELLS. SUZUKI, T. Dept. of Oral Physiology, Matsumoto Dent. Coll., Shiojiri, Nagano 399-07

Effects of dibutyryl cyclic AMP (dbcAMP) applied extracellularly or cAMP injected intracellularly on submandibular ganglion cells were examined by intracellular recording. Adiministered cAMP caused long lasting biphasic or monophasic responses of the membrane potential. The former was composed of a slow gradual depolarization (DP) followed by a slow gradual hyperpolarization (HP) (Rm increase by 11-65%). The latter was composed of alow gradual HP or slow gradual DP (Rm decrease by 10-29%) alone. Rhythmic HP (0.8-3.4/min in frequency) and/or evoked HPs resembling those induced by caffeine were generated during the slow gradual HP. EPSP after-hyperpolarization (AHP) and the late component of spike-AHP were enhanced markedly in both amplitude and duration during the slow gradual HP. Injected 5'-AMP was ineffective under comparable conditions. Therefore, cAMP action was similar to the caffeine action. In some cells tested, cGMP caused a slow depolarization of membrane potential and abolished generation of rhythmic HPs activated spontaneously or by caffeine. Furthermore, cGMP may be involved in the generation mechanism of pacemaker potential observed in a small number of submandibular ganglion cell. In contrast, injected 5'-GMP was ineffective.

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LONG LASTING HYPERPOLARIZATION INDUCED FOLLOWING ACTION POTENTIAL OF RABBIT OTIC GANGLION CELL. YOSHIZAKI, K., HOSHINO, T. AND KOYANO, H. Dept. of Physiol., Akita Univ. Sch. of Med., Akita 010

The duration of an afterhyperpolarization recorded from otic ganglion cells was of 380±160 msec (n=77). In some of these cells, the afterhyperpolarization was followed by a long lasting hyperpolarization (LLH) of which duration has the mean value of 4.7± 1.7 sec (n=22) in the course of reverting of the resting membrane potential. All of the LLH has a refractory period of 2 to 5 min. The equilibrium potential of LLH estimated was equal to that of the afterhyperpolarization and was in the range from -82 to -90 mV. Under the conditions of passing inward current through the cell, the LLH was also induced following the somato-dendritic spike by direct stimulation of the cell, but the initial segment spike produced by antidromic stimulation was not followed by the LLH. The above results suggest that the LLH is induced by increase of G_K of a somato-dendritic membrane. The LLH was obliged to disappear by perfusion of low Ca^{++} and high Mg^{++} solution (Ca^{++}, ≃0mM, Mg^{++}, 4.0mM) and/or Co^{++} solution (2.1mM). In addition, the LLH was evoked by iontophoretic injection of Ca^{++} ion into the cell. The LLH following the action potential or the afterhyperpolalization, therefore, seems to be caused by increse of Ca^{++}-induced G_K.

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DIFFERENTIAL EFFECT OF ANTIDROMIC VOLLEYS IN DEPRESSING THE SYNAPTIC TRANSMISSION THROUGH B AND C NEURONS IN THE BULLFROG SYMPATHETIC GANGLION. TASAKA, J. and TOSAKA, T. Department of Physiology, Tokyo Medical College, Shinjuku-ku, Tokyo 160

The compound fast excitatory postsynaptic potential (f-epsp) in B neurons of bullfrog sympathetic ganglion has been shown to be depressed for more than 1 hour by applying supramaximal antidromic stimulation (20 Hz for 15 sec) to the sciatic nerve for activating both B and C ones (J. Physiol. Soc. Japan, 45, 422, 1983). In C neurons, antidromic volleys did not affect the f-epsp, whereas they eliminated almost completely the slow inhibitory postsynaptic potential. The antidromic tetanic stimulation abolished the posttetanic potentiation in both B and C neurons but did not affect the facilitation in the second epsp of paired ones. The depression of f-epsp was not inducible when the antidromic stimulation was applied in the low Ca/high Mg medium, indicating a release of transmitter of some kind may be involved. However, putative transmitter substances and their antagonists tested so far, such as 10-100 μM adrenaline (n= 2), 1-10 μM phenoxybenzamine (n=10), 10 μM phentolamine (n=2), 10 μM yohimbine (n=5), 1, 3 μM α-melanocyte stimulating hormone (n=3), 10 μM met-enkephalin (n=2), 0.1-10 μM naloxone (n=10), 0.001-0.1 μM avian pancreatic peptide (n=2), 10, 1000 μM indomethacin (n=3), 0.01-100 μM γ-aminobutyric acid (n=8), 10, 100 μM d-bicuculline (n=8) and 1.4 μM atropine (n=5) were all ineffective in antagonizing the observed depression of f-epsp.

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LONG-TERM-ENHANCEMENT (LTE) OF SLOW (S-)EPSP, INDUCED BY A PHYSIOLOGICAL PREGANGLIONIC CONDITIONING TRAIN. MOCHIDA, S. AND LIBET, B.* Dept. of Physiol., Tokyo Med. Coll., Tokyo 160; *Dept. of Physiol., Univ. of California, San Francisco, CA. 94143.

The muscarinic slow depolarizing response to ACh or methacholine, MCh (Libet&Tosaka, 1970) and the equivalent s-EPSP (Ashe&Libet,1981), had been found to exhibit LTE after a brief exposure to dopamine, DA, in presence of COMT-inhibitor.

S-EPSP test responses at 35°C in rabbit SCG are readily increased, 50 to 100%, for >3 hours, following a conditioning preganglionic train (10 pps-2 min, or 3 pps-7 min). No COMT-inhibitor is needed. The enhanced s-EPSP is abolished by the muscarinic antagonist QNB ($5X10^{-8}$M). In cat stellate ganglion, a conditioning stimulus train (10 pps-2 min) on the sympathetic chain below T-4 induces an LTE of s-EPSP, elicited heterosynaptically in preganglionic ramus T-4 as well as homosynaptically at the chain. Specific DA antagonists (3 to 7μM) d-butaclamol, spiroperidol, and flupenthixol strongly depress the LTE. Adrenergic antagonists, whether α (dihydroergotamine, 37μM) or β (sotalol, 10μM), were without effect. Thus, LTE of the muscarinic s-EPSP can be easily induced by physiological neural inputs; the induction can be heterosynaptic and is not due to presynaptic PTP; and LTE is probably mediated by intraganglionic release of a second transmitter, namely DA in at least the rabbit SCG. (Supported by USPHS grant NS-00884.)

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MECHANISM OF THE POTENTIATION OF TRANSMITTER RELEASE BY ADRENALINE IN BULLFROG SYMPATHETIC GANGLIA. KUMAMOTO, E., KUBA, K., MINOTA, S., NOHMI, M. Dept. Physiology, Saga Medical School, Nabeshima, Saga 840-01

The mechanism was further examined of the long-term potentiation of the fast excitatory postsynaptic potential (fast e.p.s.p.) by an adrenaline-cyclic AMP system. The facilitatory action of adrenaline (10 μM) was blocked by dibutyryl cyclic GMP (100 μM). Cholera toxin (2 μg/ml) enhanced the quantal content of the fast e.p.s.p., indicating the involvement of Ns protein (bearing the guanyl nucleotide regulatory site) in the mechanism of the activation of adenylate cyclase. Adrenaline did not prolong the synaptic delay in spite of increasing transmitter release, being different from the actions of K-channel blockers (TEA, 4-AP, Cs). The facilitation of the fast e.p.s.p. induced by a paired stimulation of the preganglionic nerve was decreased by the pretreatment with adrenaline. The magnitude of the potentiation by adrenaline was independent of the Ca^{2+}/Mg^{2+} ratio in a low Ca^{2+}-high Mg^{2+} solution. These results suggest that adrenaline neither suppresses the voltage-dependent K^+-conductance nor enhances the voltage-dependent Ca^{2+}-conductance at the presynaptic terminals, but elevates the basal level of the intracellular free Ca^{2+} concentration in the presynaptic terminals through an increase in endogenous cyclic AMP concentrations and that this mechanism is blocked by endogenous cyclic GMP.

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EFFECT OF MASTOPARAN ON THE NERVOUS SYSTEM (I) YOSHIKO OHGUCHI, HARUO KOBAYASHI* AND YOICHIRO KURODA. Dept. of Neurochemistory. Tokyo Metropolitan Institute for Neurosciences, Tokyo 183 and *Dept. of Physiology, Tokyo Medical College, Tokyo 160.

Mastoparan is a tetradecapeptide isolated from a japanese hornet (Vespula lewisi) and release histamine from mast cells (Nakajima et al. 1979), also catecholamines from adrenal chromaffin cells with an induction of Ca^{2+}-influx (Kuroda et al. 1980). Recently, mastoparans have appeared to bind with high affinity to calmodulin, an important Ca^{2+}regulating protein and antagonize the calmodulin activity. Since there has been no work for the effects of mastoparan on the nervous system, applied this toxin to various nerve preparations. (1) Olfactory cortex slices of guinea pig; inducing a complex response to the post synaptic potentials, presumably due to the indirect release of transmitter and/or modulator with different spatial and temporal parameters. (2) Frog sympathetic ganglia; depolarizing the resting potential about 8 mV within 2 min after the addition. (3) Cultured sympathetic neuron-like cells; changing a Ca^{2+} component in their action potentials.

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STIMULATING EFFECT OF CHOLINE OR CDP-CHOLINE ON EXOCYTOSIS-ENDOCYTOSIS CUOPLING IN PRE-SYNAPTIC TERMINALS DURING TRANSMITTER RELEASE IN CAT SYMPATHETIC GANGLIA. KADOTA, K. AND KADOTA, T.* Div. Neurochemistry, Psychiatric Res. Instit. of Tokyo, Setagaya, Tokyo 156 and Dept. Anat. Sch. of Med., Chiba Univ., Chiba 280

The effects of choline or CDP-choline (cytidine diphosphate choline) on the post-ganglionic potential (PGP) and morphology of cat sympathetic ganglia were tested in vitro or in situ. The ganglia, stimulated for 30 min at 10 Hz, were maintained or perfused in or with Krebs solution containing the testing substance (10 mM). The PGP died out 10 - 20 min after stimulation in vitro, decreased but not vanished in the perfused ganglia, and slightly reduced in amplitude in the ganglia maintained under normal supply of blood. The administration of choline or CDP-choline protected the reduction in amplitude of the PGP in the ganglia in situ, perfused with Krebs solution or preserved under normal blood. Electron micrographs showed the depletion of synaptic vesicles (SV) and the expansion of terminal surface membrane in the ganglia in vitro. The SV decreased in the number but not depleted in perfused ganglia. The reduction of SV number was slight in the extent in the ganglia preserved in blood, in which endocytotic vacuoles or coated vesicles (CV) were often observed. Choline or CDP-choline exerted its effect by increasing the number of SV, endocytotic vacuoles and CV.

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EFFECTS OF TETANIC STIMULATION ON THE PREGANGLIONIC NERVE FIBRES ON THE SYMPATHETIC GANGLION CELL PROPERTIES UNDER INHIBITION OF CHOLINERGIC TRANSMISSION. MORIMOTO, K., CHEM, P. W., MATSUOKA, Y., YONEMURA, K. AND TANAKA,I. Dept. of Physiol., Kumamoto Univ. Med. Sch., Kumamoto 860

The effects of preganglionic nerve stimuli on the ganglion cell were studied in the isolated superior cervical ganglia of guinea pigs, in which actions of Ach as a chemical transmitter from preganglionic nerve terminals were eliminated by using hexamethonium (C_6, 10^{-3} M), C_6 (10^{-3}M) + atropine (10^{-5} M) or Ach (10^{-3} M). Under those conditions, EPSPs were almost completely depressed but membrane potential of the cell was still depolarized after tetanic stimuli of preganglionic nerve fibres. KCl-responses were enhanced more or less, and magnitude of after-potentials in the ganglion cells were reduced after the tetanic stimulation. In some cells the tetanic stimuli caused hyperpolarization instead of depolarization, both KCl-response and after-potential were enhanced by the stimuli. Those effects of the tetanic stimuli were resemble to the effects of exogenously applied enkephalin as found in our previous study. Terefore, we will further try to identify the enkephalinergic-like component in the chemical transmission in the ganglia.

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THE SYNAPTIC MECHANISM UNDERLYING THE NON-CHOLINERGIC AFTERDISCHARGE OF CAT LUMBAR SYMPATHETIC GANGLIA (LSGs). SUGIYAMA, K., HIGASHI, H., INOKUCHI, H. AND NISHI, S. Dept. Physiol., Kurume Univ. Sch. Med., Kurume.

In an attempt to characterize the synaptic potential and its transmitter responsible for production of the non-cholinergic afterdischarge of cat LSGs, we have recorded intracellularly from deeply curarized and atropinized LSG neurons. Tetanic preganglionic stimulation evoked a long-lasting depolarization (late slow EPSP) in about 15 % of LSG neurons. The late slow EPS-current was associated with an increased membrane conductance (Gm). LHRH and substance P (SP) produced Gm-increasing depolarizations, while 5-HT induced a Gm-decreasing depolarization. Late slow EPSP and LHRH- and SP-induced depolarizations were reduced in amplitude by membrane depolarization and nullified at -20 to -10 mV but could not be reversed. Their amplitude-membrane potential relationships were not significantly altered by reduction of external K or Cl to 1/10, whereas reduction of external Na to 1/10 reversibly abolished LHRH- and SP-depolarizations. During LHRH-depolarization, which showed almost no desensitizing phenomena, late slow EPSP was markedly reduced or nullified. In contrast, late slow EPSP persisted after desensitization of LSG neurons to SP. The results suggest that LHRH or a related substance could be the non-cholinergic transmitter candidate for the feline LSGs and that their late slow EPSP is likely produced by an increased Na conductance.

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STATISTICAL ESTIMATION OF SHAPE OF PSPs: COMPUTER SIMULATION OF THE CODING PROCESS.
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In order to establish a statistical estimation of the shape of postsynaptic potentials (PSPs) elicited in human motoneurons, we simulated, on a computer, the coding process of a motoneuron, based on results obtained from animal experiments. In the simulation, we adopted a monosynaptic impulse response excitatory PSP (IR-EPSP), a linear returning gradient to an augmentative EPSP level due to polysynaptic activity, and an inhibitory PSP (IPSP). These PSPs were summated by each other. As soon as the summated membrane potential attained the critical level for firing, the model neuron fired, and the potential fell down the resting level. This process was repeated and a sequence of motoneuronal spikes was generated. We also calculated a distribution of time lags from input to output and a distribution of a bias potential level, from which motoneuronal spike-triggering IR-EPSP started to rise. When there are no IPSPs, the lag-time distribution showed a peak if lag times from onset of the IR-EPSP to output were measured, whereas the lag-time distribution revealed a trough when IPSPs were added and lag times from onset of IPSPs to output were measured. From this simulation and theoretical analysis, we discussed whether it is possible to estimate the shape of PSPs from the shape of lag-time distribution between input and output.

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MINIATURE INHIBITORY SYNAPTIC POTENTIALS IN RAT SPINAL MOTONEURONS.
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In the new born rat spinal cord *in vitro*, spontaneous potentials were recorded with KCl electrodes, from motoneurons, after blocking nerve impulses by tetrodotoxin (10^{-6} g/ml), or after abolishing synaptic transmission by replacing extracellular Ca^{2+} with Mg^{2+}. The amplitude of the miniature potentials was less than 1 mV and was increased by intracellular injection of Cl^-. Strychinine (2-25 μM) abolished these spontaneous potentials. It is suggested that these potentials are produced by the spontaneous release of packages of inhibitory transmitter at synapses on motoneurons. An increase of osmolarity linearly increased the frequency of miniature inhibitory synaptic potentials (m.i.s.p.). An increase of extracellular K^+ concentration also increased m.i.s.p. frequency. Replacement of Ca^{2+} with Mg^{2+} abolished the K-dependent increase of m.i.s.p. frequency, suggesting that such an increase of transmitter release is caused by depolarization-induced Ca^{2+} influx at the nerve terminals. Frequency of m.i.s.p. increased as the temperature was increased, with a Q_{10} of about 3 between 25 and 35 °C.

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THE EFFECT OF ENKEPHALIN AND SUBSTANCE P ON THE RAT DORSAL HORN NEURONS IN VITRO.
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Intracellular recordings were made from substantia gelatinosa (SG) and lamina IV neurons of adult rat spinal cord slices. Application of the enkephalin analog, D-Ala-D-Leu-Enkephalin (100 nM-2 μM) by perfusion or Methionine-Enkephalin (M-ENK) by pressure ejection from micropipette produced dose-related hyperpolarization associated with an increased membrane conductance in about 50 % of the SG cells tested. The hyperpolarization became smaller when the membrane was hyperpolarized and eventually reversed in polarity. The reversal potential followed the external potassium concentration as expected from the Nernst equation. Naloxone abolished the hyperpolarization. Substance P (SP) applied by perfusion (1-5 μM) or by brief pressure ejection produced no changes in membrane potential or conductance in 12 of 12 SG cells but it increased excitatory postsynaptic potentials in amplitude and frequency of about 50 % of the SG cells. In 13 of 14 lamina IV neurons, SP produced a concentration-dependent depolarization associated with a decreased membrane conductance. The depolarization decreased in amplitude with membrane hyperpolarization and was nullified at about -90 mV but not reversed with more negative membrane potential. SP induced depolarization was not affected by tetrodotoxin, while it was blocked by Co which blocks Ca channels.

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SLOW SYNAPTIC POTENTIALS OF RAT DORSAL RAPHE NEURONS IN VITRO. HIGASHI, H., YOSHIMURA, M. AND NISHI, S. Dept. Physiol. Kurume Univ. Sch. Med., Kurume

Intracellular recordings were made from neurons in the dorsal raphe nucleus (DRN) in the rat brain slices. A single focal stimulus elicited a slow IPSP following a fast EPSP. The slow IPSP was associated with a decrease in membrane resistance (R_N). Application of 5-HT to DRN neurons by superfusion or from 5-HT-containing microcapillary by pressure induced a slow hyperpolarization which was also associated with a decrease in R_N. The slow IPSP and the 5-HT-hyperpolarization were reversed at the same level of membrane potential (-95 mV). LSD or methysergide (100 $\sim$ 200 nM) reversibly abolished both the slow IPSP and 5-HT-hyperpolarization, whereas imipramine (1 $\sim$ 20 μM) markedly enhanced and prolonged the responses. A train of repetitive stimuli elicited a slow EPSP following the slow IPSP. The slow EPSP was associated with either an increase or a decrease in R_N. In neurons in which the slow EPSP was a R_N-decreasing type, noradrenaline (NA) also caused a R_N-decreasing slow depolarization. In other neurons both the NA-induced depolarization and slow EPSP were of R_N-increasing type. Phentolamine or prazocin (0.1 $\sim$ 1 μM) abolished both the slow EPSP and NA-depolarization while propranolol and yohimbin (1 μM) were without effects. It may be concluded from these results that the slow IPSP and the slow EPSP are mediated, respectively, by 5-HT and NA.

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EFFECTS OF EXCITATORY AND INHIBITORY AMINO ACIDS ON CULTURED CEREBELLAR NEURONS FROM CHICK EMBRYO. MORI-OKAMOTO,J., ASHIDA,H. AND TATSUNO,J. Department of Physiology, National Defense Medical College, Tokorozawa, Saitama 359

The susceptibility to amino acids of cultured cerebellar neurons (25-60 days) were intracellularly investigated using KAc-electrodes. In almost all neurons, iontophoretically applied GABA and glycine suppressed spontaneous spikes, decreased the membrane input resistance and produced either hyperpolarization or depolarization. The mean reversal potentials for GABA and glycine were -47 and -43mV respectively. In a low Cl^- medium or with the use of KCl-electrodes, these reversal potentials were shifted toward OmV, and sometimes their effects were converted to be excitatory. The effects of GABA and glycine were antagonized by picrotoxin or bicucullin, and strychnine, respectively. Cultured neurons were also sensitive to L-glutamate, DL-homocysteate and L-aspartate (least potent), and the depolarizations thus induced were associated with the decrease or occasionally with the increase of the membrane resistance. Glutamate and homocysteate showed similar reversal potentials (-25mV). Glutamate diethylester and D-α-aminoadipate antagonized the actions of these excitatory amino acids. These results suggest that cultured cerebellar neurons have acquired diffuse sensitivities to neurotransmitter candidates in the course of neuronal development.

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QUANTUM NATURE OF MINIATURE SYNAPTIC POTENTIALS IN CEREBELLAR PURKINJE CELL DENDRITES. ASHIDA,H., MORI-OKAMOTO,J., TATSUNO,J. AND OKAMOTO,K. Department of Physiology and Department of Pharmacology, National Defense Medical College, Tokorozawa, Saitama 359

Miniature synaptic potentials were recorded intracellularly from Purkinje cell dendrites in guinea pig cerebellar slices without any external stimulation. Amplitude histograms of them had regularly spacing peaks. Autocorrelations of them indicated clearly that they were composed of the units of about 0.4 - 0.5 mV. Computer simulations using random numbers generated according to the positively confined truncated normal distribution with the same statistical features as the observed miniature potentials demonstrated indistinctly periodic peaks in amplitude histograms on rare occasions, but autocorrelations of them never indicated periodicity. This fact asserted that the regular peaks in amplitude histograms of the miniature potentials were not due to statistical fluctuation error, but due to the quantum. The estimation of the parameters of binomial distribution by the successive peak counts in amplitude histogram showed that total number of the quanta was 10 - 40, probability that each quantum is released was 0.1 - 0.3 and the mean number of quanta released (mean quantum content) was 3 - 4. By the similar estimation of the Poisson parameter, the mean quantum content was 3 - 8.

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LONG-TERM POTENTIATION OF CORTICORUBRAL SYNAPTIC TRANSMISSION AND CAMP-INDUCED SLOW DEPOLARIZATION. TSUKAHARA, N. AND YONEMORI, F. Dept. Biophys. Eng. Fac. Eng. Sci. Osaka Univ., Toyonaka and Natl. Int. Physiol. Sci. Okazaki

Tetanic stimulation of 100 Hz for 3 minutes induces potentiation of corticorubral synaptic transmission lasting for an hour or so. The potentiation was in both in vivo kitten red nucleus and in vitro slice preparation using kitten brain stem containing the red nucleus. Further tetanic stimulation induced a later slow depolarization with a peaktime of about 10-20 msec, which persisted several tens seconds after tetanic stimulation. This slow depolarization is induced without tetanic stimulation, but by applying dibutyryl c-AMP extracellularly. Furthermore, during slow depolarization, the membrane resistance was increased. It is likely that the slow depolarization was produced by the decrease of potasium conductance. Indeed, after application of dibutyryl c-AMP, the later component of afterhyperpolarization of the red nucleus neurons was reduced. This later component of afterhyperpolarization has been shown to be due to Ca-activated potasium conductance.

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MEMBRANE CURRENTS PRODUCED BY L-GLUTAMATE IN RAT HIPPOCAMPAL NEURONS IN DISPERSED CELL CULTURES.
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The properties of the membrane currents produced by L-glutamate in hippocampal neurons prepared 17- to 19-day-old fetal rats in dispersed cell cultures were studied with the patch electrode voltage clamp technique. In the majority of neurons tested, the relationship between the glutamate-induced current (I) and the membrane potential (V) was non-linear, the slope conductance being much smaller in the negative potential range (< -40 mV) than in the more positive range. The removal of Mg ions from the extracellular solution increased the amplitude of the glutamate-induced current in the negative potential range, making the I-V relationship almost linear. It can therefore be concluded that Mg ions suppress the glutamate current in a voltage-dependent manner. The reversal potential of the glutamate current was -3.6 ± 1.5 mV (mean ± S.E., n=10) when the interior of the cell was perfused with 150 mM KCl. The permeability ratios (P_x/P_{Na}) for Li, K, Rb, Cs and Tris ions were 1.2, 1.1, 1.1, 1.2 and <0.1, respectively.

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IONIC CHANNELS ACTIVATED BY GLUTAMATE AND HOMOCYSTEATE IN HIPPOCAMPAL NEURONS OF THE GUINEA PIG. YAMAMOTO, C. AND SATO, H. Dept. of Physiol., Facul. of Med., Kanazawa Univ., Kanazawa 920

In the presence of Mn^{2+} and tetrodotoxin, depolarizations induced by L-glutamate or D-homocysteate (DH) in CA3 neurons were accompanied by increases in membrane potential fluctuation in thin hippocampal sections of the guinea pig. The properties of the Glu and DH-induced noises suggest that the excitatory amino acids depolarize hippocampal neurons by their gating actions on the ionic channels. The amplitude of the Glu noise was larger than that of the DH noise. In hippocampal neurons, Glu and DH seem to activate identical channels. The amplitude difference between Glu noise and DH noise may be explained by the assumption that an average open-gate time for DH is shorter than that for Glu.

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EFFECTS OF GLUCOSE DEPRIVATION ON ELECTRICAL ACTIVITIES OF GUINEA-PIG HIPPOCANPAL PYRAMIDAL NEURONS. MIYAKAWA, H.,KANEKO, K. AND KATO, H. Dept. of Pysiol., Yamagata Univ. Sch. of Med., Yamagata 990-23

The effects of glucose deprivation on electrical activities of CA1 pyramidal neurons were studied by recording field, extracellular and intracellular potentials using in vitro slice preparations. The population spikes elicited by the st. radiatum stimulation were blocked in 10-25 min after the glucose deprivation. They were irreversible after 60 min deprivation. The population spikes elicited antidromically by stimulation of the st. alveus maintained for more than 60 min after glucose deprivation. Extra- and intracellular recordings revealed that orthodromically elicited action potentials and EPSPs were blocked after glucose deprivation, while direct and antidromic stimulation could elicite action potentials even after the synaptic block. These obserbations indicate that synaptic regions are the primary responsible sites for the cellular disfunction in the early period after the glucose deprivation.

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ACTIONS OF 4-AMINOPYRIDINE ON NEURONAL ACTIVITIES STUDIED IN HIPPOCAMPAL SLICE PREPARATIONS. MATSUDA,Y. AND NAKAMURA,M. Dept. Physiol., Nagasaki Univ. Sch. Med., Nagasaki 852

Modulations of the synaptic transmission and the neuronal excitation by 4-aminopyridine (4-AP) were studied by means of intracellular recordings from pyramidal neurones in the CA1 and CA3 regions of hippocampal slice preparations. The results were as follows.

1) In the presence of 4-AP in concentrations of 100-500μM, the elicitation by an afferent shock as well as the spontaneous occurrence of EPSPs were remarkably facilitated while IPSPs were apparently suppressed. At the beginning of perfusion with a 4-AP solution, however, neurones frequently showed a barrage of hyperpolarization (probably IPSPs).

2) Application of 4-AP reduced the adaptation of cell firing which evolved during the injection of depolarizing currents into pyramidal neurones. Concomitantly, the hyperpolarizing potential following repetitive spikes was suppressed by 4-AP, especially in CA3 pyramidal neurones.

Possible mechanisms for the above-mentioned phenomena are discussed.

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EFFECT OF DENERVATION ON THE INSECT NEUROMUSCULAR JUNCTION. WASHIO, H. AND NIHONMATSU, I. Lab. Neurophysiol., Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194.

Functional and structural changes at the denervated neuromuscular junction have been studied in cockroach leg muscles. About 2 days after nerve section, the spontaneous release of transmitter disappeared together with blockage of nerve-muscle transmission. The time course of the degenerated process depended largely on temperature. At 12°C the failure occurred about two weeks after nerve section. A few weeks later sometimes the miniature potential activity was again resumed at the denervated junction at a very low rate. The present work was also undertaken to find out whether this functional change was associated with any structural changes following denervation. Electron-microscopic study showed that synaptic vesicles were clearly reduced in number and the glial wrapping around an axon terminal already took place by 48 hours after nerve section. By five days almost all terminals were depleted of the vesicle and glial cells moved completely into the synaptic cleft in close contact with the muscle fiber. Thus, the cessation of miniature junctional activity was found to be correlated with glial cell engulfment of axon terminals and loss of synaptic vesicles in the nerve terminals.

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THE ELIMINATION OF POLYNEURONAL INNERVATION IN THE MOUSE GASTROCUNEMIUS MUSCLES

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Light(silver and ChE stains) and electron microscopic studies were performed on the end-plates of Gastrocunemius muscles in the neonatal mouse: 1) The period of elimination extended from 4 to 12 days old in lateral parts containing only fast muscle fibres, and from 5 to 20 days old in medial parts containing slow muscle fibres. 2) 89 end-plates in a lateral part of lateral head from 5 and 8 days old mice(75 and 13%polyneuronal innervation, respectively) were examined by electron microscopy. Specialized Schwann processes as have reported previously were observed to indent excess terminals in the normal appearance(11 end-plates). Drastic invaginations appeared the associated terminal's plasma membrane and abundant vacuoles laid near. 3) In 13 end-plates, terminals in the appearance of electron-lucent degeneration laid on basement membrane. 4) In 12 of 26 vesicle-filled retraction bulbs, an enhancement in the electron-density of cytoplasma or autophagies appeared but not in their axons. Of these, 5 examples were sequestered in Schwann cells of end-plates. These results suggest that the time-course of elimination,so far as hind leg muscles in the same position, may depend on the property of muscle fibre types or motor units, that specialized Schwann processes may play a role in a reduction in the size of excess terminals and that the elimination in over 15% end-plates may be involved in degeneration of terminal per se.

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THE APPLICATION OF TIMESCALING TECHNIQUES TO THE ANALYSIS OF TIMING OF SPONTANEOUS SYNAPTIC POTENTIAL OCCURRENCES.

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Statistical methods for testing the Poisson and non-Poisson hypothesis of the occurrences of spontaneous synaptic potentials were proposed and discussed. Notion of the Poisson hypothesis has been extended so as to allow the nonstationarity in the data. The timescaling technique has been introduced for the test of such an extended Poisson hypothesis. The application of the method to the occurrences of miniature end-plate potentials (m.e.p.p.s) observed at frog neuromuscular junctions suggested that the large deviations of various statistics from the Poisson expectations would be mainly due to nonstationarity of the data. The stochastic timescaling method has been introduced to test the non-Poisson process with aftereffect. Computer simulation confirmed the methodological validity. The method was applied to m.e.p.p. occurrence times under the application of Theophylline (1mM). The appearence of biphasic probability excesses, positive followed by negative, after the occurrence of m.e.p.p. were implied by the analysis. The possibility of the appearence of stochastic fluctuations of the probability of m.e.p.p. occurrences was also noted and discussed.

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AN ANALYSIS OF ACTION OF Ca^{++} AND Mg^{++} CONCENTRATION CHANGES ON SPONTANEOUS TRANSMITTER RELEASE.

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Effects of concentration changes of Ca^{++} and Mg^{++} in external solution on the frequency of miniature end-plate potentials (m.e.p.p.) were investigated at neuromuscular junction of the frog. Preparations were continuously superfused with flowing solution and tonicity, pH and temperature of the solutions were kept constant. In normal condition (K^+ 2.5mM), the relation between log Ca^{++} concentration — log m.e.p.p. frequency was sigmoid and apparent halfmaximum point was close to 0.7mM. When Mg^{++} (0.6 — 15mM) was added to the solution, no appreciable change in the frequency was observed in the normal concentration of Ca^{++}, but in lower concentration of Ca^{++} raised Mg^{++} acted to increase in the m.e.p.p. frequency. This effect of Mg^{++} was dose-dependent. Statistical analysis of the m.e.p.p. occurrence time sequence was adopted in these cases. In low Ca^{++} and high Mg^{++} solution, the intensity function showed some significant deviations from the range expected from the independent nature of the m.e.p.p. release. These results suggest that the effect of Mg^{++} in low Ca^{++} condition is, at least partly, caused by a release of Ca^{++} from intracellular storage sites.

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ACTION OF DIVALENT METALS IN TETANIC POTENTIATION OF MINIATURE END-PLATE POTENTIAL FREQUENCY AT FROG NEUROMUSCULAR JUNCTION. NARITA, K., KITA, H. AND YASUGI, E. Dept. of Physiol., Kawasaki Med. Sch., Kurashiki, Okayama 701-01

The effects of divalent metals on the time course of a tetanic rise in miniature end-plate potential (MEPP) frequency were studied by using the frog sciatic nerve-sartorius muscle preparation. Tetanic stimulation (50 Hz, 2-3 min) of the motor nerve increased the frequency of MEPPs in saline solutions containing a different divalent metal like Ca^{2+}, Sr^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+} or Ni^{2+}, of which Ca^{2+} and Sr^{2+} can support synchronous acetylcholine release. Following Barton et al.(1983), the ratio of Ca^{2+} bound to receptor, b, at intervals during the tetanus to the Ca^{2+} bound before the beginning of stimulation, b_0, was calculated from the MEPP frequencies. The increase in b/b_0 during the tetanus in solutions made with 1mM MgEGTA and without added Ca^{2+} followed a linear or convex time course to reach an asymptote. The results obtained in solutions containing Mg^{2+}(1-11 mM), Mn^{2+}(0.2-5), Co^{2+}(1-5) or Ni^{2+}(1-5) were similar to those in OCaMGEGTA solutions. In Ca^{2+}(0.05-0.3)- or Sr^{2+}(0.1-0.3)-containing solutions, b/b_0 rose in a concave or sigmoidal manner and an asymptote was reached during the tetanus. It is suggested that these divalent cations enter the terminal during stimulation and elevate the intracellular [Ca^{2+}] directly or indirectly.

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ACCELERATING ACTIONS ON EVOKED TRANSMITTER RELEASE OF TETRAETHYLAMMONIUM AND ITS ANALOGS. MASUO, K.* AND MAENO, T. Dept. of Surg.* and Dept. of Physiol., Shimane Med. Univ., Izumo, Shimane 693

Facilitatory presynaptic effects of tetraethylammonium (TEA) and its derivatives were investigated using the fractional release (P) as an index. P was calculated from the endplate potentials elicited with a train-of-three stimulation. Chemicals tested were TEA, triethylbutylammonium (C_4), triethylcholine (TEC), triethylmethylammonium (C_1), triethylphenylammonium ($\emptyset$), and triethylpropylammonium (C_3), $\emptyset$ was found to be about 2.5 times stronger than TEA in augmenting P, whereas effectiveness of C_1 was less than about 1/17 of that of TEA. The order of facilitatory presynaptic effectiveness determined in the present experiments was as follows:

$$\emptyset > \text{TEA} = C_3 > \text{TEC} = C_4 > C_1$$

Although Hill constant (n) of dose-release relation of TEA was 1.4-1.7 in neutral Ringer solution (pH=7.2), n is decreased to 1.0 in basic condition (pH=9.2). Rest of chemicals had n of 1.0-1.1 in both neutral and basic solutions. These suggested a presence of two distinct receptive sites (pH sensitive and insensitive sites) in the TEA action. The pH sensitive site has an affinity almost specific to TEA, and would probably locates at the outer surface of presynaptic terminal membrane.

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EXTRACT OF SEA URCHIN FROM FIJI INCREASED THE FREQUENCY OF MINIATURE ENDPLATE POTENTIALS. ANRAKU, M., KIHARA, H. and HASHIMURA, S. Dept. of Physiol., Fac. of Med., Kagoshima Univ., Kagoshima 890

The purification procedure of the crude extracts from the sea urchin collected at Fiji Islands were carried out, and the substance (echinotoxin, ETX) which increased MEPP frequency of frog was separated from the saponin. ETX increased the MEPP frequency instantly logarithmically relating to the ETX concentration. The increasing effect of ETX on the MEPP frequency was proportional to the external Ca, Mg and Na ion concentrations, but appeared even when the external divalent cations were depleted. ETX augmented the increase of MEPP frequency by hypertonicity even when the external calcium was replaced by magnesium. The increase of MEPP frequency by the depolarization in high K was augmented only when the external calcium existed. From these results, the increasing effect of ETX on the MEPP frequency was accounted for, at least in part, the increased permeability to the external cations.

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EFFECTS OF VARIOUS CHEMICAL MODIFYING REAGENTS ON THE ENDPLATE POTENTIALS IN FROG MUSCLE. TANABE, N., MARUHASHI, J., Daiichi College of Pharm. Sci. Fukuoka, Fukuoka 810

Maruhashi et.al. have suggested that the N-terminal amino acid concluding carboxyl groups (i.e. either glutamic acid or aspartic acid) of the molluscan acetylcholine and glicine receptors may be critical to the Na transport function of these receptors. In this experiment our objective was to decide whether the N-terminal is glutamic or aspartic acid residue. The endplate of M.sartorius of Rana catesbeiana was used. Various enzymes and chemical modifying reagents were tested for their effects on epp and conductance. Aspartase, leucineaminopeptidase and trinitrobenzene sulfonic acid caused a decreace in epp and conductance. However purified glutamic acid decarboxylase and pyridoxisal 5'-phosphate had no effect. From these results, we conclude that the N-terminal of the endplate protein is an aspratic acid residue, and that this residue plays an important role in ion transport.

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DISCHARGE MODULATION OF SPINDLE SECONDARIES IN EXTERNAL ANAL SPHINCTER DUE TO ANAL CANAL PRESSURE CHANGE. TAMURA, K. AND TAKAHIRA, H. Dept. of Physiol., Tokai Univ. School of Med., Isehara, 259-11

The anal canal in dogs and cats is surrounded by double muscle layers; internal and external sphincters. The former is the terminal portion of intestinal circular smooth muscles and the latter is said to be a tonically active, striate muscle. In previous experiments, it was found that the most definite and reproducible action in the anal canal was the 'recto-anal reflex'; an inhibition of internal sphincter that causes a fall of anal canal pressure, induced by rectal distension.

The aim of the present experiment is to find sacral afferent units, being active during the recto-anal reflex. The decerebrate cats were used. The twenty-six units, identified by electrical stimulation of pudendal nerve, revealed the following characteristics; (i) tonic discharges with rates of 30/sec, (ii) conduction velocities between 24-69 m/sec, (iii) discharge modulation by alteration of anal canal pressure or by manual deformation of anal canal. It is very likely that these units are the spindle secondaries embeded in the external sphincter, but signalling the event due to the internal sphincter activity.

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AMPLITUDE OF EXCITATORY POST-SYNAPTIC POTENTIALS DEPENDS ON FREQUENCY OF Ia DISCHARGES. MIZOTE,M. AND HOMMA,S., Dept. of Physiol.,Sch. of Med., Chiba Univ.,Chiba 280

EPSPs of motoneurone and Ia discharges which were elicited by muscle stretches to the gastrocnemius muscle were recorded simultaneously in immobilized cats by gallamine, after the cats were operated under anesthesia of Urethane and Chloralose.

Large amplitude of EPSPs and many Ia spikes are recorded at low frequency of vibration, and they are decreased as the frequency is increased. Most of Ia discharges are produced in two kinds of phase, early and late phase of vibration cycle, at low frequency of vibration, but in only early phase at high frequency.

Size of EPSPs corresponds to the total number of Ia spikes during each vibration cycle.

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REFLEX ACTIONS ON MOTONEURONES INNERVATING FOOT MUSCLES IN THE CAT. HONGO, T., *JOHANNISSON, T., MATSUDA, J. AND YOSHIDA, K. Dept. of Physiol., Inst. of Basic Med. Sci., Univ. of Tsukuba, Ibaraki 305 and *Dept. of Physiol., Univ. of Göteborg, Göteborg

Reflex actions of group Ia muscle afferents on motoneurones identified as innervating the following foot muscles were investigated electrophysiologically; M. flexor digitorum brevis (FDB), Mm. lumbricales superficiales (LS), Mm. interossei (IO) and muscles of the Vth digit (abd. brev. and add. long., DQ). Afterhyperpolarizations of these motoneurones were generally long-lasting (167±28 msec), comparable to those of soleus and much longer than those of gastrocnemius motoneurones previously reported. The amplitude of monosynaptic Ia EPSPs evoked by the maximal Ia volley of each dissected muscle nerve (maximal Ia EPSP) was measured in individual motoneurones. The aggregates of the maximal Ia EPSPs evoked from various hindlimb muscle nerves in motoneurones of the foot muscles were generally larger than in motoneurones of the shank and thigh muscles, and the largest of the mean aggregates mounted to 20 mV (LS). Foot muscles provided more than 90% of the aggregate Ia EPSPs. Among the Ia inputs from the different foot muscle nerves the homonymous one always predominated. Each species of motoneurones exhibited its own specific pattern of convergence of Ia input. It was further indicated that FDB motoneurones with axons in the lateral and the medial plantar nerve had different convergence patterns.

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RECOVERY OF LOCOMOTION IN CATS WITH BILATERALSERIAL HEMISECTION OF THE SPINAL CORD.
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When the spinal cord of the cat was bilaterally hemisected at different levels of thoracic and cervical cord, locomotion recovers after a period of flaccid and spastic palsy. In the present experiments, we have investigated changing patterns of spinal reflexes and spontaneous movements of the hindlimbs in the chronic cats. Experiments were performed on the following four groups; 1)cats with unilateral hemisection at around T_{13}, 2) cats with serial hemisection at around T_{13} followed by contralateral mid-thoracic hemisection at intervals of 37-126 days, 3) cats with unilateral hemisection at C_2 and 4) cats with serial hemisection, first at C_2 followed by contralateral mid-thoracic hemisection at intervals of 21-73 days. EMGs were picked up from bilateral vast.lat., sartorius and/or biceps femoris muscles by intramuscular wire electrodes when tendon tap or cutaneous pinching was applied. After the first hemisection no pathological pattern in spinal reflexes was observed. However, after the second hemisection, crossed extensor reflex was easily elicited by pinching skin of the toe, spontaneous alternating movements developed, and several days later the cat could stand up with the four laegs. The ipsilateral hindlimb to the first hemisection showed earlier changes than the other side, suggesting some plastic changes develop in the cord.

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SPINAL SEGMENTAL LEVELS OF PINNA AFFERENTS RESPONSIBLE FOR SCRATCHING REFLEX IN THE CAT.
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We previously reported that electrical (50Hz, 5-10V) as well as tactile stimulation applied to pinna elicited scratching movement of approximately 4Hz in the ipsilateral hindlimb in C1 spinalized cat. In the present study, we examined the segmental levels of pinna afferents from the receptive areas effective in initiating the scratch reflex by utilizing the HRP method. 30-40% HRP solution (5-20 mg) was injected into the receptive areas confined to the edge of the scapha and bursella. Labeled cells were found in C1-C3 dorsal root ganglia in most cases (21/26) and in several exceptional cases also in C4 ganglion. Labeled cells were most numerous in number in C2-C3 ganglia and much fewer in C1 ganglion. The diameters of the labeled cells were about 45-54 μm in long axis and 30-38 μm in short axis. For comparison, when HRP was injected into concha, stimulation of which elicited no scratching rhythm, labeled cells were very scarce in cervical dorsal root ganglia but numerous in cranial nerve (VII,X) ganglia.

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MODULATORY ACTION OF LOCAL NON-SPIKING INTERNEURONS ON THE DESCENDING EQUILIBRIUM REFLEX PATHWAY IN CRAYFISH.
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The equilibrium reflex of uropods is released by body roll stimulus provided that the animal is performing abdominal posture movement when the stimulus is given. During the abdominal movement, uropod motoneurons increase their excitability showing the sustained depolarization(<5-10mV). The motoneuron EPSPs evoked by descending statocyst interneurons are usually subthreshold (<5mV). During the abdominal movement, however, statocyst interneurons can evoke motoneuron spikes by summation of the subthreshold EPSPs with the sustained depolarization. We concluded that this sustained depolarization is produced by local non-spiking interneurons (LNSNs) from the following results. (1) In 14 LNSNs, sustained membrane potential change was observed during the abdominal movement. (2) Artificial membrane potential change of these LNSNs by current injection in the same direction with that during the abdominal movement increased the motoneuron activity as in actual abdominal movement. (3) Artificial cancellation of LNSNs' membrane potential change during the abdominal movement also canceled the increase in motoneuron activity.

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GROUP Ia AND Ib DISCHARGES IN RELATION TO THE LONG-LASTING HYPERPOLARIZATION OF SOLEUS ALPHA-MOTONERUON IN THE ACUTE DECEREBRATE, STANDING CAT. SAKAMOTO, T., OHTA, Y., MORI, S. AND ATSUTA, Y*. Dept. of Physiol., Dept. of Orthoped.*, Asahikawa Med. Coll., Asahikawa 078-11

In the precollicular-postmammillary decerebrate, standing cat, stimulation of the dorsal part of the mid-pontine tegmental field (DTF) terminates spontaneous discharges of soleus alpha-motoneurons (MNs), and also elicits membrane hyperpolarization which outlasts DTF stimulation. The stimuli consisted of rectangular (10 to 40 uA) pulses of 0.2 ms duration that lasted 5-10 s. By a series of DTF stimulation delivered with inter-stimulus interval of 30 to 40 s, bilateral hindlimb forces decreased in a staircase manner from about 0.5 kg to 0.1 kg. Suppression of hindlimb force was closely related with that of motoneuronal activity. Twenty group Ia (GIa) and 9 group Ib (GIb) afferents innervating soleus muscle were intraaxonally identified by intramuscular stimulation, maintaining dorsal and ventral roots intact. All these muscle afferents discharged tonically during standing of the animal, but decreased their firing frequency from about 50 to 2 spikes/s gradedly with the graded decrease in the hindlimb force. The results of the present study has demonstrated that long-lasting membrane hyperpolarization of soleus MNs is not due to the increase in the inhibitory inputs by way of GIb fibers, but is partly due to the decrease in the excitatory inputs by way of GIa fibers upon them.

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THE CELLS OF ORIGIN PROJECTING THEIR AXONS TO THE DORSAL PART OF THE CENTRAL TEGMENTAL FIELD IN THE PONS. OHTA, Y., SAKAMOTO, T. AND MORI, S. Dept. of Physiol., Asahikawa Med. Coll., Asahikawa 078-11

Stimulation of the dorsal part of the central tegmental field (DTF) in the pons (P3 to P7, LRO, H-4.5 to H-6) decreases the tone of the hindlimb extensor muscles in acute precollicular-postmammillary decerebrate cats. The effective DTF area corresponds to the caudal portion of the nucleus centralis superior, where there are rich crossing fibers but few cell bodies. Using the retrograde tracer technique with HRP, attemps were made to determine the cells of origin projecting their axons to the DTF area. To determine DTF area and to inject HRP to this area, a double-barreled microelectrode was employed. It was made of a metal-filled stimulating and of a HRP-filled injecting micropipette. HRP was injected to the DTF area in two groups of preparations, one without (group A) and the other with microlesion (group B) within the DTF area. In the group A preparations, only a few neurons were labeled in the dorsolateral pontine tegmental field. In the group B preparations, labeled neurons were observed in the locus coeruleus complex (LC, LCα, LSC), cuneiform nucleus and laterodorsal tegmental nucleus. In both groups of preparations, many neurons in the pontomedullary reticular formation were also labeled. These results suggest that there are neurons in the LC complex which send their axons to the DTF area.

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PATTERN GENERATION IN THE BRAIN STEM UNDERLYING THE FORELIMB STEPPING OF THALAMIC CATS. SHIMAMURA, M., KOGURE, I.* AND FUWA, T. Dept. Neurophysiol., Tokyo Metropol. Inst. for Neurosci., Fuchu, Tokyo 183

We recorded the neuronal activities from, and stimulated, various regions in the brain stem of thalamic cats. Neuronal activities were recorded during stepping on a moving treadmill, and were averaged by using the flexor EMG as a trigger. In wide regions of the brain stem, an averaged response was obtained with similar latency as a ipsilateral flexor EMG, however, in paralemniscal pontine reticular formation (PLRF) the averaged response preceded the flexor EMGs. When repetitive electrical stimulation was applied to the PLRF on one side, coordinated movements of bilateral forelimbs were elicited: The ipsilateral forelimb was flexed and the contralateral forelimb was extended. When train pulse stimulation was alternately applied to the PLRF on bilateral sides, alternating forelimb movement as in stepping was evoked, depending on each stimulation. A small unilateral lesion in the PLRF reduced forelimb stepping on a motor-driven treadmill, particularly the flexor muscle contraction in the forelimb ipsilateral to the lesion. Bilateral PLRF lesions, abolished forelimb stepping on both side. These results suggest that PLRF exerts a patterned control of the bilateral forelimb muscles and play a role in initiating and maintaing forelimb stepping.

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JAW REFLEX RESPONSES ELICITED BY MECHANICAL STIMULATION IN MAN. YAMADA, Y. Dept. of Physiol., Nagasaki Univ. Sch. of Dent., Nagasaki, 852

Jaw opening and closing reflex respnses were studied in six healthy subjects electromyographically during weakly clenching on a rubber stamp placed between upper and lower incisors and during active opening effort. A short latency (mean 9.5 ms) opening (digastric) response which may have a disynaptic pathway was elicited by weakly tapping on all four central incisors during clenching. This response was accompanied by a jaw closing (masseter) response (jaw jerk reflex). A closing response was elicited in the masseter muscle by tapping on the upper incisor during active opening. This closing reflex was characterized with the longer latency of 12 ms than that of the jaw jerk reflex (mean 8.5 ms), and was associated with depressed activity in the jaw opening muscle. Furthermore, the long latency closing response was found only in ipsilateral muscle to the stimulated tooth, although the jaw jerk reflex was observed bilaterally.

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SPINAL ASCENDING PATHWAYS CONCERNING CUTANEOUS STIMULATION EFFECTS ON JAW OPENING REFLEX. NISHIGORI, A., YASUMO, W., KAWAMOTO, M. and YAMAGUCHI, Y., Laboratory of Clinical Physiology, College of Biomedical Technology, Osaka University, Osaka

Jaw opening reflexes (JOR) can be modified by preceding cutaneous stimulation (CS): a short period of JOR facilitation appears immediately after CS and then inhibition follows. In the present study, ascending spinal pathways involved in these effects have been examined using rats anesthetized with pentobarbital sodium. JORs were evoked by electrical stimulation of the dental pulp (mainly nociceptive) and that of the infraorbital nerve (more non-nociceptive), and the reflex EMG's were recorded from the digastric muscle. Single pulse electrical stimulation via needles inserted in the skin of various parts was used as CS. Both facilitatory and inhibitory effect of CS, given to the sciatic nerve or its innervated areas, on the pulp induced JOR was entirely suppressed by dorsal column section at the 12th thoracal segment. Some inhibitory effects reappeared when intensity of CS was augmented. On the other hand, the effects of CS on infraorbital JOR remained to some extent even after the section. These results suggest that the dorsal column is concerned more in affecting the nociceptive components of JOR and other pathways including the anterolateral tract participate more in influencing the non-nociceptive components.

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SYNAPTIC MECHANISM OF GLOSSOPHARYNGEAL-HYPOGLOSSAL REFLEX IN THE TOAD. MATSUSHIMA,T., SATOU,M. & UEDA,K. Fac. of Sci., Univ. of Tokyo, Hongo, Tokyo 113

Intracellular potentials were recorded from primary afferent fibers in the solitary tract (ST fibers) and from hypoglossal motoneurons innervating tongue muscles (protractor and retractor motoneurons: PMNs and RMNs), following electrical stimulation applied to lingual branch of glossopharyngeal nerve on either side (ipsilateral or contralateral nerve: iIX or cIX) in the Japanese toads. (1) Action potentials of ST fibers in response to the iIX stimulation had peak latencies with a bimodal distribution (3.6 and 4.7ms respectively; range:2.8-9.1ms; n=81). (2) The iIX stimulation evoked EPSPs in both PMNs and RMNs. The mean onset latencies of these EPSPs in PMNs and RMNs were 9.4ms (± 2.7ms; range:4.5-25.0ms; n=102) and 6.0ms (± 1.5ms; range:4.2-10.4ms; n=43) respectively. (3) Following the cIX stimulation smaller EPSPs with longer latencies were evoked in both PMNs and RMNs. The mean onset latencies of these EPSPs in PMNs and RMNs were 10.4ms (± 2.4ms; n=46) and 11.4ms (± 3.7ms; n=20) respectively. Facilitation of these EPSPs were often observed when repetitive stimuli were applied at short intervals (10ms).

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BULBAR NEURONAL MECHANISMS OF SWALLOWING IN THE RABBIT. Sakai, Y.[1] and Takazawa, A.*[2]
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Systematic exploration of bulbar swallowing-related units(SWUs) were performed in vagotomized, paralyzed and artificially respirated rabbits which were locally anesthetized. The first branch of the superior laryngeal nerve was stimulated for evoking the pharyngeal phase of swallowing, which was identified by the relevant activities of both phrenic and pharyngeal nerves. (1) Of 452 units, 35 pharyngeal and 3 esophageal SWUs were recognized because of their high-frequency burst discharge. Many of the former were found either in the ambiguous, retrofacial and hypoglossal nuclei or in these vicinity, while 7 cells were in the solitary tract nucleus and the medial reticular formation. The esophageal ones were in the retrofacial region. Of 85 respiration-related units, 72 were "true" respiratory, because their firing was markedly inhibited. During the pharyngeal phase, there seemed to occur a short, small inspiratory activity followed by a suppressed expiratory activity. These findings may delineate a neuronal organization within the swallowing center having a coordinating mechanism with the respiratory center. (2) Intravenous dose(0.5mg/kg) of thiopental shortened both the activity of all SWUs tested and the pharyngeal phase, as if the drug blocked a tonic descending influence on all portions of the swallowing center. This effect has been already reported to be abolished by a midbrain transection.

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SIMILARITIES AND DIFFERENCES BETWEEN THE COMMON TRIGEMINAL SYSTEM AND INFRARED SENSORY SYSTEM OF CROTALINE SNAKES IN TRANSGANGLIONIC HRP TRANSPORT AND HISTOCHEMISTRY. TERASHIMA, S. Dept. of Physiol., Ryukyu Univ. Sch. of Med., Nishihara-cho, Okinawa 903-01

Crotaline snakes possess a lateral descending nucleus (DLV) in the trigeminal infrared sensory system. The similarities and differences between the DLV and the caudal nucleus of the common descending nucleus (DVc) in Agkistrodon blomhoffi were noted as follows. Similarities: a) the nuclear structure of each was organized into a marginal zone for fine fiber input, and a nucleus proper for coarse fiber input; b) both had topical organization with large overlapping, although no overlapping was found in the periphery; c) no interaction was seen between the two (in HRP transport); and d) both received input of fine fibers from the vagus (in HRP transport). Differences: a) in the DLV there were terminals 3 times larger than those in the DVc; b) in the DVc there were serotonin-containing fibers, but not in the DLV; and c) there was higher activity in the DLV than in the DVc (in [^{14}C]deoxyglucose uptake and succinate dehydrogenase distribution; the same activity is also noted in the nucleus reticularis caloris, which relays afferent input from the DLV).

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ALTERATION OF JAW MOVEMENTS AFTER SECTION OF TRIGEMINAL SENSORY NERVES. INOUE, T., NAKAMURA, T*, AND MORIMOTO, T. Dept. Oral Physiol. & Dept. Dent. Prosth., Faculty of Dent., Osaka Univ., Osaka 565.

To understand a role of oral sensation in the control of jaw movements, the change in the jaw movement patern following section of the bilateral maxillary and inferior alveolar nerves was analysed in the rabbit. The jaw movements were recorded using the laser-position detector simultaneously with emgs of masticatory muscles. The results are summarized as follows: 1) the number of chewing strokes increased significantly after denervation, 2) magnitude of vertical and lateral jaw movements decreased and the movement traces became complicate in comparison with those of the intact animals, 3) the chewing rhythm increased a few days after denervation but slightly decreased 1 week later, and 4) in spite of losses of a periodontal sensation, the chewing pattern differed among foods with different consistency. It is concluded that an oral sensation, particularly a periodontal sensation, contributes greatly to the control of jaw movements during mastication.

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NEURONS RELAYING CORTICAL INPUT TO MASTICATORY RHYTHM GENERATOR IN GUINEA PIG. IRIKI, A., NOZAKI, S. AND NAKAMURA, Y. Dept. Physiol., Fac. Dent., Tokyo Med. Dent. Univ., Tokyo 113.

It was reported that the masticatory rhythm generator (MRG) was located in the bilateral lower brain stem reticular formation and activated by cortical inputs via pyramidal tract (PT) to produce rhythmical jaw movements (RJM). We studied the localization of the neurons relaying cortical inputs to MRG as well as cortical neurons directly projecting to them, with the following results: 1) Microstimulation (MS) of the cortical masticatory area (CMA), where MS (100 μA, 0.3 ms, 30 Hz) produced RJM, evoked a negative field potential (latency 2.5 ms) in the dorsal portion of the contralateral nucleus reticularis paragigantocellularis (dPGCl). 2) MS (70 μA, 0.1 ms, 30 Hz) of dPGCl evoked RJM similar to that by MS of CMA. 3) Unilateral dPGCl lesion abolished RJM induced by MS of the contralateral CMA, leaving that by MS of ipsilateral CMA unaltered. 4) MS of dPGCl evoked a surface-positive depth-negative antidromic field potential in CMA via PT axons conducting at 10 m/s. The negative potential was largest in lamina V of CMA, being larger contra- than ipsilaterally. 5) HRP injection to dPGCl labeled pyramidal cells 3-4 times as many in the contra- than ipsilateral cortex with most of the population in lamina V of CMA. 6) During CMA-evoked RJM, rhythmically firing cells in the nucleus reticularis gigantocellularis were found to be driven by dPGCl cells. We conclude that a population of cells in dPGCl relays direct inputs via PT cells of the contralateral CMA to MRG.

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CHARACTERISTICS OF SNEEZES EVOKED BY MICROSTIMULATION TO THE MEDULLA OBLONGATA IN THE MESENCEPHALIC CAT. NONAKA, S., OHTA, Y.*, MORI, S.* AND UNNO, T. Dept. of Otolaryngol., Dept. of Physiol.*, Asahikawa Med. Coll., Asahikawa 078-11

It was tested whether or not microstimulation to the brain stem evokes sneezes in the decerebrate cats. Subglottic pressure was recorded by a pressure transducer introduced into the subglottis through the mouth, along with EMGs of intrinsic laryngeal muscles (thyreoarytenoid : TA, posterior cricoarytenoid : PCA) and respiratory muscles (diaphragma : DIA, rectus abdominis : RA). For stimulating the brain stem, glass microelectrode filled with Woods metal was employed. The stimuli consisted of 20-50 uA rectangular pulses of 0.2 ms duration with 10 pulses/s that lasted 5-10 s. The stimulation to the lateral medulla oblongata elicited a series of sneezes characterized by an abrupt increase in the subglottic pressure (pressure height 70-100 cmH_2O, duration 50 ms) which was followed by forceful, active expiration. Just before the onset of abrupt increase in the subglottic pressure (10-30 ms), the activity of diaphragma was terminated, and RA muscle was reciprocally activated. Just before the peak subglottic pressure (20-40 ms), the activity of TA muscle was terminated, and with this PCA muscle was reciprocally activated. EMGs recorded during stimulus-induced sneezes were similar to those recorded during sneezes without microstimulation. Effective stimulus areas were located along the spinal tract of the trigeminal nerve and along the nucleus of the spinal trigeminal tract.

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SOME PHYSIOLOGICAL PROPERTIES OF AMBIGOUS MOTONEURONS IN THE RAT. YAJIMA, Y. AND HAYASHI, Y. Dept. of Physiol. Hyogo College of Med., Nishinomiya, Hyogo 663

Axonal conduction velocity (c.v.) of antidromically identified motoneurons in the nucleus ambibuus (NA) was determined by the two point stimulation of the recurrent laryngeal nerve (RLN). The c.v. histogram has bimodal distribution with the peaks at 25 and 45 m/sec, respectively (mean, 51.2 ±31 m/sec), in accordance with the distribution of antidromic response latencies of NA motoneurons to RLN stimulation. Spontaneous activities were classified into three types: rhythmic burst discharge with the mean frequency of 68.7 ±23 Hz (46 neurons; 58%), continous maintained activity with the mean rate of 22.7 ±17 Hz (18; 23%) and sporadic discharge of 2.3 ±1.3 Hz (15; 19%). Not a few NA burst neurons fired synchronously with artificial ventilation (2Hz). For some NA neurons both RLN and the superior laryngeal nerve stimulation induced antidromic spikes, suggesting that respective NA neurons have axonal ramification and supply both laryngeal nerves. Conditioning stimulation of RLN caused suppression of antidromic invasion for more than 10 msec, which is likely to be attributed to the recurrent collateral inhibition.

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ON THE TECTO-FACIAL REFLEX PATHWAY IN THE CAT. TANAKA, T., ASAHARA, T. AND NISHIMURA, Y. Dept. Physiol., Sch. Med., Mie Univ., Tsu, Mie-ken, 514

Synaptic potentials in the facial motoneurons innervating the posterior auricular muscle were recorded on stimulation of the contralateral paralemniscal zone, and both the ipsi- and contralateral superior colliculi. EPSPs with latencies of 1.0-1.3 msec (1.2±0.1 msec, N=58) and IPSPs with latencies of 2.0-5.0 msec(3.1±1.0 msec, N=17) were elicited in the facial neurons by stimulation of the contralateral paralemniscal zone. Single volleys in the contralateral superior colliculus evoked EPSPs with latencies of 1.3-2.5 msec(1.8±0.3 msec, N=67) and IPSPs with latencies of 3.0-5.0 msec(3.7±0.7 msec, N=33). Stimulation of the ipsilateral superior colliculus also produced both EPSPs and IPSPs with similar ranges of the latencies to those induced by stimulation of the contralateral superior colliculus in the facial motoneurons. These findings show the existence of the disynaptic excitatory projections from the superior colliculus bilaterally to the facial motoneurons via the contralateral paralemniscal zone. Further they indicate that there are inhibitory polysynaptic pathways in the link between the tectum and facial motoneurons. Experiments also show that, in comparision with facial motoneurons eliciting EPSPs to the tectal stimulation, the neurons responding with IPSPs have the higher input resistance, that they need weaker stimulating currents to reach the threshold for firing.

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POSSIBLE NEURAL CIRCUIT MEDIATING EXPERIMENTAL TREMOR IN MONKEYS. OHYE, C., SHIBAZAKI, T., HIRAI, T., WADA, H., HIRATO, M. AND KAWASHIMA,Y. Dept of Neurosurg., Gunma Univ. Sch. of Med., Maebashi, Gunma 371.

Since about 10 years, we have been studying on the neural mechanism of the experimentally produced, spontaneous and persistent tremor in monkeys with a mesencephalic ventromedial tegmental lesion. Recording from the spinal cord ipsilateral to the peripheral tremor in awake monkeys revealed that the rhythmic grouped discharge of descending nature time-locked to the tremor was found in the area of the pontine reticulospinal tract. Based on the other series of experimental study, the spinothalamic tract contralateral to the tremor, projecting to the thalamic VPL_O-VPL_C area (equivalant of the human Vim nucleus) is considered to be the possible ascending limb mediating the tremor. As Vim projects to cortical 3a area, connection between 3a and pontine reticular formation (RF) may complete the neural loop mediating the tremor. In fact, electrical stimulation of 3a area induced response in the contralateral pontine RF which may project to the spinal cord. It is our hypothesis that spinothalamic tract-Vim-3a-contralateral pontine RF-reticulospinal tract consists tremor mediating neural circuit.

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MORPHOPHYSIOLOGICAL STUDY ON MONOSYNAPTIC CONNEXIONS BETWEEN RETICULOSPINAL NEURONES AND DORSAL NECK MOTONEURONES IN THE CAT. IWAMOTO,Y., SASAKI,S. AND SUZUKI,I. Dept. of Neurophysiology, Institute of Brain Research, School of Medicine, University of Tokyo.

Dorsal neck motoneurones received disynaptic EPSPs from pyramidal and tectoreticular fibres. These EPSPs were found to be mediated by reticulospinal neurones(RSNs). A large portion of the tectal disynaptic EPSPs was suggested to be mediated by RSNs which were located in the nucleus reticularis pontis caudalis and sent their descending collaterals to the brachial segment through the MLF but not to the lumbar cord(C cells). Physiologically, monosynaptic connexions between C cells and neck motoneurones were demonstrated by using spike-triggered average of unitary postsynaptic population potentials electrotonically from C2 ventral root and of unitary EPSPs recorded intracellularly from dorsal neck motoneurones. Morphologically, intraaxonal staining of C cells with HRP revealed that main collaterals emerged from stem axons in the ventral funiculus entered the ventral horn from its venromedial border to terminate in laminae IX(motor nuclei) and VIII of Rexed. Terminals of single collaterals were distributed in a fairly restricted area extending about 200um rostrocaudally, 300um dorsoventrally, and 400um mediolaterally. Terminals were found to be in contact with somata and/or proximal dendrites of large motoneurone-like cells.

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FIRING CHARACTERISTICS OF SECONDARY VESTIBULAR NEURONS AND ABDUCENS MOTONEURONS IN THE ALERT CAT. YOSHIDA, K., BERTHOZ, A., VIDAL, P. P. AND DROULEZ, J. Dept. of Physiol., Inst. of Basic Med. Sci., University of Tsukuba, Ibaraki and Lab. de Physiol. Neurosensorielle du C.N.R.S., Paris.

Single unit activities of identified second order vestibular neurons were quantitatively analyzed in relation to eye movements and compared with those of identified abducens motoneurons. Based on the rate-position relationships during fixations, the mean firing rate at central gaze position (Fo) and sensitivity to eye position (k) for motoneurons were found to be 33 spikes/sec and 4.4 spikes/sec per degree respectively. The mean sensitivity to saccadic eye velocity (r) was 0.8 spikes/sec per degree/sec. The mean values of Fo and k for vestibular neurons related to horizontal eye movements were 51 spikes/sec and 2.4 spikes/sec per degree. The velocity sensitivities r for saccades in on and off directions were often quite different and were, on the average, 0.34 and 0.46 spikes/sec per degree/sec. Several vestibular neurons related to vertical eye movements had a high position sensitivity with a mean of 4.2 spikes/sec per degree, and negligible sensitivity to saccadic velocity in any direction. Vertical neurons having characteristics similar to those of horizontal neurons were also observed.

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DIRECT SYNAPTIC INPUTS FROM THE CAMPUS PRERUBRALIS AND NEIGHBORING AREAS TO SUPERIOR AND INFERIOR OBLIQUE MOTONEURONS IN THE CAT OCULOMOTOR NUCLEUS. NAKAO, S. AND SHIRAISHI, Y. Dept. of Physiol., Tottori Univ. Sch. of Med., Yonago 683

The nature of synaptic inputs from the campus prerubralis (CPR) and neighboring areas to superior oblique motoneurons (SO Mns) and inferior oblique motoneurons (IO Mns) in the oculomotor nucleus was investigated in anesthetized cats, using intracellular recording techniques. Electrical stimulation of the CPR induced EPSPs in nearly all the IO Mns on both sides with latencies ranging from 0.6 to 1.0 msec, suggesting their monosynaptic nature. Systematic tracking with the stimulating microelectrode around the CPR and monitoring the extracellular field potential also suggested the presence of monosynaptic excitatory connections of the interstitial nucleus of Cajal (INC) and posterior commissure with IO Mns. In SO Mns on each side, CPR stimulation produced usually EPSP-IPSPs or EPSPs. Latencies of the EPSPs and EPSP components of the EPSP-IPSPs ranged from 0.5 to 1.0 msec. One exceptional SO Mn showed a pure IPSP with a monosynaptic latency of 0.8 msec. The above postsynaptic potentials in SO Mns were elicited by INC stimulation as well.

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REVERSIBLE SUPPRESSION OF SACCADIC EYE MOVEMENTS BY LOCAL INJECTION OF MUSCIMOL AND XYLOCAINE IN THE MONKEY SUPERIOR COLLICULUS. HIKOSAKA, O. AND WURTZ, R.H. Dept. of Physiol., Toho Univ. Sch. Med., Ota-ku, Tokyo 143 and Lab. of Sensorimotor Research, National Eye Institute, N.I.H., Bethesda, MD. 20205, U.S.A.*

To reevaluate the oculomotor function of the superior colliculus (SC) we tested the effects of local and reversible inactivation of the monkey SC by injecting a small amount of muscimol (GABA agonist) or Xylocaine (local anesthetic). Two rhesus monkeys were trained to fixate a spot of light on the screen and make a saccadic eye movement if the spot jumped to another location. A glass pipette for drug injection was introduced into the intermediate layer of the SC through a stainless steel guide tube. The pipette contained a fine tungsten microelectrode which was used for recording of single cell activities and microstimulation. Following a muscimol injection contralateral saccades with a certain range of vector (direction and amplitude) became delayed, slowed and shortened. These saccades corresponded to those elicited by microstimulation and those preceded by a burst of cell spikes recorded at the injection site. One monkey became unable to make saccades with such particular vectors; even at this stage ipsilateral saccades were completely normal. The effect of Xylocaine injection was qualitatively similar but less pronounced and short-lasting. These results indicate that the SC must be intact for the initiation of normal saccades.

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THE PROPERTIES OF THE POTENTIAL CHANGES IN THE EYELID ELICITED BY FLASH STIMULATION IN MAN, ESPECIALLY RELATED TO THE INTENSITY OF FLASH STIMULI.

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The averaged photically evoked lid potential changes(P.C.) were studied polygraphically from the standpoint of the stimulus strength in healthy subjects and patients. In the P.C. obtained by random flash stimulation to a single eye of healthy adults, the EMG and EOG components in the occluded side were observed and increased in amplitude according to the intensity strengthened, although the ERG components disappeared. During natural sleep, the EMG and EOG components disappeared in the stage 2. The ERG components remained and were increased in amplitude according to the intensity strengthened. In a patient with an artificial eyeball, the EMG components in the healthy and extirpated side were increased in amplitude according to the intensity strengthened respectively. The ERG and EOG components disappeared in the extirpated side. In the affected side of patients with peripheral facial paralysis, the EMG and EOG components were decreased or disappeared, although the ERG components were observed. Strengthening the stimulus intensity, decreased EMG components were increased and shortened in amplitude and latency respectively. The above results suggest that the EMG and EOG components of the P.C. may be modified by the activities of brain stem indicated by the EEG and lid MV responses.

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PROPERTIES OF SLOW COMPONENT OF PHOTICALLY EVOKED LID POTENTIAL CHANGES ESPECIALLY RELATED TO STIMULUS FREQUENCY.

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In order to clarify a relationship between the slow component of the photically evoked lid potential changes and the late response of the visually evoked responses(VER), the properties of the evoked lid potential changes were studied from the standpoint of the stimulus frequency in rabbits. The average summated responses of the photically evoked potential changes, EEG, EOG, ERG and MV responses obtained by rhythmic flash stimulation ranging from 1 to 20 Hz were studied polygraphically under various conditions, such as adaptation, anesthesia, occulsion of a single eye and extirpation of unilateral eyeball. The slow component of the photically evoked lid potential changes was gradually decreased according to stimulus frequency increased and disappeared in higher frequencies than 8 Hz. In various conditions, this slow component was found out to appear in corresponding very well to the late response of the VER. Under the extirpation of the unilateral eyeball, in addition, the slow component and the late response of the VER in the extirpated side were observed dominantly in amplitude than those obtained in the contralateral side. From the above results, it was concluded that there was a clear correlation between the slow component of the photically evoked lid potential changes and the late response of the VER.

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RESPONSES OF CEREBELLAR PURKINJE CELLS TO GUSTATORY STIMULI IN THE FROG. HANAMORI, T., NAKASHIMA, M., NAKACHI, T. AND ISHIKO, N. Dept. of Physiol., Miyazaki Med. Coll., Miyazaki

Single Purkinje cell activities during taste stimulation of the tongue were studied using glass microelectrodes filled with sodium acetate into which pontamine sky blue had been dissolved. To express changes in the Purkinje cell activity, the peristimulus time histogram being constructed from the complex and simple spike discharges before, during and after repetitive applications of a given taste solution was used. Responses of 42 Purkinje cells to NaCl showed that the complex spike discharges were facilitated and inhibited in 10 and 3 cells, respectively, the remaining 29 had no detectable change. The simple spike discharges, on the other hand, were facilitated in 17 cells, inhibited in 5, and not affected in the remaining 20. Fourteen of 17 Purkinje cell responses to quinine failed to show any detectable change in the spike activity. Of the remaining 3, one responded with an increase in the simple spike discharges, one showed facilitatory response and the last one an inhibitory response in the complex spike discharges.

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INTRADENDRITIC RECORDING FROM CEREBELLAR PURKINJE CELLS IN VIVO AND PROBABLE SITE OF GENERATION OF DENDRITIC SPIKES. TAKAHIRA, H. Dept. of Physiology, Tokai Univ. Sch. of Med. Isehara, 259-11

It is now apparent by slice experiment and by in vivo experiment on mammalian cerebellar cortex that the slow spikes could be recorded intracellularly from dendrites of Purkinje cells, however, the actual generation site of those spikes has not been definitely explained. Llinas & Nicholson (1971) proposed that the dendritic slow spikes are due to summation of small spikes generated at multiple sites in branching trees. On the other hand, several authors noted the identical patterns of repetitive slow spikes, even if they were recorded by unexpected puncture anywhere in the molecular layer. The present author's results are in agreement with the latter observations. Further, the interaction experiments between two, evoked 'tripple spike complexes' indicate that those triplets are due to repetitive firing of single site by single generator action. In consideration of tremedously arborizing structure such as Purkinje-dendrite, the single generation site could be only located in the basal stem of primary branch. The other parts of arborization could be regarded as passive, unleaky cable, thus, one can observe the identical pattern of response, if the microelectrode penetrated into any branch in the molecular layer of cerebellar cortex.

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AFFERENT PROJECTIONS TO THE CEREBELLAR NODULUS FROM THE CARDIOVASCULAR AFFERENTS IN RABBITS. NISIMARU, N.,*ARAMAKI, H. AND*WATANABE, Y. Dept. of Physiol., Medical College of Oita Oita 879-56

The sympathetic renal nerve activity is inhibited by the Purkinje cells in the A zone of cerebellar anterior and posterior lobules I, II, III, VII and VIII (Nisimaru, N. and Yamamoto, M., 1977, Nisimaru, N. and Shimoyama, I., 1979). In the present study, the projections to the cerebellar cortex from the cardiovascular afferents were studied in α-chloralose-urethane anesthetized rabbits. The cervical vagal and aortic nerves were electrically stimulated. The responses recorded from the cerebellar surface were found in the lateral region of nodulus in a contralateral sagittal strip perpendicular to the longitudinal axis of the folia. The latency of the responses evoked by electrical stimulation of the cervical vagal and aortic nerves was 5-10 ms. Profiles of these potentials recorded from the cerebellar nodulus indicate that they are transmitted through both the climbing and the mossy fiber systems. The electrical stimulation of these regions of nodulus produces a marked depression of sympathetic nerve activity to the contralateral kidney.

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CLIMBING FIBER PROJECTIONS FROM THE LIMB CUTANEOUS AFFERENTS TO THE CEREBELLAR PARAMEDIAN LOBULE. INUI,N. and DEURA,S. Dept. of Anat., Gifu Univ., Sch. of Med., Tsukasa-machi, Gifu 500.

Climbing fiber projections to the cerebellar paramedian lobule were investigated electrophysiologically by means of the bilateral fore- and hindlimb cutaneous nerve stimulations in the cats anesthetized with pentobarbitone (30mg/kg,i.p.), and the data were compared with the results obtained in the lobule Ⅳ and V of the anterior lobe.

The sites in which responses to four limb nerve stimulations were evoked ("four limbs area") were located up to 4-5 mm laterally from the midline in the rostral and intermediate folia of the paramedian lobule and lobule V of the anterior lobe. High amplitude responses to the same stimulations were observed in the medial part of "four limbs area" in the intermediate folia of the paramedian lobule, while in the central part of this area in the lobule V of the anterior lobe.

This study suggested that climbing fiber projections in the paramedian lobule "four limbs area" were different from those in the anterior lobe.

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DIRECT PROJECTIONS TO THE CEREBELLAR NUCLEI FROM THE PONTINE AND INFERIOR OLIVARY NUCLEI IN CATS. YOSHIDA, K.*, YAMAMOTO, T. AND OKA, H. Dept. Psychol., Dept. Physiol., Fukui Medical School, Matsuoka, Fukui 910-11

The direct projections to the three cerebellar nuclei from the brainstem precerebellar nuclei were investigated using retrograde HRP labeling in cats. HRP was injected into the cerebellar nuclei iontophoretically(5 μA, 10 min.) after recording antidromic responses evoked by the thalamic stimulation(VL or VM). In the case of HRP injection into the dentate nucleus, the labeled cells in the pontine nuclei were significantly large in number and distributed more contralaterally to the injection site. However the labeled cells in the dorsolateral subdivision of the nuclei were chiefly located ipsilaterally. The labeled cells in the inferior olivary nuclei were located almost contralaterally in the principal olive. Following HRP injection into the interpositus nucleus, the labeled cells in the pontine nuclei were remarkably small in number, while those in the lateral reticular or vestibular nuclei were moderately large and found dominantly in the ipsilateral side. The labeled cells in the olivary nuclei were also large and distributed throughout the rostral one-half of the contralateral medial accessory olive. The distribution of the labeled cells following HRP injection into the fastigial nucleus was almost same as that into the interpositus nucleus besides the labeled cells in the medial accessory olive were in the more caudal parts.

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INPUTS FROM THE CEREBRAL CORTEX TO DENTATE NUCLEUS NEURONS IN THE CAT. FUTAMI,T., MITOMA,H. AND SHINODA,Y., Dept. of Physiol., Sch. of Med., Tokyo Medical and DentalUniversity, Yushima, Bunkyo-ku, Tokyo.113

To determine whether the dentate nucleus (DN) receives inputs from the cerebral cortex, intracellular recording was made from DN neurons in Nembutal-anesthetized cats. Stimulation of the contralateral cerebral pedunclus (CP) produced EPSPs in DN cells and these EPSPs had latencies of 1.8 - 5.0 msec. EPSPs were also evoked from the contralateral pontine nucleus (PN) at latencies of 0.8 - 3.4 msec and from the contra-inferior olive nucleus (IO) at latencies of 1.6 - 3.8 msec. Most of these EPSPs were monosynaptically induced from these nuclei. Simultaneous stimulation of CP and PN at an appropriate interval produced larger EPSPs than algebraic summation of EPSPs evoked from CP and PN separately. The same was also true in case of combined stimulation of CP and IO. These results indicated that CP-induced EPSPs were relayed to DN by way of PN and IO. In addition to these relay nuclei, N.reticularis tegmentis pontis (NRTP) was also considered a relay nucleus, since stimulation of the contralateral NRTP produced monosynaptic EPSP.

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RELAY CELLS IN THE PRETECTUM FOR VISUAL SIGNALS TO THE CEREBELLAR FLOCCULUS IN THE RABBIT — DOUBLE LABELLING STUDY WITH RETROGRADE TRANSPORT OF FLUORESCENT TRACERS.
MAEKAWA, K. AND TAKEDA, T. Dept. of Physiol., Jichi Med. Sch., Tochigi, 329-04.

Visual signals reach the cerebellar flocculus through two routes; the one via climbing fibers is transferred through the dorsal cap (dc) of the inferior olive and the other via mossy fibers, through the nucleus reticularis tegmenti pontis (Nrtp). HRP studies showed visual signals to the dc were relayed in the dorsal and lateral terminal nucleus (DTN & LTN) of the accessory optic tract as well as in the nucleus of the optic tract (NOT), while those to the Nrtp were relayed mainly in the NOT. Double labelling study with fluorescent tracers, PI in the Nrtp and DAPI in the dc, demonstrated that the dc was innervated by more axons from the DTN-LTN than from the NOT, while the Nrtp was innervated by more axons from the NOT than from the DTN-LTN. About 7 % of DTN-LTN cells and 10 - 14 % of NOT cells were double-labelled, indicating their axons bifurcated to innervate both the Nrtp and the dc. In the DTN-LTN, cells projecting to the dc were distributed along the whole rostro-caudal extent of it, while those to the Nrtp were mainly in the central part of it. In the NOT, cells to the dc were in the rostral half of it and those to the Nrtp were scattered along the whole rostro-caudal extent of it. Spatial distributions of double-labelled cells coincided with those of cells projecting to the dc both in the DTN-LTN and in the NOT.

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ADAPTIVE CHANGES OF FLOCCULAR PURKINJE CELL RESPONSIVENESS INDUCED BY SUSTAINED OPTOKINETIC AND VESTIBULAR STIMULATION IN PIGMENTED RABBITS.
Nagao,S. Dept.of Physiol.,Fac.of Med.,Univ. of Tokyo,Bunkyo-ku,Tokyo 113

Twenty pigmented rabbits were prepared chronically for unit recording from the flocculus. While the rabbit was subjected to continuous(for 1-2 hours)optokinetic stimulation with drum rotation(0.33Hz,2.5°peak-to-peak) or combined with various optokinetic and vestibular stimulation with turn table rotation(0.1Hz,5°),Purkinje cells were recorded in the floccular area where the microstimulation induced the abduction in the ipsilateral eye. A continuous drum rotation around the stationary rabbit increased the amplitude of modulation of simple spikes relative to drum velocity,and decreased that of complex spikes.The amplitude of simple spikes relative to turntable velocity increased in outphase combination of drum and table rotation, and decreased after inphase combination.But after stimulation with rotating the drum 10° in phase with 5°table rotation, there was a shift of phase in modulation. These changes paralled those on the gain and phase of eye movements and support the view that the flocculus controls the eye movement through the modification of Purkinje cell responsiveness to visual and vestibular stimuli.

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EFFERENT PATHWAY FROM THE CEREBELLAR FLOCCULUS RELATED TO VERTICAL EYE MOVEMENTS IN CATS.
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The cat's cerebellar flocculus can be divided anatomically into three zones on the basis of differences in their efferent projection sites. The caudal zone of these three zones projects to the group y nucleus of the vestibular nucleus. Electric microstimulation of the group y nucleus evoked a slow and smooth upward eye movement with a latency of 14.7 ± 4.7 msec at stimulus intensity of 40 μA and frequency of 200 Hz. Electrolytic lesion of the group y eliminated a slow and smooth downward eye movement evoked by stimulation of the caudal zone of the flocculus, while horizontal smooth eye movements and vertical saccadic eye movements remained normally. These data suggest that Purkinje cell activity in the caudal zone of the flocculus can be evoke vertical smooth eye movements by inhibiting the activity of neurons of the group y nucleus and that the group y nucleus lies in a pathway from the flocculus of the cerebellum to the oculomotor nucleus driving vertical eye movements.

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INFLUENCE OF FLOCCULAR STIMULATION ON EXCITATORY RELAY NEURONES OF THE VESTIBULAR REFLEXES OF ANTERIOR SEMICIRCULAR CANAL ORIGIN IN THE CAT. HIRAI, N., UCHINO, Y. Dept. Physiol., Kyorin Univ. Sch. of Med., Mitaka-shi, Tokyo 181

Floccular influence on the excitatory vestibular reflex arcs of anterior semicircular canal origin was examined in the anaesthetized cat. A total of 116 vestibular neurones were identified as vestibuloocular neurones by orthodromic and antidromic activation from the anterior semicircular canal nerve and contralateral oculomotor nucleus, respectively. On the basis of their ascending course and whether they further responded antidromically to stimulation of the spinal cord in the C1 segment, the vestibuloocular neurones were grouped into 1) VO(vestibuloocular proper) neurones in the superior vestibular nucleus, ascending in the depth of the brain stem, 2) VO neurones in the other vestibular subnuclei, ascending in the medial longitudinal fasciculus and 3) VOC(vestibuloocular neurones with descending axon to the cervical segment) neurones. Floccular stimulation suppressed the activity of all VO neurones of first group (N=19) and one fourths of VO neurones of the second group (12/48). None of VOC neurones (N=49) received inhibition from the flocculus. It is concluded that, from the physiological as well as anatomical aspect, the anterior canal-related excitatory vestibuloocular reflex arc is not composed of a homogeneous neurone group.

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TWO GROUPS OF VESTIBULAR NUCLEAR NEURONS WHICH PROBABLY MEDIATE HORIZONTAL CANAL SIGNALS TO THE THIRD NUCLEUS REGION. KAWAGUCHI, Y. Dept. of Physiol., Fac. of Med., Univ. of Tokyo, Bunkyoku, Tokyo 113

In both urethane-α-chlorarose anaesthetized rabbits and decerebrate, lightly urethane anaesthetized rabbits, the localization of vestibular nuclear neurons that mediate horizontal canal signals to the medial rectus motoneurons(MR) was investigated. These neurons are characterized by their specific inhibition that they receive from the ipsilateral retina via the flocculus. To identify the relay neurons, four criteria were used;(1)they were activated monosynaptically by vestibular stimulation;this excitation was depressed by (2)retinal and (3)floccular stimulation; (4)they were activated antidromically from the third cranial nucleus region. Neurons fulfilling these criteria were found in the rostrolateral part of the medial vestibular nucleus and also in the ventral part of the lateral vestibular nucleus. In decerebrate rabbits these neurons in both of these loci responded well to horizontal rotation of the whole body, being excited by rotation from cotralateral to ipsilateral side(type 1 vestibular neurons).

These results suggest that horizontal canal signals are conveyed to MR by two vestibular neuron groups which are of functionally different characteristics.

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Neuronal events correlated with long-term adaptation of vestibuloocular reflex in the primate flocculus.

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The change of acivity of floccular Purkinje cells was examined in monkeys during sustained visual-vestibular stimulation which caused adaptation of the horizontal vestibuloocular reflex(HVOR). In the floccular area related to the HVOR by microstimulation, Purkinje cells consistently changed their simple spike responsiveness to head rotaion in parallel with the HVOR adaptation. Similar changes occurred even after the HVOR had been extinguished by lesioning the bilateral vestibular nuclei. On the other hand, complex spike discharge modulated during vestibular-visual stimulation with the pattern expected to change the simple spike modulation. These results support the hypothesis that the flocculus adaptively modifies the HVOR under visual-vestibular mismatching stimuli.

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NEWLY IDENTIFIED ORGANIC ACIDS ACTING AS HUNGER AND SATIETY SUBSTANCES IN RATS. SHIMIZU, N., OOMURA Y., INOKUCHI, A., NEMOTO, S., SAKATA, T. and Arase, K. Dept. Physiol. and Dept. Int. Med., Fac. Med., Kyushu Univ. 60, Fukuoka 812.

The endogenous organic acid 2-deoxytetronate (2-DTA) lactone was found to be increased from a normal value of 38.4 μM to 1.5mM and 2.5mM, after food deprivation for 36 and 48 hrs respectively. The serum concentration of the another organic acid, 3-deoxypentonic acid (3-DPA) lactone of normal rats was 73 μM which did not change so dramatically like 2-DTA. S and R type of 2-DTA were both effective. As compared to the control rats, infusion of the racemic type of 2-DTA into the third ventricle (2.5 μmol) reduced food intake for 24 hrs, whereas the racemic type of 3-DPA transiently elicited feeding. Four kinds of 3-DPA isomers were further tested for the elicitation of feeding. 2S,4S-type isomer induced feeding with a latency of 8 to 12.5 min, while 2R,4S,2S,4R or 2R,4R-types did not. These results indicate that 2S,4S-type of isomer is the active form in the living body, which is also confirmed by GC/MS analysis. Activity of the glucose-sensitive neurons in the lateral hypothalamus (LHA) were also significantly depressed by 2-DTA but were increased by 3-DPA . A good correlation was seen between the LHA glucose-sensitive neuronal activity and the feeding behavior elicited by 2-DTA and 3-DPA, indicating that these effects is mediated through the LHA neurons.

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GLUCOSE RESPONSIVE NEURONS IN RAT HYPOTHALAMIC TISSUE SLICES. FUKUDA,M., ONO,T., TOHYAMA,M*, INAGAKI,S.*, TAKAGI,H.* and YAMANO,M.* Dept. of Physiol., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01 and *Dept. of Neuroanat., Inst. Higher Nerv. Activ., Osaka Univ., Osaka.

The effects of glucose were investigated in vitro in the ventromedial nucleus (VMH), dorsomedial nucleus (DMH) and lateral area (LHA) in rat hypothalamus. Structures of glucose responsive neurons in the VMH and DMH were examined by intracellular horseradish peroxidase (HRP) injection. In the VMH, 19% (94/520) responded to glucose (5.5-20 mM/l), 32% (69/213) in the DMH and 39% (24/61) in the LHA. In the VMH and DMH, most (15% in the VMH, 25% in the DMH) were excited, and in the LHA most (23%) were inhibited. Glucose responsive neurons identified by HRP were mainly multipolar with large cell bodies and richly branching main dendrites in the VMH, and bipolar with small cell bodies in the DMH. The ultrastructure of VMH glucose responsive neurons was also observed by electron microscopy. Synaptic terminals including small vesicles and large dense core vesicles were richly connected in dendrites. These suggest that the glucose responsive neurons in the hypothalamic area have independent glucose receptive properties and receive various synaptic transmitter inputs on their dendrites.

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LATERAL HYPOTHALAMUS AND VENTROMEDIAL NEURON RESPONSES TO FEEDING AND DRINKING IN FREE MOVING RATS. SHIBATA, R., SASAKI, K. ONO, T., NISHINO, H. AND FUKUDA, M. Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01.

Using 7 chronically implanted teflon coated platinum iridium electrodes (dia. 25 um) long-term recordings of single unit activity from lateral hypothalamic area (LHA) and ventromedial nucleus (VMH) were made in freely moving rats. Their responses to feeding and drinking behavior were analysed. Ten (43%) of 23 LHA neurons recorded showed clear-cut responses, mostly inhibitory, to individual acts in either feeding or drinking or both. None of the remaining 13 exhibited any feeding- or drinking-related activity changes although 3 displayed marked circadian rhythmicity. In contrast, none of 34 VMH neurons recorded showed significant response to individual feeding or drinking acts. Background activity of 5 of 32 neurons increased during feeding and this activity increase was attenuated by drinking. Furthermore, these neurons increased activity after intraventricular or intraperitoneal injection of 2M NaCl solution. Remaining 27 neurons did not exhibit any significant feeding- or drinking-related activity changes. These results suggest that LHA neurons might be more important in neural, somatosensory information processing while VMH neurons contribute more to humoral, chemical information processing.

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LATERAL HYPOTHALAMIC NEURON RESPONSES ASSOCIATED WITH REWARDS AND ANTICIPATORY CUE SIGNALS IN RATS. ONO, T., NAKAMURA, K., NISHINO, H., SASAKI, K. and FUKUDA, M. Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01.

Lateral hypothalamic (LHA) neurons are involved in feeding and intracranial self-stimulation (ICSS), and are concerned with learning of cue signals, but contributions to each process remain obscure. To relate activity of the LHA neurons to natural and artificial reward and anticipatory cues, the same licking task was used to obtain both rewards. Glucose solution or ICSS to the posterior LHA was given, if rat licked a spout after any of three discriminative cue tones: 1200 Hz for glucose, 4300Hz for ICSS and 2800 Hz for no reward. Of 318 LHA neurons analyzed, 212(66.7%) responded to one or both rewarding stimuli. Only reward responsive neurons (81/212) acquired response to cue tones associated with glucose or ICSS reward. Latency of neural responses was 20-80 msec; latency of licking was 140 to 270 msec. Neurons inhibited by glucose or ICSS reward were diffuse in LHA; exitated ones were predominant in dorsal region, especially near zone between fornix and internal capsule under zona-incerta, and fewer in antero-ventral region. Neurons responding to cue tones for glucose or ICSS were diffuse, but tended to be posterior. Results suggest that reward responsive LHA neurons might be important in reward related learning processes.

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MONKEY BAR PRESS FEEDING BEHAVIOR AND DORSAL AND VENTRAL PALLIDAL NEURON ACTIVITY. NISHINO, H., ONO, T., SASAKI, K., FUKUDA, M., MURAMOTO, K. and TAKAHASHI, J. Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01

The activity of 358 neurons was recorded in the dorsal and ventral pallidum during three stages of monkey bar press feeding behavior : 1) food discrimination, 2) bar press and 3) food ingestion. Correlation between firing variations and behavioral events was investigated. In the dorsal pallidum one third of recorded neurons responded during bar press and ingestion, and half correlated with feeding motor acts such as arm extension, flexion, bar pressing, grasping, chewing etc. Responses were often function or direction dependent in that firing increased during arm extension and decreased during flexion, and vice versa. In the ventral pallidum, especially in the rostro-ventral part (subcommissural region), responses were a little different from those in the dorsal part. During bar press, firing change depended on the situation, i.e. it changed much for a preferable food, moderately for regular food, but not significantly for non-food objects. In the case of a brand new food or a false food, responses appeared or disappeared within a few trials. Results suggest that the dorsal pallidum is intimately related to motor performance while the ventral pallidum is not, but it might be important in relating motivational factor with motor output.

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FEEDING-RELATED ACTIVITY OF MONKEY LATERAL HYPOTHALAMIC OPIATE-SENSITIVE NEURONS. OOMURA, Y., NISHINO, H., AOU, S. AND LENARD, L.* Dept. of Biological Control, Nat. Inst. Physiol. Sci., Okazaki 444, Dept. of Physiol., Fac. Med., Kyushu Univ. 60, Fukuoka 812, Dept. of Physiol., Toyama Med. and Pharmaceu. Univ., Toyama 930-01, Inst. of Physiol., Univ. Med. School, Pecs H-7643, Hungary.

To elucidate contributions of monkey lateral hypothalamic (LHA) opiate-sensitive neurons to behavior, correlation between iontophoretic application of morphine on monkey LHA neurons and their feeding-related activity changes was investigated. Orbitofrontal cortical (OFC) neurons were also investigated. Firing rates of about 40% and 25% of LHA and OFC cells tested, respectively, were suppressed by morphine. LHA morphine-sensitive cells changed feeding-related activity more often than insensitive cells. These were divided into 2 group which showed: (i) sustained decrease of activity from bar press/food procurement phase to eating phase, or (ii) sustained increase during the same period. The task-related activity changes in morphine-sensitive cells was similar to that in insensitive cells in the OFC, but during the bar press and eating phases OFC morphine-sensitive cells decreased firing more often than insensitive neurons. The results suggest that morphine-sensitive neurons in the LHA and OFC are involved in tonic regulation of food procurement and consumption.

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SOMATOSTATIN OR DOPAMINE INHIBIT THE PANCREATIC SECRETORY RESPONSES TO INTRACEREBROVENTRICULAR INJECTION OF THYROTROPIN-RELEASING HORMONE

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A previous study in our laboratory showed that i.c.v.injection of TRH (50 pmoles/100gB.W.) induced the pancreatic secretory response via the vagus nerve. The present study demonstrated that the i.c.v.injection of somatostatin (1.6 nmoles/100gB.W.) or dopamine (50 nmoles/100gB.W.) significantly inhibit the pancreatic secretory responses to preceding i.c.v. TRH injection. Intravenous injection of the same dose of somatostatin caused no inhibitory effect, suggesting that somatostatin acts in the C.N.S..Intravenous injection of dopamine inhibited the i.c.v. TRH induced pancreatic secretory responses, whereas the injection did not abolish the pancreatic secretory responses to efferent electrical stimulation of vagus nerve. These results suggest that dopamine in the systemic circulation passes through circum ventricular area in the brain, and acts on the TRH sensitive center of the vagus nerve.

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HYPOTHALAMIC CONTROL OF ADRENAL NERVE ACTIVITY IN THE RAT.

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The modulatory role of hypothalamus on the adrenal gland was studied by examining changes in the efferent discharge rates of the central cut end of the adrenal nerve on bilateral stimulation and lesioning the lateral hypothalamic area (LHA) and the ventromedial nucleus (VMH) in rats. Effects of intraventricular injection of 2-deoxy-D-glucose (2-DG) on the nerve activity were also examined. The discharge rate was found to be increased during stimulation (0.1 mA, 0.1 ms, 30 s) of the middle part of the LHA (LHAm), while it decreased on stimulation of the more rostral part of the LHA (LHAa). Lesion (2 mA, D.C., 15 s) of the LHAm caused a decrease in the nerve activity, while that of the LHAa caused an increase. On the other hand, lesion of the VMH caused an increase in the nerve activity, though stimulation showed no change. Intra-III-ventricular injection of 2-DG (1.2 mg/rat) resulted in an increased discharge rate which was suppressed by the LHAm lesion or VMH stimulation, while the VMH lesion caused no change. These results indicate that the LHAm has probably an excitatory influence on the adrenal nerve, while the LHAa has an inhibitory one. The VMH on the other hand might exert a tonic inhibitory control, which is apparent during levels of increased activity induced by intraventricular injection of 2-DG.

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HYPOTHALAMIC CONTROL OF GASTRIC ACID SECRETION AND PARAVENTRICULAR NEURON PROPERTIES.

SHIRAISHI,T. Dept. of Physiol. Tokai Univ. Sch. of Med. 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 heretofore been attributed to specific neurons in the lateral hypothalamus (LHA). The present study was initiated to investigate the character of r-PVN neurons. In 22 rats, 145 r-PVN neurons were observed. Duration of discharge was 22.3 ± 17.8 min. There were four patterns of r-PVN discharge: high frequency regular, hi-freq. irregular, low freq. regular and lo-freq. irregular. About half of both regular and irregular were phasic. Electrical stimulation of r-PVN evoked responses in 407 LHA units. Latencies of responses ranged from 4.7 to 78.1 ms; indicating mono- and polysynaptic, and myelinated and unmyelinated fiber connections. Neuronal activity increase in r-PVN frequently suppressed gastric type LHA neurons and both spontaneous and 2-DG induced GAS. Chemosensitive neurons have recently been found in r-PVN by electrophoretic application of various chemicals; such as glucose, 2-DG, NE, DA, and TRH and its related peptides. These results suggest that r-PVN might modulate GAS, whether directly or through the LHA is still uncertain.

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THERMOSENSITIVITY OF NEURONS IN THE PARAVENTRICULAR NUCLEUS OF RATS. INENAGA, K., OSAKA, T. and YAMASHITA, H. Dept. of Physiol., Univ. of Occup. Environ. Health Sch. Med., Kitakyushu, 807

It has been suggested that the central control of thermoregulation is influenced both by humoral mechanisms and the autonomic nervous system. We studied the thermosensitivity of neurones in the paraventricular nucleus (PVN) which is thought to be an important site for the integration of neuroendocrine and autonomic functions. In rat hypothalamic slice preparations, 19 out of 65 PVN neurons were considered warm-sensitive ($Q_{10}>2$ and thermal coefficient>0.2 imp./sec $^{\circ}C$), since spontaneous discharge increased with increase of temperature in the perfusion medium. Nine were cold-responsive neurons ($Q_{10}<1$ and thermal coefficient<-0.1 imp./sec $^{\circ}C$). The thermosensitivity of neurons in the suprachiasmatic nucleus was tested for comparison with that in the PVN. Only one out of nine neurons was judged as a warm sensitive neuron. The thermoresponsive neurons were distributed diffusely throughout the PVN. Even after blockade of synaptic transmission by low Ca^{2+} and high Mg^{2+} perfusion medium, some neurons still, showed thermosensitivity. The present results indicate that PVN neurons are themselves thermosensitive.

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THE NEURAL CONNECTIONS BETWEEN THE PERIVENTRICULAR AND THE VENTRAL PREMAMMILLARY NUCLEI IN RATS —— SPECIAL REFERENCE TO SOMATOSTATIN NEURONS. KATO, M., SUZUKI, M. and KAKEGAWA, T. Dept. of Physiology, Inst. of Endocrinology, Gunma University, Maebashi 371

Electrical stimulation of the ventral premammillary nucleus (PMV) suppressed the plasma growth hormone (GH) rise which was induced by the intravenous injection of 10μg growth hormone releasing factor. Similar but stronger effect was observed when the periventricular nucleus (Pe) was stimulated. These inhibitions were probably ascribed to the activation of somatostatin (SRIF) neurons, whose somata reside in the Pe. If this is the case, the PMV must be connected to the Pe or more specifically to SRIF neurons. After retrograde tracer (WGA) injection into the Pe and surrounding areas, many labeled cells were found in the PMV. Thus many PMV neurons project directly to these areas. Electrophysiological study demonstrated that 9% of 450 Pe units responded antidromically to the stimulation of PMV, 11% were orthodromically excited and 11% were inhibited. Among these neurons, which responded to the PMV stimulation, 1% of them responded antidromically to the median eminence stimulation. These results showed that some Pe neurons, which project to the median eminence, received some kinds of inputs from the PMV. Although more work is necessary, elucidation of these neural circuits may explain the mechanism of hypothalamic control of GH secretion.

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EFFECTS OF ELECTRICAL AND CHEMICAL STIMULATION OF THE PARAVENTRICULAR NUCLEUS (PVN) ON NEURONS IN THE SUBFORNICAL ORGAN (SFO) OF CATS. YAMASHITA, H., OSAKA, T., KANNAN, H. and INENAGA, K. Dept. of Physiol., Univ. of Occup. Environ. Health Med., Kitakyushu, 807

It has been postulated that the SFO and the PVN are involved in the control of drinking behavior and water balance. The present study was undertaken to clarify the functional connections between the SFO and the PVN which were suggested by anatomical studies: specifically projection from the SFO to the PVN based on retrograde transport of horseradish peroxidase and the presence of immunoreactive fibers for vasopressin and oxytocin in the SFO.

We recorded the single unit activity of SFO neurons in hemispherectomized cats following both electrical and chemical stimulation in the PVN. A total of 132 neurons in the SFO were tested for their responses to electrical stimulation of the PVN. Forty eight neurons (36%) were inhibited, three (2%) excited and two (2%) antidromically activated. The remaining 72 neurons showed no response. In separate experimetns the wall of the third ventricle near the PVN was locally perfused with glutamate solution (0.5M, pH 8) using a concentric push-pull canula. Of 35 SFO neurons tested, six were inhibited, five facilitated and 24 neurons did not respond to chemical stimulation. The results indicate that the SFO receives mainly inhibitory synaptic inputs from cells in or near the PVN.

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ELECTROPHYSIOLOGICAL EVIDENCE FOR THE INVOLVEMENT OF NEURONS IN THE VENTROLATERAL MEDULLA IN MEDIATING BARORECEPTOR INPUTS TO THE PARAVENTRICULAR NUCLEUS OF THE RAT. KANNAN, H. and YAMASHITA, H. Dept. of Physiol., Univ. of Occup. Environ. Health Sch. Med., Kitakyushu, 807

Extracellular recordings were made from neurons in the ventrolateral medulla (VLM) in urethane-chloralose anesthetized male rats. Forty six neurons could be antidromically activated following stimulation of the paraventricular nucleus (PVN). From their antidromic spike latencies, these neurones could be divided into two different groups (38.0 ± 7.8, n=26 and 10 ± 0.49 ms, n=20). The threshold for evoking antidromic spikes in slowly-conducting neurons (first group) was significantly lower than that in rapidly-conducting neurons (second group). Many neurons of the first group had active irregular firing, while almost no neurons of the second group had spontaneous activity. Baroreceptor activation induced by i.v. administration of phenylephrine inhibited spontaneous activity in half the tested neurons of the first group (n=18). The results suggest the existence of two functionally different populations among VLM neurons which project directly to the PVN, and that VLM neurons with slowly conducting axons mediate afferent information from cardiovascular baroreceptors to the PVN.

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EFFECTS OF A1 NORADRENERGIC REGION STIMULATION ON THE ACTIVITY OF NEURONS IN THE PARAVENTRICULAR NUCLEUS. TANAKA, J., KABA, H., SAITO, H., OTSUKA, K. AND SETO, K. Dept. of Physiol., Kochi Med. Sch., Nankoku, Kochi 781-51

Electrical stimulation of the A1 noradrenergic region induced excitatory(23% and 24%), excitatory-inhibitory(24% and 7%), or inhibitory(35% and 24%) responses of spontaneous activity of units(phasically and continuously firing units) in the paraventricular nucleus of the rat hypothalamus. There was significant difference between the response distributions of the phasically and continuously firing units. Both latency and duration of excitatory responses were significantly shorter in the excitatory responses that were followed by inhibition, suggesting the existence of two distinct excitatory pathways. Neither latency nor duration of inhibitory responses, however, were different between the primary and post-excitatory inhibitory responses.

Effects of α- and β-antagonist on responses of phasically firing units to A1 region stimulation were tested. Intraperitoneal phenoxybenzamine significantly attenuated the excitatory responses that were not followed by inhibition, but not the other responses. Intravenous propranolol, on the other hand, significantly attenuated both primary and post-excitatory inhibitory responses, but not the excitatory responses. These results suggest that the A1 noradrenergic influence contain both α-adrenoceptor (excitatory) and β-adrenoceptor (inhibitory) components.

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EFFECTS OF CATECHOLAMINE SYNTHESIS INHIBITORS ON NEURONS PROJECTING FROM THE VENTROLATERAL MEDULLA OBLONGATA TO THE PREOPTIC AND ANTERIOR HYPOTHALAMIC AREA. KABA, H., SAITO, H., OTSUKA, K. and SETO, K. Dept. Physiol., Kochi Med. Sch., Nankoku, Kochi 781

At least two types of neurons ('fast' and 'slow' cells) projecting from the rat ventrolateral medulla oblongata to the preoptic and anterior hypothalamic area were distinguished on the basis of their antidromic spike latencies and waveforms. Mean antidromic spike latency for the fast cells was 7.3 ms, while the value for the slow cells was 45.5 ms. The occurrence of antidromic responses of 22 out of 32 slow cells was completely abolished by the injection of 6-hydroxydopamine (6-OHDA) directly into the medial forebrain bundle, whereas the other slow cells remained unaffected, indicating the existence of substantial numbers of not only 6-OHDA-sensitive but also 6-OHDA-resistant slow cells. The fast cells tested (n = 6) were not affected by 6-OHDA. Diethyldithiocarbamate (600 mg/kg, ip), a noradrenaline synthesis inhibitor, increased the spontaneous activity of both 6-OHDA-sensitive and 6-OHDA-resistant slow cells. LY134046 (40 mg/kg, ip), an adrenaline synthesis inhibitor, increased the spontaneous activity of 6-OHDA-resistant but not 6-OHDA-sensitive slow cells. The results strongly suggest that the 6-OHDA-sensitive slow cells are A1 noradrenergic, the 6-OHDA-resistant slow cells are C1 adrenergic, and the fast cells are non-aminergic.

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MEDIAL PREOPTIC NEURONAL RESPONSES TO CONNATURAL FEMALE IN THE SEXUALLY ACTIVE AND INACTIVE MALE MONKEYS. AOU, S., OOMURA, Y. AND YOSHIMATSU, H. Dept. of Biological Control, Nat. Inst. Physiol. Sci., Okazaki 444; Dept. of Physiol., Fac. Med., Kyushu Univ. 60, Fukuoka 812

Neuronal responses to connatural female were investigated in the medial preoptic area (MPO) of two sexually active and two sexually inactive male monkeys. In the sexually active male monkeys, neuron activity of about 70% of MPO cells tested decreased in relation to acquisition of the receptive female and mating behavior. Firing rates of these neurons decreased more after ejaculation than befor and gradually recovered. On the otherhand, the presentation or presense of the female monkey elicited a excitatory response in about 20% of MPO neurons of the sexually inactive male monkeys. In the same schedule of sexual behavior, increased neuronal activity in the dorsomedial hypothalamic nucleus (DMH) was found to synchronized with each mating act which includes mounting, intromission and thrusting. These findings suggest that the MPO is involved in coding of the presense of the female in different mannar between sexually active and inactive male monkeys and the DMH is related to the actual performance of mating act.

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SIGNIFICANCE OF AMYGDALOID NEURON RESPONSES TO COMPLEX SENSORY STIMULI. NISHIJO, H., ONO, T., NISHINO, H., FUKUDA, M., YAMATANI, K.* AND SASAKI, K. Department of Physiology, and Neurosurgery*, Faculty of Medicine, Toyama Medical and Pharmaceutical University, Sugitani, Toyama 930-01, Japan.

The amygdala (AM) receives all sensory information that is highly processed by the neocortex. From recent studies, the AM is proposed to play a role in sensory-affective association in all modalities. In this study we recorded unit activity in the monkey AM during discrimination of objects and feeding of various foods. Neuronal responses in the AM were of six general types. 1) Attention or arousal neurons indiscriminatly responded to any sensory stimuli. 2) Specific food dominant, and 3) specific nonfood dominant neurons responded primarily at the sight of food and certain nonfood including human and monkey faces respectively. 4) Attention-evaluation neurons responded to introduction of new food, salting of food, and experimenter's approach to the animal. Background activity also increased in these trials. 5) Anticipatory food related neurons responded to the sight of food, and in food anticipation situations. 6) Reward perception neurons responded only in ingestion phase after putting food into the mouth. Last response was attenuated by heavily salting food. Results suggest that the AM might be important in complex information processing to recognize the significance of an object.

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DIFFERENT DISTRIBUTION OF NEURONS SENSITIVE TO NEUROCHEMICALS AND ITS BEHAVIORAL SIGNIFICANCE IN THE MONKEY AMYGDALA. NAKANO, Y., OOMURA, Y.,LÉNÁRD, L., AOU, S., NISHINO, H. AND YAMAMOTO, T., Dept. of Biological Control, Nat. Inst. Physiol. Sci., Okazaki 444; Inst. of Physiol., Univ. Med. School, Pécs, Hungary.

Extracellular neuronal activity was studied in monkey amygdala and the effects of electrophoretically applied noradrenaline (NA), dopamine (DA), acetylcholine (ACh) and morphine (Mo) were examined. The behavioral paradigm was a high fixed ratio bar press task (FR 20). Out of 186 neurons, 68% of neurons exhibited firing changes during food task. The predominant effect of DA (23%) and ACh (30%) was firing increase, while that of NA (31%) and Mo (30%) was firing decrease. The number of NA sensitive neurons was larger in the lateral part of amygdala than in the centromedial part. On the other hand, DA, ACh and Mo sensitive neurons were found more often in the centromedial part. The distribution of catecholamine sensitive neurons was comparable to the anatomical data. The population of cells which responded to the different phases of the task was almost similar between centromedial and lateral part. But the inhibitory response to the different phases of the task was observed more often in the centromedial part than in the lateral part. These results suggest that amygdalar neurons may possibly be related to the food-related response in the different manner between centromedial and lateral part.

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PATHWAY FROM THE PIRIFORM CORTEX TO THE DEEP AMYGDALOID NUCLEI. OHTA, N. AND MOTOKIZAWA, F. Dept. of Physiol., Nara Medical College, Kashihara, Nara

Neurons of the deep amygdaloid nuclei(AMYGd) are orthodromically activated by electrical stimulation of the piriform cortex(PC) in the cat. On the basis of the HRP studies it has been deduced that there are two routes for projection from PC to AMYGd; one via the lateral entorhinal area(EA), the other via the agranular insular cortex (AI). In the present experiment, neuronal chains of these routes were analysed. Spike potentials of EA and AI following electrical stimulation of PC and AMYGd were extracellularlly recorded in the cat anesthetized with pentobarbital sodium. The location of electrode tips was marked with the fast green FCF.

Neurons of EA and AI were activated orthodromically or antidromically both from PC and AMYGd. Out of 230 units recorded in EA, 16 units were activated antidromically from AMYGd as well as orthodromically from PC. Neurons of this type were not detected in AI. The latencies of the orthodromic responses in EA and AI from PC were about 20 msec and 15 msec respectively. Since the differences in latency between orthodromic responses in EA and antidromic responses induced in PC following stimulation of EA were 2-5 msec, the pathway from PC to EA is considered to be multisynaptic. Orthodromic responses from PC and antidromic responses from AMYGd were distributed in superficial and deep cortical layers of EA respectively.

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THE INFLUENCE OF THE KINDLING EFFECT ON THE ELECTROPHYSIOLOGICAL PHENOMENA. IKEDA, H., OKAMOTO, M., NAITO, H. AND YASUHARA, M. Dept. of Physiol. Kansai Medical Univ., Moriguchi, Osaka 570

The amygdaloid nucleus(AMG) or hippocampus(HPC) of the rabbit was repeatedly stimulated electrically for 1 sec once a day and the PPR, MV and ECG simultaneously recorded continuously over a period of about 2 months. The threshold of the evoked muscular discharge on stimulation of the cerebral cortex was also examined. In the unstimulated control group, no remarked changes were observed in the PPR, MV and ECG of the continuous record. In the AMG kindling group, an increase in amplitude of MV occurred following initiation of stimulation, reached a maximum at 3 days and then declined. Little change was seen in the early components PPR1-PPR4, the ERG components of PPR, but the amplitude of the late components PPR5-PPR8, which are believed to appear via the brain stem reticular formation, gradually increased. In the HPC kindling group, on the other hand, an increase was observed in the MV following initiation of stimulation and no trend for decrease was seen later. There was no change in the early components, PPR1-PPR4, the late ones, PPR5-PPR8 showed no change or increased. The threshold of the evoked muscular discharge showed no change or a rise in the unstimulated group while the threshold declined in the AMG kindling group. The heart rate showed no changes with the kindling.

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THE MECHANISMS OF THE HIPPOCAMPAL THETA-GENERATION IN CATS: TWO GENERATOR ZONES AND CORRELATIONS. KASABA, T., OTSUKA, K. and AOKI, M. Dept. of Physiology, Sapporo Medical College, Chuoku, Sapporo 060.

We previously demonstrated that electrical stimulation of the lower part of the septum elicited the hippocampal theta waves in cats anesthetized with urethane. Depth profile analysis using a bundle electrode confirmed two amplitude maxima; one smaller peak around the pyramidal layer and the other larger peak in the dentate gyrus. In the present study, we examined the correlations of the two theta waves. Septal stimulation produced a reciprocal potential change, a positive wave around the pyramidal layer and a negative wave in the dentate gyrus in 300-600msec. Rhythmic theta waves were observed to succeed these potential changes. These two theta waves were synchronized but were approximately 180° out of phase. There was no correlation between the amplitudes of these two theta waves (r=0.01-0.3). We also analyzed correlations between the theta waves of bilateral hippocampi. Theta waves of bilateral dentate gyri or pyramidal layers were synchronized and in phase. However, there were only weak correlations between the amplitudes of bilateral theta waves; pyramidal layers (r=0.12-0.43) and dentate gyri (r=0.41-0.51). These results suggest that there may exist a common rhythm generator for synchronizing the theta waves in two generator zones and bilateral hippocampi, though the amplitudes could vary independently.

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SPONTANEOUSLY OCCURRING SYNCHRONOUS ACTIVATION OF PYRAMIDAL CELLS IN THE KINDLED HIPPOCAMPUS. FUJITA, Y., SATO, H., MINAMI, S. AND TAZAWA, Y. Department of Physiology Nippon Medical School, 1-1-5, Sendagi, Bunkyo-ku, Tokyo 113, Japan.

Enhancement of inhibition is one of the features of the kindled hippocampus of the rabbit (Fujita et al.: Jpn. J. Physiol., 31 (1981) 879-889, ibid., 33 (1983) 227-238, Brain Res. 278 (1983) 313-317). The inhibition occurred in synchrony with the interictal EEG spike. The interictal inhibition consisted of the Cl-dependent and the Cl-non-dependent hyperpolarization. This was assumed to be occurring in the dendrites, while that was identified as the somatic IPSP. By observing the depth profiles of interictal field potentials, slow positivity was shown to occur in the dendrite layer corresponding in time to the Cl-non-dependent component. Of significance was the fact that the population spike or an action potential led the interictal episode. This implies that the inhibition took place through synchronous activation of recurrent inhibitory circuits. On the other hand, the interictal hyperpolarization was similar in magnitude to that produced by supramaximal fornix stimulation. It is therefore concluded that enhancement of inhibition occurred not through that in the individual recurrent inhibitory circuits, but through spontaneous appearance of synchronous activation of a number of recurrent inhibitory circuits.

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THE FIRING PATTERN OF THE PUTATIVE HIPPOCAMPAL INTERNEURONS DURING ACUTE SEIZURE. KOGURE, S. Dept. of Physiology, Nippon Medical School, Bunkyo-ku, Tokyo 113.

Single unit activities were studied in the hippocampal CA1 area of lightly anesthetized and immobilized rabbits. The units which responded with repetitive firing to the supramaximal contralateral hippocampal stimulation, were recorded as the putative interneurons and examined during the electrically induced seizure discharge. Thirty-five putative interneurons were classified into two types. TYPE I units (N=21) which were situated in the stratum pyramidale or the superior subzone of the stratum oriens were elicited with relatively shorter latencies (7.1 $\pm$ 1.5 msec), while TYPE II units (N=14) which distributed in the stratum oriens were elicited with longer latencies (11.7 $\pm$ 1.8 msec). When acute seizure was induced by means of tetanic stimulation to the contralateral hippocampus, TYPE I ceased to fire during the early part of the afterdischarges, subsequently followed by re-firing during the late part. On the other hand, TYPE II showed a sustained firing without any depression during the whole course of the afterdischarge. These differences in behavior between TYPE I and TYPE II suggest that there are two groups of putative interneurons in the hippocampal CA1 area.

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MORPHOLOGICAL INVESTIGATION OF THE HRP-INJECTED THALAMIC NEURONS IN THE CAT. YAMAMOTO,T. AND NODA,T. Dept. of Physiol., Fukui Medical School, Matsuoka, Fukui 910-11

Morphological as well as electrophysiological analysis of intracellularly HRP-injected thalamic neurons was performed in the cat. Seventy-seven neurons were stained in the VA(n=27), VL(n=25), VM(n=23) and CL(n=2)nuclei. About a half of the neurons(n=36) were identified as projection neurons by their axonal trajectory and/or electrophysiological responses. Four neurons were considered as interneurons by their characteristic dendritic morphology and axonal trajectory.

The pattern of dendritic arborization of the projection neurons could be classified into three groups; radiate type, obliquely-spreading type and tufted type. These dendrites were covered with scattered filamentous spines. There were some varieties in the shape of the spines, i.e., the length of stalks and the size of the spine heads. Concerning axon collaterals, most of projection neurons seemed to give collaterals in the reticular thalamic nucleus. Some neurons gave collaterals near the somata or within their dendritic domain(n=5), though no thalamo-cortical neurons(n=18) could be found to give such collaterals.

Three types of interneurons could be found in the present study. They had aspinous and beaded dendrites, and some of the distal dendrites were very fine and similar to the axon terminals of 'en-passant' type.

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UNIT RESPONSES TO WHISKER DEFLECTION IN THE THALAMIC RELAY NUCLEUS. ITO,M. Aichi Colony for the Handicapped, Kasugai, Aichi 480-03

Whisker-related neuronal responses were recorded under urethane anesthesia from the rat thalamic relay nucleus. Measurements were made of the receptive-field (RF) size (in terms of the number of effective whiskers), threshold velocity of whisker deflection, latency of response and tuning curve slope (determined on a log-log plot of threshold amplitude vs. frequency of whisker deflection), and they were compared with the data obtained for the cortex previously. The RF size (and naturally the latency value) was smaller than that for cortical layer IV, the smallest value among different cortical layers. When thalamic neurons were divided into sustained- and transient-types, the former had a mean slope value of -0.36 that was close to -0.39 of cortical layer IV neurons whereas the latter assumed a mean value of -0.80 comparable to -0.68 of cortical layer Vb neurons. Thalamic points of recording were marked by dye and examined in sections incubated for cytochrome oxidase activity. Different section orientations were adopted but thalamic "barreloids" were much more difficult to visualize than cortical "barrels".

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MODULATION OF VB NEURON DISCHARGE SYNCHRONIZED WITH HEART BEAT. AIKAWA, S., NODA, K., AKITA, H., IWASHITA, Y., OCHIAI, N. and KOBAYASHI, S.* Dept. of Physiol., Sch. of Hygenic Sciences, Kitasato Univ., Sagamihara, Kanagawa and Section of ME, Seishin Igaku, Inst., Itabashi-ku, Tokyo.*

Unitary discharges were recorded extracellularly in the thalamic ventrobasal complex in the cat. 25 of 379 units responded to not only somatic stimuli but also heart beats (HBS). These receptive feilds were distributed on every part of body surface including face, ear, hindleg, etc. Applying electric stimulation to the receptive fields of these neurons, complete or partial inhibition of HBS spike trains were observed.

After Nembutal injection (6.25 mg/kg i.v.) repeatedly the HBS discharges were vanished, but the unitary responses to somatic electric stimulus were still remaind.

Electrical stimuli to the body surface other than the receptive feilds did not show these HBS inhibitions.

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SOMATOTOPIC DISTRIBUTION OF NOCICEPTIVE NEURONS WITHIN THE VENTROBASAL COMPLEX OF THE THALAMUS. YOKOTA, T., UMEHARA, H., KOYAMA, N. AND TAGUCHI, H.* Dept. Physiol., Med. Coll. Shiga, Seta, Otsu 520-21

Trigeminal nociceptive neurons were found within the shell region of nucleus ventralis posteromedialis (VPM) proper and spinal nociceptive neurons were found within the shell region of nucleus ventralis posterolateralis (VPL). Nociceptive neurons were not found along the border between VPM proper and VPL. Hence, nociceptive neurons were found within the shell region of the VPM proper-VPL complex. They were nociceptive specific (NS) neurons and wide dynamic range (WDR) neurons. Trigeminal NS neurons were found within the caudal third of the VPM proper. Trigeminal WDR neurons were found within a narrow zone of about 300 micrometer wide just rostral to the NS region. Similar spatial segregation was found in the VPL. Within the shell region of the VPM proper, nociceptive neurons having a receptive field in the opthalamic division were located dorsolaterally. Nociceptive neurons having a receptive field in the maxillary division were located dorsomedially. Nociceptive neurons having a receptive field in the mandibular division were located ventromedially.

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CROSS-CORRELATION ANALYSIS OF CONNECTIVITY BETWEEN THALAMIC RETICULAR AND VENTRO-BASAL COMPLEX NEURONS. SHOSAKU, A. Dept. of Neurophysiol., Inst. of Higher Nervous Activity, Osaka Univ. Medical School, Osaka 530.

The thalamic reticular nucleus (TR) is thought to be a source of recurrent inhibition to relay cells of the ventro-basal complex (VB). Cross-correlation analysis of spontaneous activities between TR and VB neurons which had receptive fields (RFs) on single vibrissa was performed in urethane-anesthetized rats. There was no significant correlation between 54 pairs of TR and VB neurons which had RFs on different vibrissae. Of 34 pairs representing the same vibrissa, 25 pairs showed three types of correlation which indicate the following connections: 1) monosynaptic excitation from VB to TR neurons (7 pairs). 2) monosynaptic inhibition from TR to VB neurons (11 pairs). 3) reciprocal connection combining the both (7 pairs). These results suggest that one TR neuron may receive excitatory inputs from about 40% of all the VB neurons with RFs on the same vibrissa and exert inhibitory effects to about 50% of them. Further, there are one-way connections from TR to VB and VB to TR neurons in addition to the reciprocal connection.

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POSTURAL CHANGE OF AWAKE UNRESTRAINED CATS INDUCED BY ELECTRICAL STIMULATION OF THE CAUDATE NUCLEUS. OHNO, T. AND TSUBOKAWA, H.* Dept. of Physiol., Institute of Basic Med. Sci., The University of Tsukuba. Niihari-gun, Ibaraki 305.

The effect of electrical stimulation of the caudate nucleus on the posture was investigated by using awake, unrestrained cats. In order to support a micromanipulator which allowed a stimulating electrode to stimulate various regions in the caudate nucleus systematically, a chamber was attached to the skull over the left caudate nucleus. The animal was trained to drink water from a tube standing with one limb on each of four plates by which the vertical force exerted by each limb was measured. When the animal was standing stably, trains of stimulating current pulses of less than 250 μA were applied at 100 Hz for 5 sec. Stimulation within the caudate nucleus evoked the head-turning toward the right which was sometimes followed by circling behavior. The caudal part of the caudate nucleus was effective in inducing these behavioral responses. Common patterns of changes in the vertical force exerted by each limb associated with the head-turning was a decrease in force exerted by forelimbs and a strong in crease in it by the left hindlimb and a weak increase in it by the right hindlimb, resulting in left backward displacement of the center of gravity. The latency of the changes in the force varied over a wide range (min., 50 msec). This method seems to be useful for analysing neuronal mechanisms of postural control by the caudate nucleus.

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SET-DEPENDENT RESPONSE OF TONICALLY ACTIVE PUTAMEN NEURONS DURING VOLUNTARY MOVEMENT.
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Neuronal activities in the putamen were recorded during 3 successive conditioned behavior of monkeys. SELF-PACED MOVEMENT (SPM): flexion-extension of elbow for juice reward followed by licking. FREE-REWARD (FR): rewards delivered with arm position fixed. NO REWARD (NR): similar to FR but solenoid click was not followed by the reward and licking extinguished after the first few solenoid clicks. A group of neurons (n=115) were virtually silent during absence of movement (average frequency less than 1 Hz) and showed intense phasic discharges during limb or orofacial movements, which confirmed results of Crutcher and DeLong (1983). The other group of neurons (n=232) had regular activity at about 5 Hz. These tonically active neurons lacked phasic discharges during movement, but they exhibited one or two impulses at 60 msec latency or synchronization of their tonic discharges by the solenoid click preceding reward in SPM and FR, but not in NR, when click-triggered licks had extinguished. These results demonstrated that characteristic responses of tonically active neurons to auditory cue for movement behavior is dependent upon the set of the animal to initiate the behavior and suggested that they may play a role in linking conditioned stimuli and responses.

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SOME PROPETIES OF AUDITORY EVOKED POTENTIALS AND DELTA COMPONENTS.
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We reported developmental propeties of the main negative(N) waves of auditory evoked potentials(AEP) mainly in relation to the alpha and theta components of children's resting EEGs. Here, we studied N waves of younger children in relation to the delta components of resting EEGs.

Subjects were 6 normal chilren ranging from 1 to 5 years of age. EEGs were led monopolarly from 6 points along the midsagittal line of the scalp. The averaged AEPs to the tone-pips(1000Hz,60dB) and auto power spectra of the resting EEGs were obtained through the digital computer.

In all subjects, addition to the alpha components at around occipital region, the theta and particulary delta components were observed at fronto-central region. In such cases, single N wave with a latency of about 200-250 msec was identified. This latency was longer in comparison with that of N waves which were observed in cases with dominant alpha component. Furthermore, a positive wave was observed clearly with a latency of about 100 msec, though it was not noticed in cases with alpha dominant component.

Considering these results, maturational aspects of the brain might be inferred from the frequency components of the background EEGs and also from the main N waves in the AEPs.

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THE TEMPORAL VARIABILITY OF DELTA COMPONENTS IN THE CHILDREN'S EEGS.
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As one of the properties of delta and slow theta components in the children's EEGs, we examined the temporal variabilities of the peak frequency components.

Subjects were 8 normal children ranging from 1 to 5 years. EEGs were led monoporaly from 6 points along the midsagittal line. The spontaneous resting EEGs of about 2 minutes were sectioned into successive epochs of 12 seconds with overlapped time of 9 seconds. The auto-power spectrum was obtained for each epoch. From these spectra, the averaged auto-power spectrum, the coefficient of variation (C.V.) and the histogram of peak frequency components were calculated through the minicomputer.

In the auto-power spectra of many subjects, alpha and theta components were recognized, but each peak in delta and slow theta frequency band was not clearly found. In these cases, the existences of delta and slow theta components were confirmed through the histogram of peak components. About the temporal variabilities of delta and slow theta components, the histograms of peak components showed broad distributions and the values of C.V.s were relatively small. These properties were different from those of alpha and theta components. From these results, delta and slow theta components might have some information on the maturational aspects of the brain.

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ON AN ANALYSIS OF THE NONLINEARITY OF THE VISUAL EVOKED RESPONSES OF CAT

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Nonlinear oscillatory characteristics of the visual evoked responses (VER) of cat were examined with investigation their steadiness and changes with the passage of time. VER were recorded from visual cortex (Cx) and lateral geniculate body (LGB) simultaneously. Light-flash stimuli were changed in frequency in increasing and decreasing order. Entrainment and hysteresis phenomena of VER in specific frequency bands were observed both in Cx and in LGB. For each stimulus frequency original data from Cx and LGB were plotted on a three dimensional phase space (X,s,dX/dt), where X; electric potential , s;normalized time. At each frequency of the entrainment bands near 20Hz, different types of steady loops were observed between increasing and decreasing course. Moreover periodic concurrent change of the shapes of loops from Cx and LGB occured at the same stimulus frequency. If these stedy loops were the limit cycles of a dynamical system concerning VER, then there existed at least two different limit cycles. Furthermore close relationships of Cx and LGB concerning this oscillation generating system of VER of the cat were also ascertained.

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POSTNATAL DEVELOPMENT OF THE THALAMOCORTICAL PROJECTION TO THE PRIMARY AUDITORY CORTEX IN THE CAT.

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Postnatal development of projection of the medial geniculate body (MGB) to the primary auditory cortex (AI) was investigated using anterograde and retrograde HRP methods. When HRP was injected into the ventral division of MGB (MGv), terminals in AI were labeled densely in layers III-IV and very sparsely in layer I in adult cats. In neonatal animals, by contrast, terminals were labeled densely in layer I and moderately in layers III-IV: the former became thinner and the latter denser with age; an adult pattern was attained by 3 weeks. After HRP injection into layer I of AI, numerous neurons were labeled in the laminated part of MGv in neonatal animals but not in adult cats while labeled neurons were scattered in the medial and dorsal divisions of MGB at any age. Distribution of labeled neurons in MGv after layer I injection was similar to that after layers III-IV injection, which was characteristic to neonatal animals. This study suggests that MGv neurons projecting to layers III-IV have collaterals to layer I which are eliminated during development.

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POSTNATAL DEVELOPMENT OF THE RETINAL AND CEREBELLAR PROJECTIONS ONTO THE MEDIAL WALL OF THE LATERAL SUPRASYLVIAN VISUAL AREA (mLS) IN THE CAT.

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Using field potential analysis and the orthograde HRP method, the postnatal development of the retinal and cerebellar projections was examined in the mLS of the cat. Optic nerve stimulation elicited a surface negative-depth positive(sN-dP) potential in adult cats. In young kittens (younger than 3 weeks old), by contrast, a surface positive-depth negative potential was induced. The response became similar to that in adult cats by 1 month after birth. The response elicited by cerebellar stimulation remained sN-dP throughout aging. These changes were in agreement with morphological findings. Namely, retinal projection, mediated by either the thalamus or areas 17 and 18, terminated mainly in deep cortical layers in adult cats, whereas it terminated mainly in superficial cortical layers in young kittens. Cerebellothalamocerebral projection, on the other hand, remained terminating mainly in layer I. The area responsive to both optic nerve and cerebellar stimulations was wider in adult cats than in kittens.

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RESPONSE PROPERTIES OF THE DENTAL MECHANOSENSITIVE NEURONS RECORDED IN THE ANTERIOR CORONAL GYRUS OF THE CAT. TAIRA, K., MATSUMOTO, N., SOMEI. K., OKUDA, K., SATO,T. AND SUZUKI, T. A. Dept. of Oral Physiol., Sch. of Dent., Iwate Med. Univ., Morioka, Iwate 020

Applying light mechanical stimuli to the canines, unitary discharges of the dental mechanosensitive neurons (DM-neurons) were recorded extracellularly in the somatosensory oral area of the anterior coronal gyrus (SI) of the cat. The oral area could be divided into four regions corresponding to the projecting site of each canine as it was proposed by the multi-unit recording. However, the DM-neurons in each region received dominant input from the particular canine, but also received minor input from other canines. The interaction between the dominant and the minor inputs was examined, delivering the conditioning and the test stimuli to two canines with several interval (Ti). The response to the test stimulus was inhibited at first (Ti=20-30msec), then facilitated (Ti=50-150msec) by the conditioning stimulus. Furthermore, evoked potential analysis suggested that the neurons distributed widely in the oral projection area were repolarized in the inhibited period. These properties of the DM-neurons were discusses in relation to the cortical information processing for the food's shape and its localization in the oral cavity.

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LOCALIZATION AND RESPONSE CHARACTERISTICS OF TRIGEMINAL PROPRIOCEPTIVE NEURONS IN SI. IWATA, K., ITOGA, H., and SUMINO, R. Dept. of Physiol., Sch. of Dent., Nihon Univ., Chiyoda-ku, Tokyo 101

Intracortical localization and response characteristics of neurons in SI, which received trigeminal proprioceptive inputs (the masseteric muscle, the temporomandibular joint and the periodontal membrane) were studied in α-chrolalose anesthetized cats. Fifty eight neurons with masseteric inputs were recorded in lamina IV-V of the anterior coronal (3a) and the anterior lateral sygmoid gyrus (6aB) (Anterior Neuron). And fifty two were in lamina II-III of the posterior coronal gyrus (3b) (Posterior Neuron). Most of these neurons received inputs from the facial skin as well as the masseteric muscle. Cutaneous receptive fields of PNs were confined to the region of the contralateral mandibular angle and the adjacent buccal skin, while those of ANs were in the contralateral or the bilateral perioral regions and some ANs had wider receptive field extending to neck, shoulder and forelimb in the contralateral side. 10 neurons of PNs received input convergence not only from the masseteric muscle and the facial skin but also from the temporomandibular joint, and 32 of ANs from the periodontal membrane. The mean latencies of ANs following masseteric stimulation were 10.1 ± 1.59 msec, and those of PNs were 14.25 ± 3.13 msec.

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MOTOR EFFECTS OF INTERCORTICAL MICROSTIMULATION (ICMS) OF BILATERAL SOMATIC SENSORY INPUT AREA (BSSI) IN THE SECOND SOMATIC SENSORY CORTEX (SII). MORI, A., HIRABA, H., MATSUURA, N., and SUMINO, R. Dept. of Physiol., Sch. of Dent., Nihon Univ., Chiyoda-ku, Tokyo 101.

We have already reported (Mori et al. Exp. Brain Res.51:108-116, 1983) that ICMS of the contralateral somatic sensory input area (CSSI) in SII produced movement of contralateral side of the body with very weak currents (2-30μA). In the present study, we examined input-output relationship of neurons in BSSI of SII in lightly sedated (Katamine, 1-2mg/Kg) cats using the same microelectrode for ICMS and recording cellular activaties. ICMS of BSSI elicited blinking, shoulder and abdomen movements and flexion of hindlimb on the bilateral side of the body. The minimum current for movement was 6μA and maximum current used was 30μA. Neurons in the area for a particular movement generally received a peripheral input from the skin overlying the muscle to which the area projected. After pyramidal tract was sectioned at the medullary level, ICMS of BSSI did not elicite any movement of the body. ICMS of BSSI in SII did not abolish movement after removal of the motor cortex (area 4γ) and the contralateral side of the SII. It is concluded that neurons of the bilateral somatic sensory input area in SII have a motor function which is independent of the motor cortex.

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LOCAL INJECTION OF MUSCIMOL IN DIFFERENT AREAS OF THE HAND REGION OF CONSICOUS MONKEY SOMATOSENSORY CORTEX (SI). IWAMURA, Y., HIKOSAKA, O., TANAKA, M., AND SAKAMOTO, M. Dept. Physiol. Toho Univ. Sch. Med., Otaku, Tokyo 143

A small amount of muscimol, a potent agonist of GABA was injected at various sites in the hand region of the conscious monkey SI, to investigate deficit in the hand manipulative behaviors caused by the reversible and localized disfunction of the cortex. We first recorded single cell activities using a glass-coated platinum-iridium microelectrode and determined their receptive field characteristics. Then we inserted a pipette for muscimol injection through the same hole on the dura resulted from the preceding electrode penetration. Changes in monkey's behavior following the injection were examined using various tasks that required the monkey to manipulate her hand and fingers in precise or complex ways to pick up small pieces of food pellets from various containers. These tests were done also without monkey's vision. A total of 18 injections of muscimol were made and changes in tactile behavior were detected following 15 injections. The behavioral changes varied depending on the injection sites and time after the injection. Injections in area 2 disrupted behaviors that required organized informations from multiple fingers, while area 3a or 3b injections interefered with the same behaviors by the disruption of somatotopically discrete information. Area 1 injections lead to symptoms similar to area 3b or 2.

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LATERAL SPREAD OF EPICORTICALLY INDUCED NEURONAL ACTIVITY IN THE CAT MOTOR CORTEX. OSHIMA, T. AND EZURE, K. Dept. of Neurobiol., Tokyo Metropolitan Inst. for Neurosciences, Fuchu-shi, Tokyo 183

In the encéphale isolé cat preparations, lateral spread of neuronal activity within the motor cortex was investigated by comparing intracellular responses to near and distant epicortical stimulation (EPICS). Excitatory postsynaptic potentials (EPSPs) were dominant responses in lamina II cells to both near and distant EPICS. These EPSPs were conveyed laterally by the impulses of superficial horizontal axons with the mean velocity of 2.4 m/sec, in good agreement with estimation by the negative wave in the direct cortical response (DCR). In most lamina IIIa cells, inhibitory postsynaptic potentials (IPSPs) dominated or preceded EPSPs for distant EPICS. Correspondingly, a positive wave preceded negative one in distant DCR. Laminae IIIb-VI cells responded with EPSPs, disinhibition, IPSPs and disfacilitation. The latter two responses were more conspicuous to distant than near EPICS. Lateral spread of activity would occur via at least two horizontal routes: one excitatory in laminae I-II and the other inhibitory in laminae II-IIIa. The newly found middle layer inhibitory route may operate as a diffuse reset mechanism in the manner that some laminae II-IIIa cells give off horizontal axon collaterals inhibiting lamina IIIa cells directly and laminae IIIb-VI cells indirectly by disfacilitation.

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INTRACORTICAL CONNECTIONS OF THE MOTOR CORTEX IN THE CAT: AN INTRACELLULAR HRP STUDY. NODA, T. AND YAMAMOTO, T. Dept. of Physiol., Fukui Med. Sch., Fukui 910-11

In the cat precruciate cortex(area 4γ), the intracortical axonal projections of the intracellularly HRP-injected neurons have been examined. The HRP-stained cells were reconstructed with a camera lucida from the DAB-cobalt-GOD-reacted serial sagittal sections of the cortex. 1) Layer II pyramidal cells: the axon branches ramified extensively in layers I and II(about 800 μm tangentially), and less extensively between layers III and VI. 2) Layer III pyramidal cells: the axon branches projected around the soma-dendritic fields between layers I and III(400-700 μm tangentially), but a few extended farther in layers II and III(up to 900 μm), including the long horizontal one in layer III(about 2 mm). The descending branches projected extensively in layer V. 3) Layer V fast and slow PTNs: the axon branches were confined mainly in layers V and VI, and, partly, up to the middle of layer III. 4) Layer VI pyramidal cells: some cells projected exclusively in layer VI with long horizontal branches, and others had also the ascending branches to the border between layers III and V. 5) A layer III aspinous stellate cell: the axon arborized extensively and gave off numerous small clusters of terminal branches between layers II and VI. This cell is supposed to be an inhibitory interneuron(probably a basket cell). In the light of these findings, the intracortical circuit of the motor cortex was discussed.

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DISTRIBUTION OF NEURONS RELATED TO DISTAL AND PROXIMAL FORELIMB MOVEMENTS IN PRIMATE PREMOTOR CORTEX AND THEIR PROPERTIES. KURATA, K. AND TANJI, J. Dept. Physiol. Hokkaido Univ. Sch. Med., Sapporo, 060.

Three monekys were trained to move proximal and distal forelimb in a temporally separated manner. They were required to perform rotation movements at the shoulder and then to press a key in response to presentation fo three different sensory signals (visual, auditory, and tactile). Recordings were made transdurally from forelimb area of the precentral motor cortex (MC) and shole area of the premotor cortex (PM) of each animal. Premotor cortex was defined as the lateral part of frontal agranular cortex where muscle contraction could not be evoked by intracortical microstimulation at the intensity below 50 μA.

Two hundred fifty-five neurons in PM were related to the distal forelimb movement (distal neurons) and 172 neurons in PM were related to the proximal forelimb movement (proximal neurons). Distal neurons were ditributed in separate foci in PM, most densely in postarcuate region. Proximal neurons, however, distributed more widely in PM. Both proximal and distal PM neurons showed less modulation of discharge frequency in association with movement than MC neurons, and 29% of distal neurons responded selectively to one or two modality of sensory signals triggering the movement.

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EFFECTS OF PREMOTOR CORTEX COOLING UPON VISUALLY INITIATED HAND MOVEMENTS IN THE MONKEY. SASAKI, K. AND GEMBA, H. Inst. Brain Res., Fac. Med., Kyoto Univ., Kyoto 606

The premotor cortex was temporarily impaired with local cooling method and effects of the cooling upon the visually initiated, reaction time hand movement and upon cortical field potentials associated with the movement were examined in the monkey. Cooling of the bilateral premotor cortex (dorsolateral part of area 6 aα and β) disorganized the well trained reaction time movement and resulted in nearly self-paced, random movements without appreciable paralysis. During the cooling, less than half of movements were in reaction to the light stimuli delivered at random time intervals, as compared with more than 90 % in the control. Unilateral (contralateral) cooling of the premotor cortex produced much less effects than the bilateral cooling. The cooling effect could be induced with the simultaneous warming of the supplementary motor area that prevented the cooling from spread to the area. The cooling effect was reversible with rewarming. However, the effect faded considerably in repeated trials on the same day and also gradually in successive experiments in several weeks, which is in contrast with the unfaded cooling effects of, e.g., the motor cortex. The present study suggests that the premotor cortex is a key station for the visuo-motor integration in the conditioned movement, and that the integrative function can be easily compensated even when impaired temporarily.

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AN ANALYSIS OF THE RHYTHMICAL JAW MOVEMENTS PRODUCED BY ELECTRICAL STIMULATION OF THE MOTOR CORTEX OF RABBITS. SASAMOTO, K., MURAKAMI, T.*, LUND, J.P.** AND OLSSON, K.Å.*** Dept. of Physiol., Fac. of Dent., Kyushu Univ., Fukuoka 812, Dept. of Oral Physiol., Nippon Dental Univ., Niigata*, Univ. de Montréal**, Univ. of Umeå***

The antero-lateral regions of the cerebral cortex of rabbits anesthetized with urethane were stimulated with either short trains of electrical pulses (3 pulses, 500 Hz) or ten-second trains of shocks at 50 Hz. The movements of the mandible and EMG activity from the muscles of mastication were recorded and analysed. Two basic types of responses are reported; twitches of the digastric muscles that followed the stimulus at short-latency (4-6.7 ms) and rhythmical masticatory movements that could only be evoked by prolonged stimulation. Different masticatory patterns are represented in separate areas of the cortex. The movements represented in the antero-medial half of the masticatory area are made mainly in the vertical plane, while stimulation of the postero-lateral part produces movements in which the jaw swings to the contralateral side during closure. Increases in stimulus intensity usually increase the frequency of the movements but other changes in the pattern depend on the site of stimulation. We concluded that the stimulus-bound short-latency responses and mastication are independent phenomena. We suggest that the basic patterns of mastication are elaborated by brain stem circuits that are themselves controlled by specific regions of the motor-sensory cortex.

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ALTERATIONS OF ADENOSINE-SENSITIVE CYCLIC AMP SYSTEM BY FORMATION OF A CHRONIC EPILEPTIC FOCUS IN RAT CEREBRAL CORTEX.

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The level of adenosine-elicited accumulation of cyclic AMP was measured in slices from the rat cerebral cortex in which a chronic epileptic focus was induced by microinjection of ferrous chloride solution into the sensorimotor area. The cyclic AMP accumulation was greater in the slices from an anterior half of the cortex showing dominant ECoG spike activity than the other side. The accumulation was not reduced by dipyridamole. Elicitation of cyclic AMP accumulation by adenosine was blocked by adenosine antagonists such as theophylline, 3-isobutyl-1-metylxanthine, and 8-phenyltheophylline. Further, there was a regional difference in 2-chloroadenosine-elicited accumulation of cyclic AMP in the presence of adenosine deaminase. Pattern of the difference was similar to that in adenosine-elicited accumulation. These results suggest that alterations of adenosine-sensitive cyclic AMP system of the cortex are involved in the process of the iron-induced epileptic focus.

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A MORPHOLOGICAL STUDY OF THE CORTICAL NEURON IN THE COBALT INDUCED EPILEPTIC CAT'S BRAIN.

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Epileptogenic foci were produced by the topical application of metallic cobalt powder to the sigmoid gyrus of cat. In the early stage of epileptogenesis, the pyramidal neuron in the focus with Golgi staining appeared abnormal, such as the nodul formation on the peripheral shaft of the apical dendrites. THe dendrite became attenuated between the nodules, and along the apical dendrite the number of spines decreased. The present study is meant to know whether or not this abnormal neuron can become hyperexcitable.

For this purpose Lucifer yellow is injected electrophoretically through recording microelectrode after the recording the activity of neuron in the focus. In cobalt focus, however, the neuron that showed the epileptic burst discharge had not stained so clearly as the neuron from intact cat's cortex, therefore the detail of neuroral-change such as the nodul-formation on the dendrite had not detected.

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ELECTROPHYSIOLOGICAL STUDIES ON THE RECIPROCAL CONNECTION OF PYRAMIDAL TRACT CELLS BY THEIR AXON COLLATERALS BETWEEN THE MOTOR AND THE PARIETAL CORTEX.

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Our previous report has shown that there are reciprocal connections between the motor (area 4γ) and the parietal (area 5) cortex by axon collaterals of PT cells, by axons of cortico-cortical neurons and by axon collaterals of corticonuclear neurons. The present study was focused on the structure and function of the reciprocal connection system by axon collaterals of PT cells between areas 4γ and 5. It was found in some instances that the location of the branching point of cortico-cortical axon collaterals of PT cells was very close to the soma, possibly in the initial segment. Microstimulation of areas 4γ and 5 often produced both antidromic spikes and EPSPs in PT cells of areas 5 and 4γ, respectively. The EPSPs showed in many cases remarkable frequency potentiation similar to recurrent EPSPs. The current intensity of the stimulation was as low as to activate a single PT cell which was identified by recording its antidromic extracellular spikes using the stimulating electrode as an recording electrode. These results suggested that pairs of PT cells in areas 4γ and 5 were reciprocally connected by their axon collaterals. This structure may function as an oscillator such as an astable multivibrator composed of two NAND gates which receive feedback inputs from one another, i.e., a fundamental logic circuit of a Flipflop.

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DISTRIBUTION OF VISUAL NEURONS WITHIN THE LAMINA OF MONKEY PREARCUATE CORTEX. AZUMA, M. AND SUZUKI, H. Dept. of Physiol., Hirosaki Univ. Sch. of Medicine, Hirosaki, Aomori 036

Laminar distribution of prearcuate visual neurons were examined with special reference to their receptive-field (RF) properties. The visual neurons were distributed in all layers of the cortex except Layer I. On perpendicular penetration of the recording microelectrode into the cortex, similar RF eccentricity and vector angle from the fovea were found in the neurons of different depths, whereas considerable variation was seen in RF size. On the tangential penetration, the RF properties of neurons were changed over their variations on perpendicular penetration: When the penetration was made posteriorly along the anterior bank of the arcuate sulcus, the eccentricity and size of the RF were found to be similar. However, the vector angle was changed so that the RF was finally located in contralateral-lower quadrant of the visual field.

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BAR PRESS FEEDING TASK INITIATED BY VOCALIZATION AND NEURONAL ACTIVITY IN THE MONKEY ORBITOFRONTAL CORTEX. YAMAMOTO,T., OOMURA,Y., NISHINO,H., AOU,S. AND NAKANO,Y. Nat. Inst. Physiol. Sci., Okazaki 444; Dept. of Physiol., Fac. of Med., Kyushu Univ., Fukuoka 812.

Single neuron activity was recorded in the monkey orbitofrontal cortex (OBF) during bar press feeding task initiated by monkey vocalization in order to elucidate the roles of OBF on feeding behavior. Chronically implanted Pt-Ir microelectrodes were used for stable recording. The task paradigm was as follows. The monkey was requested to vocalize to turn a cue light on (initiation of the task). Then the monkey pressed a bar 30 times, and a reward (food) was presented with a cue tone stimulus. The neuronal activity during conventional feeding task in which the monkey began to press a bar in response to a cue light on was also studied as a control. A micro-computer was used to sense the vocalization and to control the task sequence. Seven out of 38 neurons analized (18%) showed activity change several sec. preceding to the onset of vocalization. Four neurons (11%) showed activity change only when the trial was initiated by vocalization. Four out of 16 neurons recorded in the lateral hypothalamic area (LHA) also showed activity change preceding to the onset of vocalization. These results suggest that the OBF and the LHA are involved in the initiation of the feeding behavior.

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NEURONAL RESPONSES IN MONKEY DORSOLATERAL FRONTAL CORTEX SUBDIVISIONS DURING BAR PRESS FEEDING BEHAVIOR. MURAMOTO, K., ONO, T., NISHINO, H., SASAKI, K. and FUKUDA, M. Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01.

The activity of 190 neurons was recorded from the dorsolateral prefrontal cortex of monkeys to determine the participation of this subdivision during operant feeding behavior consisting of discrimination of food, bar pressing to get food and ingestion. In area 8, fairly many responded in both the visual discrimination (37%) and motor initiation (35%) phases. Neurons in area 8 showed some functional heterogeneity in that visual discrimination responses were rostral, visuokinesis was central and motor initiation was in the caudal bank of the arcuate sulcus. Neurons in area 9 responded primarily (37%) during the bar pressing phase and less during the visual discrimination phase. Neurons in area 10 responded variously during most phases of the task -- food discrimination, bar pressing, and ingestion. Neurons in the periprincipal sulcal area usually responded in the visual discrimination phase, and in particular, some responded to meaningful visual or auditory cues that were related to food reward. The data suggest that neurons in the dorsolateral prefrontal cortex have multiple and heterogenous functions related to complex, learned feeding behavior.

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SINGLE UNIT ACTIVITY IN ORBITAL GYRUS DURING FOOD INGESTION IN CATS. TAIRA, M., HIRABA, K. AND NAKAMURA, Y. Dept. Physiol., Fac. Dent., Tokyo Med. Dent. Univ., Tokyo 113

In 16 unanesthetized chronic cats, single unit activity was recorded from the orbital gyrus, where repetitive stimulation (40 Hz, 0.2 ms, $\leqq$40 μA) by recording microelectrodes induced a rhythmical jaw movement (RJM) resembling mastication. Thirty-two neurons showed spike firing related to some aspect of RJM during ingestion of canned fish and milk, but none of them responded with antidromic spikes to stimulation of the cerebral peduncle. Thirteen neurons changed the firing rate (FR) rather tonically during food ingestion (non-phasic group): 12 neurons increased FR throughout a series of masticatory strokes, while 1 neuron decreased FR during initial masticatory strokes. The change in FR started preceding the onset of RJM by 500-900 ms. The remaining 19 neurons rhythmically modulated FR in the same rhythm as RJM (phasic group): the firing or the highest incidence of spikes occurred during the jaw-opening (JO) phase in 9 neurons, while they coincided with the jaw-closing (JC) phase in the remaining 10 neurons. FR of 4 opening-type neurons showed a positive correlation with the velocity of JO movement, and in 2 of them it was also positively correlated with the jaw position in JO phase. FR of 1 closing-type neuron negatively correlated with the velocity of JC movement and the jaw position in JC phase. The results suggest that there are neurons in the orbital gyrus which participate in the initiation and/or execution of masticatory jaw movement.

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ACTIVITY OF NECK MUSCLES IN SYMMETRICAL GAITS OF QUADRUPEDS. TOKURIKI, M. Dept. Vet. Physiol., Facul. Agri., Yamaguchi Univ., Yamaguchi, Yamaguchi 753

Activity of neck muscles was investigated by electromyogram in locomotion of cats, dogs, horses. Epaxial neck muscles such as M. splenius, the biventer cervicis and the complexus major of M. semispinalis capitis, and M. multifidus cervicis occurred in the activity at the same phase with the strong activity in the stance phase of a contralateral fore limb and the weak one in that of an ipsilateral fore limb. Activity of neck muscles which appeared twice in a stride preceded the stance phase of a fore leg in walk and trot of the cat, occurred in around landing of a fore leg in walk and trot of the dog, and appeared in the middle of the stance phase in walk of the horse while it occurred just after landing of a fore leg in trot. These data show that activity of neck muscles in symmetrical gaits had no direct relation to the movement of fore legs. The possibility of reflex control in activity of neck muscles was investigated by removing the dorsal ganglions from L1 to L6 in the cat. Activity of neck muscles after the ganglionectomy was different from activity of trunk muscles which disappeared after the ganglionectomy in symmetrical gaits and occurred in the phasic activity in walk. This means that the neck muscles in quadrupedal locomotion may not be controlled by afferent inputs.

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SPEED-RELATED CHANGES IN KINEMATICS AND EMG ACTIVITY OF RUNNER'S LOWER EXTREMITY. SUZUKI, S., SUZUKI, M.[1] AND WATANABE, S. Dept. Physiol., Sch. Med., Kyorin Univ., Mitaka, Tokyo 181, [1]Inst. Health & Sports Sci., Univ. Tsukuba, Sakura, Niihari 305

Telemetric surface EMG recordings from the human lower extremity muscles during running were analyzed in 6 subjects. The kinematic analysis in 2 elite athletes were also applied. The muscle activity and the movements relative to varied running speeds were studied. The increase in running speed from 1.6 to 6m/s was achieved by reduction in stance phase, whereas the swing phase remained almost constant. However, in higher speed (6-10.6m/s), shortening of both stance and swing phases occurred. As the speed increased: (a) the total amount of EMG activity in gastrocnemius(G) showed almost no change, but the activity before ground contact was significantly increased in elite athletes (b) the activity of tibialis anterior(TA) tended to enhance with the phase shift and appeared just before landing. In elite athletes ongoing activity of TA became suddenly inactive at beginning of the stance phase (c) extremely large enhanced activity of rectus femoris(RF) was observed during early swing phase in elite athletes (d) a similar enhancement was seen in biceps femoris(BF) at the end of swing phase. These enhanced phenomena showed a parallel relationship, i.e., the higher the running speed, the greater the EMG activity. The activity during stance phase in RF and BF showed almost no change though speed increased.

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ESTIMATION OF MUSCULAR FORCE OF REFLEX-ORIGIN IN RESPONSE TO STRETCHING HUMAN FINGER MUSCLE. AKAZAWA, K. and FUJII, K. Dept. of Elect. Eng., Osaka Univ., Suita, Osaka 565

Being strethced, a muscle exerts an excess force. The excess force consists of three components originated from (1)passive elements such as connective tissue, skin, membrane etc,(2)viscous-elastic properties of contracting muscle, (3)stretch reflex. The present work is to present an analytical method of estimating the force of reflex-origin. First, human flexor pollicis longus muscle which was relaxed or maintaining a steady tonic force was ramp-stretched(dynamic phase;15-20 ms) by a small amount(2-3 mm at the tip of thumb). The reflex-mediated force was not developed until approximately 50 ms after the onset of stretch, because of latencies of the neural and contractile mechanisms. Secondly, mechanical parameters of the resting and contracting muscles were determined, by analyzing dynamical relations between length and force observed during the 50 ms-period after the onset of stretch. Thirdly, force originated from two components(1) and (2) was calculated by using the determined parameters over the whole observation time. Finally, force of reflex-origin was estimated by subtracting the calculated force from the observed force. The force of reflex-origin increased linearly with increasing tonic force. Peak of the reflex-mediated force was 6-20% of MVC(maximum voluntary contraction) when the tonic force was 50% of MVC.

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INFLUENCE OF EXERCISE ON NEURO-MUSCULAR FUNCTIONS BY ESTIMATION OF M, H WAVES AND EVOKED TORQUE.

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11 young, healthy and asymptomatic subjects were tested before and after exercise for changes in their reflex responses to electrical stimulation. The method of Angel and Hoffmann was used for the electrical stimulation. The instrumentation and procedure were identical to the previous study in order to make the experimental conditions as similar as possible between before and after exercise for the monosynaptic reflex and for the voluntary reaction time(light stimulus measurement).

Previous work has shown a significant reduction in the Hoffmann reflex response(H/M retio) to result from a single moderate aerobic exercise bout. But the exercise used in this study was composed from a minute short anaerobic isometric and peripheral muscular contractions. Then, this investigation was appeared to the excitable contribution level of the neuro-muscular function, increasing in the spinal reflex activation and muscular contractive levels by the load effects of this easy short exercise.

After exercise, these responses to the electrical stimulation and the light voluntary stimulation were increased on the average by 12.0% of H/M ratio($P<0.05$). The evoked torque after exercise with H or M waves generated to significant increase on 48.2%($P<0.001$) remarkedly. While, the spinal cord delay and electrical mechanical delay were shown no changes($P>0.05$) and in contrast, neuro-muscular conjunction delay was reduc ed.

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MOTOR UNIT PROPERTIES IN MASSETER MUSCLE AND THE FIRING PATTERNS DURING TOOTH TAPPING.

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An inhibitory phase is clearly induced in surface masseter EMG during jaw movements with tooth contacts, and this phenomenon is often used in dental fields. However, there have been few quantitative analysis of motor unit activities in the masseter inhibitory phase. In the present study, we have investigated the firing patterns of masseter motor units during tooth tapping with the aim to determine the unit types which disapper immediately after tooth contacts. The masseter motor unit EMG was recorded from 15 subjects with normal occlusion by a depth needle electrode. Occlusal force was monitored by a strain-gauge between teeth, and occlusal sound was recorded by microphone. Using spike triggered averaging method, twitch contraction time and peak tension were measured in each unit. The units, which disappeared in the masseter inhibitory phase, were recruited when the occlusal force reached about 3 kg. The twitch contraction time was 40 $\pm$ 11 ms (n=36) and peak tension was 2.9 $\pm$ 0.3 g (n=36) in these units. Based on pulse density variation before and after tooth contacts, the inhibitory phase was found to be 12.2 $\pm$ 1.6 ms (n=36) in duration, and start 10.2 $\pm$ 0.4 ms (n=36) after tooth contacts. The synchronized increase of the unit activity was often observed before the inhibitory phase.

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THE CONTRACTILE PROPERTIES OF SINGLE HUMAN MOTOR UNITS IN ABDUCTOR DIGITI MINIMI AND ANTERIOR TIBIAL MUSCLES.

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To examine the contractile properties of human single motor units, single human motor units(MUs) of the abductor digiti minimi(ADM,upper limb muscle) and the anterior tibial (AT,lower limb muscle) muscles were examined during voluntary contractions using coil-shaped inserted and the surface electrodes. The contraction time(C.T.), the peak tension (P.T.) and the recruitment threshold(Fth) of MUs were determined similar to previous report and additionary the conduction velocity(C.V.) of MUs determined. For the determination of the C.V. of MUs, the surface electrodes which are nine platinum leads, each 1 mm in diameter and 3 mm apart was used. The surface electrodes were adjusted the position parallel to fiber direction. The C.V. of indivisual MUs were determined from surface EMG wave obtained by averaging method, shile triggered by action potentals of each MUs detected by the inserted electrode. There was no significant correlation between Fth and C.T. in ADM, but the C.T. was shorter when the Fth was higher in MUs of AT. MUs in ADM muscle showed a various range of P.T. in the C.T. ranged from 60-90 ms. In AT muscle, the MUs which had shorter C.V. had the higher Fth, the shorter C.T. and the larger P.T. It is suggested that the contractile properties were different in the upper limb muscle;ADM and in the lower limb muscle;AT.

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VOLUNTARY SINGLE HUMAN MOTOR UNITS. KURATA, H., OGAWA, Y. AND MASUDA, M. Lab. Sports Med., Jikei Univ. Sch. Med., Chofu, Tokyo 182, and Dept. Physiol., Jikei Univ. Sch. Med., Minatoku, Tokyo 105.

To examine the property of muscular activities at different knee joint angles (20° -135°), integration values of surface EMG (IEMG) and action potentials of single motor units (MUs) from the medial vastus, lateral vastus and rectus femoris muscles were recorded. The values of torque output during a maximum isometric contraction at each knee angle were recorded by force-displacement transducer which secured to the lower leg. The force threshold (FT) in each MU was measured. The decreased IEMG at the extended position of the knee in the medial and lateral vastus muscles, and at the flexed position in the rectus femoris muscle was observed in the school children (7-12 yrs) and in the adults. Ratio of the FT in %MVC relative to maximal FT increased from 10-40% to 100% in the medial vastus muscle, increased from 30-50% to 100% in the lateral vastus muscle, and decreased from 100% to 20-40% in the rectus femoris muscle when the knee angle changed from 120° to 20°. These data support that the inhibition to α motoneurons of medial and lateral vastus mescles is powerful at the extended position of the knee, and it is prominent at the flexed position in the rectus femoris muscle.

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DISCHARGE FREQUENCY OF MOTOR UNITS DURING VOLUNTARY CONTRACTIONS IN VARIOUS SPEEDS. OISHI, K., YONEDA, T. and ISHIDA, A. Dept. of Physiol. Sch. of Physical Edu., Juntendo Univ., Narashino, Chiba

Frequency responce of motor unit to force developing was investigated in man. Motor unit discharges were recorded during voluntary isometric force exertion in various speed(time to peak: from 90 to 5000msec). Target force was 3kg only. Bipolar fine wire electrodes which embedded in acryl-regin were inserted into the adductor pollicis muscle via needle. Discharge frequency was derived from the interspike intervals of the motor unit.

Two findings about motor unit activities were obtained as followes; 1) in ramp contractions, the frequency rise to force were almost constant even when different speeds, 2) in ballistic contractions, increase of firing rate and different mode of discharge frequency were observed. It is suggested that the different variation of surface e.m.g. amount between ramp and ballistic contraction in previous our report (Brain Res., 275:305-309, 1983) was supported partly by present results.

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Dependency of Conduction Velocity on Spike Interval during Voluntary Muscle Contraction in Human Motor Units. MORIMOTO, S., UMAZUME,Y. and MASUDA, M. Dept. of Physiol. Jikei Univ. Sch. of Med. Minato-ku, Tokyo.

The dependency of the conduction velocity of the action potential on the spike interval from preceded one was studied on human single motor units during voluntary contraction. Spike potential was recorded from the skin surface overlying m.vastus medialis by use of surface electrode. The results were as follows: 1) The conduction velocity increased with the decrease in spike interval. 2) After arterial occlusion, there was weak or no relation between two parameters. This deformed relation did not restored to the initial form within 15 min. 3) Similar effect as the occlusion could be seen in the effect of prolonged isometric contraction. But the deformed relation was restored to initial form within 15 min recovery. 4) At low muscular temperature, the conduction velocity decreased as the spike interval shorteded. After the ice removed, the relation restored to initial form for 30 min.

The mechanism of these changes are still uncertain but some possible factors can be considered, for example, composition of extracellular fluid, pH, temperature and permiability of the membrane.

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MOTOR UNIT PROPERTIES OF THE RAT MEDIAL GASTROCNEMIUS MUSCLE. KANDA, K., NOMOTO, E. AND ASAKI, S. Dept. Physiol., Tokyo Metrop. Inst. Gerontol., Itabashi, Tokyo 173

Isometric contractile properties of motor units were measured in medial gastrocnemius (MG) muscle of the adult rat (Fischer, male, 12-month-old). The ventral root was dissected untill it was ensured that it contained only one motor fiber to MG muscle. The criterion was an all-or-none response of mechanical twitch and EMG. As in cat hindlimb muscles, rat MG motor units were classified into the categories, type F and type S units, using a criterion (presence or absence of the "sag" property). Most of the type F units exhibited significant post-tetanic potentiation of twitch tension and a corresponding lengthening of twitch time to peak (TwTP). TwTPs of type F and S units were 15.7 ± 2.5 ms and 30.0 ± 4.7 ms, respectively. Tetanic tension (TetT) of type F units was much larger than that of type S (11.3 ± 5.2 gwt vs. 1.5 ± 0.6 gwt). All of type S units were very fatigue resistant. Fatigue index (FI) of type F units showed a bimodal distribution. The positive correlation between TwTP and TetT, and the negative correlation between TwTP and FI were observed among type F units. Histochemical staining of the whole muscle was also performed. Type I muscle fibers were distributed only in a limited portion in the MG muscle. Number of type I fibers was estimated at about 6 % of the total MG fibers. Histochemical profiles and cross-sectional area of each fibers were further analyzed by using the image analysis system (Quintmet 900).

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ELECTROMYOGRAPHIC ANALYSIS OF INHIBITORY EFFECT OF ACUPUNCTURE ON VIBRATION-INDUCED FINGER FLEXION REFLEX. TOMA, S*., NAKAJIMA, Y. AND HOMMA, S. Dept. Physiol., Sch. of Med., Chiba Univ., Chiba 280

Inhibitory effect of acupuncture on vibration-induced finger flexion reflex was examined in 10 normal subjects by recording single fiber EMGs provoked by vibratory stimulation of triangular waveform applied to the volar side of the index finger at random intervals. Cross-correlograms between the vibratory stimuli and the motor unit spikes revealed two types of reflex responses. In eight subjects, cross-correlations showed a sharp peak of time-locked spikes to the vibratory taps at a latency of 25 ms, which indicates monosynaptic activity. The cross-correlograms also consisted of time-unlocked spikes, which do not contribute to the peak and imply polysynaptic activity. This type of response was supposed to be elicited by the tonic vibration reflex (TVR). Cross-correlograms obtained from the remaining two subjects revealed no peaks, which indicates that only time-unlocked spikes to the taps were generated and that these spikes were evoked via polysynaptic pathway. This type of response was supposed to be elicited by exteroceptive vibration-induced finger flexion reflex. An acupuncture inhibited the both types of reflex responses, i.e. mono- and polysynaptic ones. It was supposed that the acupuncture depresses the level of motoneuronal excitability in the spinal cord.

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INFLUENCE OF PAIN STIMULATION ON THE BODY SWAY IN MAN. ASADA,H., SANADA,T*, MIURA,T*, NISHIO,S* AND IWASE,Y. Dept. of Physiology, *Dept. of Health and Physical education, Meiji College of Oriental Medicine, Hiyoshicho, Funaigun, Kyoto 629-03

Somatosensory inputs play important roles in maintaining the upright standing posture. This study examined the influence of cutaneous pain stimulation on standing posture. Body sway was measured with the gravicorder as the deviation of gravity center on standing posture with eyes closed. The repetitive square wave pulses for 10 sec at 50Hz, duration of 10 msec were given through the bipolar surface electorodes held in contact with the skin over the lateral gastrocnemius or the tibialis anterior of both lower limbs. The results were as follows; 1) The sway alteration was not observed during the subthreshold or threshold pain stimuli. The effects of suprathreshold pain stimuli on body sway were classified into three types; foward deviation type, backward deviation type and no alteration type. These shifts of gravity center were often accompanied with the increase of body sway. 2) The body sway was increased with the strength of suprathreshold pain stimulation. 3) There was no particular difference among the sway shift patterns during stimulation to motor points,non motor points and meridian points in lower limbs. These results suggest that pain inputs from the skin greatly influence not only the nociceptive reflex system in the spinal level but also the posture control system in the brain stem or the higher level.

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PATHOLOGICAL CHANGES IN MUSCLES OF GENOTYPICALLY DYSTROPHIC-DWARF MICE.

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In order to elucidate the nature of dystrophic process at preclinical stages, we studied muscles of genotypically dystrophic-dwarf(dy-dw)mice. Since dytrophy was latent in dy-dw mice, they could not be distinguished from dw ones. Recently, we found that amplitudes of muscle potentials in gastrocnemius evoked by 5-Hz stimulation of sciatic nerves decreased markedly with time in normal and dw mice but almost not in dy(and possible dy-dw)ones. Thus, dy-dw mice were identified.

Fiber-size variation and central nucleation were pronounced in the forelimb triceps of dy-dw mice. Interestingly, central nuclei were observed not only in small-sized but also in hypertrophied fibers. This strongly suggests that central nucleation might not result from degeneration-regeneration because regenerating fibers must be very small.

It was concluded that fiber-size variation and central nucleation might be characteristic of mouse muscular dystrophy at preclinical stages.

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ADAPTIVE STRATEGY OF THE CIRCADIAN LOCOMOTOR RHYTHM OF PARAMECIUM - II: ROLE OF EXTRACELLULAR Ca^{2+}. HASEGAWA, K. AND TANAKADATE, A. Dept. Physiol., Sch. Med., Kitasato Univ., 1-15-1, Kitasato, Sagamihara, Kanagawa 228

Effects of extracellular Ca^{2+} on circadian variations of following three parameters of a population of a ciliate protozoan, Paramecium, were examined: 1) hourly number of the specimens which traverse a small point in an experimental vessel (traverse frequency = TF), 2) average swimming speed (V/mm), and 3) the frequency (F_{AR} %/hr) of avoiding reaction. These parameters were measured by a "microcomputerized close-up video photoamplifier system" (Hasegawa et al, Photochem. Photobiol, 1984 in press). In a hay infusion, to which 8 mM of $CaCl_2$ was added, TF transiently increased immediately after the onset of light, and transiently decreased immediately after the offset of light. The transient increase and decrease, both of which lasted for about 1 hr, seemed to be superimposed on rhythmic TF observed in the control infusion. Under constant darkness, TF was observed to be sustained almost similarly to the "control TF", with a free-running period of 24.5 $\pm$ 1.4 hr. If compared with the control, V seemed to be suppressed during the light period, with a transient increase immediately after the onset of light. F_{AR} fluctuated almost similarly to the TF.

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OFF-SUPPRESSION TO COLORED LIGHTS IN LOCOMOTOR ACTIVITY OF THE GIANT LAND SNAIL, ACHATINA FULICA. OGASAWARA, T. AND TASAKI, K. Dept. Med., Tohoku Univ., Sendai, Miyagi 980

An light-off suppression of locomotor activity of giant land snail, Achatina fulica, was studied in the dark period of LD 12:12 cycle in which blue or red light pulses were presented. This off-suppression was reproducible to each imposed light pulse with a longer period of time than 15 minutes. Absolute light sensitivity at threshold of the animal was examined by the behavioral test using the colored light pulses over 15 minutes. Threshold intensities were about 10^{-8} $\mu W/cm^2$ for the blue light (a peak wavelength, 475 nm) and about 10^{-4} $\mu W/cm^2$ for the red light (two peak wavelengths, 630 and 660 nm). The ratio of the blue light sensitivity to the red light one is nearly consistent with those obtained by electrophysiological measurements of optic nerve discharges of the animal.

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EFFECTS OF RHYTHMICITIES OF THE NURSING MOTHER RAT ON PUPS CIRCADIAN SYSTEM. SHIRAKAWA, T.*, OIKAWA, K*, HONMA, K., HONMA, S. AND HIROSHIGE, T. Dept. of Pediat. Dent., Sch. of Dent.,* Dept. of Physiol., Sch. of Med. Hokkaido Univ. Sapporo 060

The role of nursing mother in phase-setting of the pups circadian system was reexamined in mother-pup exchange experiments by measuring the spontaneous locomotor activity of pups. Five males in a litter were blinded by bilateral ocular enucleation on the day of birth and nursed together (2 litters). The light-dark cycle was the same during the pregnant and nursing periods. A strong correlation was observed between the phase of the circadian rhythm at weaning and the free-running period of the rhythm, suggesting a coincidence of the phase in individual rhythms around the perinatal period. The area covered by the regression line with 3SD was defined as the pregnant zone and that shifed by 180° as the nursing. When 6 blinded pups, 3 of them born to a mother in the reversed light cycle, were nursed together (2 groups), the circadian phases of unexchanged pups was located in the pregnant zone, whereas those of exchanged pups was not in either of the zones. In contrast, when 2 pups, one from a reversed mother, were nursed together (6 pairs), the phases were located within the nursing zone in the half of exchanged pups and those in the rest half were between two zones. It is concluded that the circadian rhythm of nursing mother is capable of entraining the circadian system of blinded pups. The potency of the maternal zeitgeber depends on nursing conditions.

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ENTRAINING EFFECTS OF RESTRICTED DAILY FEEDING ON THE CIRCADIAN OSCILLATOR OF INFANTILE RATS. HONMA, S., HONMA,K. AND HIROSHIGE, T. Department of Physiology, Hokkaido University School of Medicine, Sapporo 060

The maternal effects of the phase of the pups' circadian system was examined by imposing restricted daily feeding (RF) on mother rats during pregnancy and nursing periods, and the circadian rhythm of spontaneous locomotor activity developed in neonatally blinded pups was measured by an Animex. Experiment I; RF was imposed on mother rats on 4 different schedules from 2 weeks prior to conception (C) till weaning at 21 days. The control group was fed ad libitum. Experiment II; RF (meal feeding from 06:00 to 10:00h) was imposed on mothers and pups during 5 different periods between C and 6 weeks after birth. A strong positive correlation was detected in the control blinded pups between the phase of the circadian rhythm at weaning and the free-running period of the rhythm, suggesting that the individual rhythms were in phase around the perinatal period. However,the phases of the rhythm of pups in Exp.I were located outside the control phase but close to the respective time of meal feeding. In Exp.II, the circadian rhythm was entrained by RF only in the group which was subjected to RF from C till 2 weeks after birth. It was concluded that the pups' circadian system can be entrained by a mother who has been subjected to RF during the early nursing period (until 2 weeks after birth). RF on pups after weaning was not effective on their circadian system.

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STRUCTURE OF THE RAT CIRCADIAN SYSTEM - ANALYSIS OF PHASE RESPONSE CURVES. HONMA, K. HONMA, S. AND HIROSHIGE, T. Department of Physiology, Hokkaido University School of Medicine, Sapporo 060

To understand the oscillatory mechanism underlying the rest-activity cycle of rats, phase response curves (PRC) were constructed for the circadian rhythm of spontaneous locomotor activity. Ten male rats were transfered to constant dark (DD) and exposed to brief light pulse (30 min) every 2 weeks. The phase shift of the rhythm yielded by the pulse was evaluated differently by measuring the phase shift on the day following the pulse delivery (immediate PRC), the shift after a steady state of the circadian rhythm was achieved (steady state PRC), the phase shift of activity onset as a phase reference (onset PRC) and the shift of activity offset as a reference (offset PRC). Significant differences were observed in PRC shape between the onset and offset PRCs as well as between the immediate and steady state PRCs. The PRC shape depended on free-running period of the rhythm in DD and on activity time. There were systemic changes in activity time after the light pulse, which depended on the circadian phase. It is concluded that the rest-activity cycle of rats is regulated by two circadian oscillators which are internally coupled and which regulate two activity components independently, in which activity onset and offset are included respectively.

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EFFECTS OF PHOTOPERIOD AND AMBIENT TEMPERATURE ON CIRCADIAN LOCOMOTOR ACTIVITY RHYTHM IN THE SMALL MAMMAL. TOKURA, H. AND OISHI, T. Div. Human Life Environ. Sci., Graduate Div. Human Culture, Nara Women's Univ., Nara 630

Effects of temperature cycle (25°C during light and 10°C during dark) on circadian locomotor activity rhythm were studied in the dark active Djungarian hamster entrained by LD 6:18. Amounts of activity /24 hr showed drastic reduction with the environmental conditions from cyclic to constant (25°C) temperature (T_a) and recovered with the environmental conditions from constant to cyclic T_a. However, no consistent changes in the amounts of activity did not occur in the animal kept for a few months under constant T_a, even when the animal was placed from constant to cyclic T_a. Phase angle difference (hr) between activity onset and light off times seemed to approach to zero value with the environmental conditions from constant to cyclic T_a. Our findings suggest that, although temperature cycles only cannot entrain circadian activity rhythm fully in this species, both LD and temperature cycles interact as Zeitgeber.

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ENTRAINING MECHANISM OF ENDOGENOUS RHYTHM OF BLINDED RAT PUPS BY NURSING MOTHER. SASAKI,Y. AND TAKAHASHI,K. Dept. of Medical Chemistry, Tokyo Metropolitan Institute for Neurosciences, Tokyo 183

By means of measurement of the circadian drinking rhythm the duration required for the entrainment of the endogenous rhythm by foster mothers with the rhythm inverted to that of the natural mother was investigated by changing the duration of nursing period by foster mother rainging from 4 to 10 days. The period of 10 days was sufficient for the entrainment, while 4 or 7 days were inadequate. This fact, together with our previous report, indicates that nursing of the foster mother must be started early stage of nursing period (before 4 days) and must be continued longer than 10 days. The fact that foster mothers 10 days after parturition could entrain the endogenous rhythm of day 1 pup, while the rhythm of pups 10 days of age was not entrained by the foster mother immeadiately after parturition, indicates that immaturity of the brain of pups is essential for the entrainment of the circadian rhythm of blinded pups by the foster mothers.

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CIRCADIAN RHYTHM OF BODY TEMPERATURE IN SPONTANEOUSLY-HYPERTENSIVE-RATS(SHR). ISOBE, Y., OHARA, K., HASEGAWA, Y.*AND AOKI, K.° 2nd and 1st* Dept. of Physiol. and Dept. of Med., Nagoya City Univ. Med. Sch., Mizuho-ku, Nagoya 467

To know the biological clock(s) inherently pre-programed or learnt, the circadian rhythms of running wheel activity and body temperature were examined on SHR and Wistar-Kyoto-rat(WKY). Body temperature was measured by a rectal thermometer at 4 h intervals throughout a 24 h period. Circadian rhythm of running wheel activity and body temperature were well entrained to light(150 lx, 0700-1900) and dark(2 lx) cycle at rearing temperature of 25 °C. The rhythms were measured on rats during warm(30 °C) or cold(16 °C) adaptation under the same lighting regime as above. During adaptation period, the running wheel activity was recorded continuously and the body temperature was measured for 4 cycles(1st, 4th, 11th & 25th day). The range of circadian rythm of body temperature was amplified by warm adaptation(25th day) mean level of body temperature was elevated in SHR while it was reduced in WKY. The shift of the zenith-arriving-time of body temperature was seen even under the light entrained condition during the thermal adaptation. These results are comparable with our former findings that the free-running period of body temperature was longer in WKY than in SHR with damping of oscillation in both kinds of rats.

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CIRCADIAN RHYTHMS IN MULTIPLE UNIT ACTIVITY INSIDE THE SUPRACHIASMATIC NUCLEUS IN THE CHIPMUNK. SATO, T. AND KAWAMURA, H. Lab. of Physiol. Psychol., Dept. of Neurosci., Mitsubishi-Kasei Institute of Life Sciences, Machida-shi, Tokyo 194.

The characteristics of the multiple unit activity (MUA) rhythms inside and outside the suprachiasmatic nucleus were examined in a diurnal rodent, the siberian chipmunk (Eutamias sibiricus). We have already reported that the circadian rhythms of wheel-running activity and sleep-waking were abolished after lesions of bilateral SCN. Circadian rhythms of MUA inside the SCN showed daytime peak in activity that paralleled MUA rhythms outside the SCN and overt activity rhythms. No phase reversal of MUA rhythms was observed. Furthermore, the onset of anticipatory activity coinsides with the beginning of significant increase of MUA. Simultaneous recording of MUA rhythms from bilateral SCN in DD condition revealed MUA rhythms of left and right SCN that were consistently in phase throughout the recording period (12 days), showing a tight coupling between left and right SCN.

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MULTIPLE UNIT ACTIVITY RELATED TO VERTICAL EYE MOVEMENTS IN OPERANT LEARNING OF EYE MOVEMENTS. IKEGAMI, S. AND KAWAMURA, H. Lab. of Physiol. Psychol., Dept. of Neurosci., Mitsubishi-Kasei Institute of Life Sciences, Machida-shi, Tokyo 194.

Operant conditioning of vertical eye movements was established in total darkness by rewarding stimulation of the lateral hypothalamus (LHT) in encéphale isolé cats under local anesthesia when visual feedback associated with eye movements was eliminated. Multiple unit activity (MUA) was recorded from the subthalamic region which included the zona incerta (ZI) and H field of Forel (HF). This region is located in the pre-oculomotor pathway. The discharge number of MUA up to 3.0 sec before and after the onset of eye movement was averaged every 16 eye movements and analyzed quantitatively using a signal processor connected to a micro-computer. The EOG was averaged similarly. The MUA recorded in the ZI and HF showed a specific enhancement of discharge rate preceding the onset of spontaneous eye movements in the control period. Such discharges gradually increased in the course of establishment of the learning which lasted 60-120 min, and gradually decreased to the control level during the extinction which lasted 60-90 min. When LHT stimulation was delivered randomly with respect to eye movements, the discharge rate preceding eye movements did not increased significantly. These results suggest that such marked increase of the neural activity in the conditioning demonstrates involvement of the subthalamic area in the operant learning process.

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

Mice of ddN-F30 strain, weighing between 20 to 30 grams, were used in this experiment. Slow bilateral injections were given at several places and different depth. Each animal received 0.2 μl of saline containing 0.075 μg of kainic acid. Two weeks after administration each mouse was tested in the open-field activity and the avoidance training. Upon complection of the avoidance trainings, all mouse brains were examined for histological preparation, and compared with training scores and histological results. It was found that there was a close association between the rate of learning deficits and the degree of degeneration of the pyramidal cells in area CA3 of the hippocampus in mice treated with kainic acid.

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CHANGES IN LEARNING ABILITY ON MULT FR 30, FI 60" REINFORCEMENT SCHEDULE AND IN MONOAMINE LEVELS IN THE CEREBRAL HEMISPHERE OF EXPERIMENTAL MICROENCEPHALIC RATS TREATED PRENATALLY WITH METHYLAZOXYMETHANOL(MAM). TAMARU, M., NAGAYOSHI, M., HIRATA, Y., MATSUTANI, T. and *SUGIMOTO, S. Dept. of Dev. Physiol., Inst. for Comprehensive Med. Sci., Fujita-Gakuen Univ., Sch. of Med., Toyoake, Aichi 470-11, *Dept. of Behavioral Sci., Fac. of Integrated Arts and Sci., Hiroshima Univ., Hiroshima 730.

Cerebral hemisphere(CH) weights and DNA content per CH in the offspring treated prenatally with MAM on days 11-13(15mg/kg/day; group I) or 15(20mg/kg; group II) of gestation markedly reduced by about 75 and 55% of those in the control at 30 days after birth, respectively. Concentrations of norepinephrine and 5-hydroxytryptamine in CH of group II markedly elevated about two times of the control, but total contents of those in CH did not change. While, concentrations of both monoamines in CH of group I did not elevate so far as the control, but total contents of those in CH significantly reduced. The learning test was started at the age of 80 days, group II was able to discriminate FR 30 from FI 60", but showed no typical pattern of response as observed for the control group, showing higher activities. While, group I showed remarkable increase of inter-response time and post-reinforcement pause and could not learn this schedule.

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VIDEOTAPE ANALYSIS OF THE RELATIONSHIP BETWEEN COPULATORY BEHAVIOR AND MPO NEURONAL ACTIVITY IN MALE RATS. HORIO, T., SHIMURA, T. AND SHIMOKOCHI, M. Dept. Human Sci., Osaka Univ., Suita, Osaka 565

We have reported that the neuronal activity in the medial preoptic area (MPO) of male rats increased during the pursuit-mounting behavior. From a simple observation, however, it is difficult to determine the exact temporal relationship between copulatory behavior and neuronal activity since the behavior consists of a sequence of very rapid acts. Therefore, we attempt to analyze it by a special video system which will take a picture with a synchronized sweep of an oscilloscope. As video- picture was 30 frames/sec, we could identify the discrete start of these acts with an accuracy of about 33 msec. The mount duration with intromission was relatively constant (300-500 msec), whereas the mount duration without intromission was variable (50-1000 msec). The mount duration with ejaculation was long (1-5 sec). The increased firing rate during intromission began to decrease within 300 msec after the dismount. Without intromission, it sometimes began to decrease by the dismount. Accompanied with ejaculation, it always began to decrease between the start of thrust and dismount.

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SYNCHRONIZATION OF SLEEP-WAKING RHYTHM TO THE LD CYCLE IN THE CAT. KUWABARA, N., AOKI, K. AND *SEKI, K. Life Sci. Inst. Sophia Univ. Tokyo. *Jap. Marine Sci. Tech. Center, Yokosuka.

We can know, by means of continuous longitudinal EEG poligraphic observation, that sleep-waking pattern of cats more or less synchronized to the light-dark cycle in the conventional LD12:12 condition. There is much total sleep time in the light phase. While in the continuous dark or light sleep-waking pattern tend to be an ultradian rhythm as time goes on. In the present study cats that are usually in LD12:12 were kept in the dark and then exposed to LDcycle again. Results show that sleep-waking pattern resynchronised after exposed to LD cycle again. In the second experiment LD cycle was advanced for 6 hrours then, after about two weeks, it was delayed for 6 hours. Results show that synchronization required for a few days, which is one of the characteristics of the circadian rhythm. From the results we are suggested that domestic cats have characteristic both of circadian rhythm and ultradian rhythm.

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AROUSAL FROM SLEEP IN CATS: A BRAIN ELECTROSTIMULATION STUDY. MATSUMOTO, H., MORITA, Y. AND SENO, H. Dept. Physiol., Sch. Med., Univ. Tokushima, Tokushima 770

We previously succeeded to induce sleep from arousal in cats and humans with inhibitory brain electrostimulation of low voltage and low frequency as a result of suppression of the sympathetic activity. The present study was undertaken to prove the hypothesis that excitation of the sympathetic activity may play an important role in arousal from sleep in cats. The ventromedial nucleus of the hypothalamus (VMH), a sympathetic center in the brain, and the cerebral cortex (F-O) were electrically stimulated with low voltage rectangular pulses (0.5-5 V, 0.2 msec) of gradual increase frequency varied from 14 to 80 Hz. Examining the effects of the brain stimulations on arousal from sleep under polygraphic recordings, we found the following results in a representative cat. In the stimulation of the VMH, EEG activation was elicited with current intensity of 0.09-0.10 mA and behavioral arousal with that of 0.10-0.13 mA. In the stimulation of the cortex, EEG activation was elicited with current intensity of 0.4-0.5 mA and behavioral arousal with that of 0.5-0.6 mA. From these, the EEG activation and arousal threshold was lower in the VMH stimualtion than in the cortex stimulation. Further studies are needed to elucidate arousal mechanism from sleep hereafter.

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PERIODICITY IN REM-NREM SLEEP CYCLE OF CATS. OIKAWA,T., FUJITANI,Y. AND HOSOGAI,M. Dept. of Physiol., Tottori University School of Med., Yonago 683

Periodicity in the sleep-wakefulness cycle was determined by autocorrelation function and power spectrum in 4 chronic cats. Binary modification was applied to the time series of sleep-wakefulness stages scored in every 1-min epoch of 24 h polysomnographic records. For the real-time data, wakefulness and NREM sleep were assigned the value 0, and REM sleep the value 1. For the compressed data in which all wakefulness stages were removed, the value 0 was assigned to NREM sleep and, again, 1 to REM sleep. The series of 0 and 1 were entered into both autocorrelation and power spectrum analyses. In almost all data of spontaneous sleep for 24 h of 4 cats, for 7 days of 1 cat, for each of 7 days after the respective 9 h total sleep deprivation of 2 cats, for each of 4 days after 12 h sleep deprivation of 2 cats and for each of 5 days after 15 h sleep deprivation, the periodicity of REM-NREM cycle was stronger in the compressed data than in the real-time data. The period lengths showed no significant difference in both except for a few. These results support sleep-dependent theory in the REM-NREM cycle.

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CENTRAL MECHANISMS OF SLEEP-WAKEFULNESS IN THE RAT. HANADA, Y. AND KAWAMURA, H. Lab. of Physiol. Psychol., Dept. of Neurosci., Mitsubishi-Kasei Institute of Life Sciences, Machida-shi, Tokyo 194.

To study brain stem sites presumably inducing polygraphic sleep-wakefulness changes, multiple unit activities (MUA) were recorded in chronically electrodes implanted male Wistar rats. Recordings were made from 62 sites in 45 rats.

Earlier MUA changes preceding polygraphic changes from wakefulness to slow wave sleep were observed mostly in the medial preoptic area. On the other hand, loci demonstrating earlier MUA changes preceding the onset of paradoxical sleep from slow wave sleep were in many cases in the posterior hypothalamus. MUA changes from slow wave sleep to wakefulness were rather prompt in these sites. In the chronic cerveau isolé rats, MUA changes preceding ECoG changes were observed in the hypothalamus but not in the locus coeruleus.

These findings strongly suggest importance of the hypothalamus rather than hind brain "sleep center" for induction of sleep-wakefulness changes.

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INTERREGIONAL RELATIONSHIPS OF THE SCALP EEG DISPLAYED AS VECTORS OZAKI, H., SUZUKI, H. AND *LEHMANN, D. Lab. Physiol. Develop. Handicap., Ibaraki Univ., Mito 310, *Neurol. Zurich Univ.

Wave form of the EEG is essentially determined by the potential difference measured between electorodes. This is important in the case of the spectral analysis and evaluation of wave form from multi-channel EEG arays. The spatial distribution of the potential changes dramatically when the reference point is changed. To avoid this ambiguity, vectorial representation of the frequency components were performed. Products of the Fast Fourier Transform of simultaneously recorded 16 channel EEGs were projected onto the two dimentional space as a sin/cos diagrams. In the diagram, the origin corresponds to the position of the reference and the distance from the origin represents the mean amplitude between reference and each another electrode. In this methods, the relative position of each another electrode does not changed wherever the reference is, and spatial distribution of the EEG could be evaluated much more effectively.

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BRAIN LATERALITY --- STUDIED BY PRINCIPAL COMPONENT ANALYSIS OF DOMINANT HAND, FOOT, EAR AND VISUAL FIELD.

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Brain laterality was studied by means of the principal compo nent analysis method on healthy adult male and female. The dichotic listening test was applied to English words as well as the addition of simple number. Computer tachistoscope was developed in the present work.

The principal compo nent analysis on enquête report of handedness revealed four components (eigen value > 1.0). The first component (56% of component loading) is tentatively named "Dynamic motion"; the second (7% of loading) "Set right and habit"; the third (6% of loading) "Consciousness and skillness"; the fourth (5% of loading) "Both hands task". Significant correlation was detected between handedness and dominant ear ($r=0.18$, $p<0.01$, $N=696$, male and female); between dominant foot and ear ($r=0.11$, $p<0.05$, $N=341$, male).

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AFFINITY PURIFICATION OF Na-CHANNELS FROM THE EEL ELECTRIC ORGAN. NAKAYAMA, T and TERAKAWA, S. Dept. Cell Physiol. Natl. Inst. Physiol. Sci., Okazaki, 444

Tetrodotoxin can be modified at position C_6 and C_{11} without affecting its toxicity. We found that tetrodotoxin is bound covalently with boric acid at the cis-diol of C_6 and C_{11} position above neutral pH. The complex is readily dissociated by lowering pH below neutral. We have prepared a complex of tetrodotoxin, boric acid and avidin to construct an affinity column for purification of Na-channels. Na-channels solubilized from the eel electric organ were applied on a biotin-agarose column, after washing the column, Na-channels were eluted with excess tetrodotoxin. SDS-polyacrylamide gel electrophoresis revealed that Na-channels purified by this method were mainly consisted of 260 - 270 K dalton polypeptides.

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PURIFICATION OF THREE AZIDE DERIVATIVES OF TETRODOTOXIN AND PHOTOAFFINITY LABELING OF ELECTROPHORUS ELECTRICUS ELECTROPLAX MEMBRANES. UEHARA, S. AND UYEMURA, K. Dept. of Physiol., Saitama Med. Sch., Moroyamacho, Irumagun, Saitama, 350-04.

Tritiated azide derivatives of tetrodotoxin (TTX), 2-nitro-4-azidepheny1-(^{3}H)-β alanine-TTX(NAP-β alanine-TTX), were synthesized and purified with ion exchange chromatography using Bio-Rex 70 resin (NH_4^+ form) and with reverse phase high performance liquid chromatography (ZORBAX ODS). Three derivatives, D1, D2 and D3 were obtained with these methods. Lethal dose for crayfish is 0.4 of that of TTX for D1, 0.07 for D2 and 0.04 for D3. D1 degraded at a rate of 10%/hr at 20 °C and 1%/hr at 4 °c in distilled water and released NAP-β alanine and TTX. D2 and D3 degraded at slower rate than D1. All three derivatives hardly showed any degradation in 0.1% acetic acid at 4 °C. TTX binding sites of Electrophorus electricus electroplax membranes were photolabeled with the derivatives in the presence or absence of TTX. D3 binded more effectively to the TTX binding sites than D1 and D2. Lubrol soluble fraction of the membranes labeled with D3 was analyzed with Sephadex G 25 column (15 x 400 mm, 0.1% Lubrol PX-50 mM ammonium acetate). The labeled component was eluted at elution volume greater than the total bed volume (Kav=2.1). The component showed high specific binding activity of up to 500 times than that of the membranes.

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CALCIUM DEPENDENCE OF MEMBRANE RESEALING PROCESS IN RUPTURED NERVE AXONS. YAWO, H. AND KUNO, M. Dept. Physiol., Kyoto Univ., Fac. Med., Sakyo-ku, Kyoto 606

Following transection of a cockroach giant axon, the nerve membrane at the cut end is resealed within 5-30 min. This membrane resealing process is highly dependent upon extracellular Ca^{2+}. When Ca^{2+} was replaced with equimolar Mg^{2+} or Sr^{2+} or Mn^{2+}, the axon failed to reseal the cut end. The obligatory extracellular Ca^{2+} concentration was 0.1-0.5 mM. When EGTA was injected ionophoretically into the axon, the membrane resealing was prevented in the presence of extracellular Ca^{2+}. When Ca^{2+} was injected into the axon, the membrane once ruptured was resealed in the absence of extracellular Ca^{2+}. These results indicate that the site of action of Ca^{2+} is the inside of the axon. Since Ca^{2+}-activated phospholipase A_2 is known to be present in the inner layer of the cell membrane, the possible involvement of this enzyme was tested by two phospholipase A_2 inhibitors, p-bromophenacyl bromide and mepacrine. Both the inhibitors prevented the membrane resealing in a dose-dependent fashion. One of the products by the action of phospholipase A_2 is lysophospholipids which may act as a fusogen for the ruptured nerve membrane. The injection of lysolecithin into the axon caused a partial resealing of the ruptured nerve membrane in the presence of an inhibitor for phospholipase A_2.

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INTRACELLULAR Ca^{2+} ELEVATION INDUCED BY SEROTONIN IN A CLONAL GLIAL CELL.
OGURA, A., SUGINO, H., KUDO, Y. AND AMANO, T. Department of Neuroscience, Mitsubishi-Kasei Institute of Life Sciences, Machida-shi, Tokyo 194, Japan.

Neuroglial cell is multifunctional. For the manifestation of a particular function, signal molecules, though chemically unidentified, are supposed to be transferred from neuron to glia. Here, we assume that neurotransmitters may act as such signal molecules. We have recently reported that newborn rat cerebral glia cells and rat clonal astroglioma (C6) cells under culture responded to serotonin by membrane hyperpolarizations. In a further analysis using a fluorescent Ca^{2+}-indicator Quin 2, we found an elevation of intracellular Ca^{2+} concentration in parallel with the serotonin - induced hyperpolarization in C6 cells. Upon an addition of 1 μM serotonin, the Quin 2-loaded C6 cell suspension showed an increase of fluorescence intensity, converted to an about two-fold rise of the intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). The magnitude of $[Ca^{2+}]_i$ rise depended both on the dose of serotonin applied and on the environmental Ca^{2+} level. This suggests that a Ca^{2+}-influx from the extracellular space was triggered by serotonin. This suggestion was supported by a ^{45}Ca uptake measurement. The $[Ca^{2+}]_i$ rise was suppressed by cyproheptadine and verapamil. Since $[Ca^{2+}]_i$ is known to regulate various cellular activities, we suggest the relevance of neurotransmitter as the signal molecule from neuron to glia.

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ANTICONVULSANT MECHANISM OF DIPHENYLHYDANTOIN AT THE CELLULAR LEVEL.
KISHII, K., ONOZUKA, M., FURUICHI, H., SUGAYA, E. AND *SUGAYA, A. Dept. of Physiol., Kanagawa Dental College, 82, Inaoka-cho, Yokosuka and Faculty of Pharmaceutical Sciences, Josai University, Saitama

To clarify the anticonvulsant mechanism of diphenylhydantoin (DPH), effects of DPH on pentylenetetrazole (PTZ)-induced bursting activity in snail neurons were investigated from the biochemical, morphological and electrophysiological viewpoints and the following results were obtained. (1) Increase in intracellular cAMP induced by PTZ was inhibited by DPH. (2) PTZ-induced calcium release from the lysosome-like granules accompanied with morphological changes was inhibited by DPH. (3) Increased activity of protein kinase in the presence of calcium was normalized by DPH. (4) PTZ-irduced bursting activity was completely inhibited by either intracellular injection or extracellular application of DPH.

These findings indicate that the anticonvulsant effect of DPH is mainly due to the inhibition of the intracellular cAMP increase as well as the inhibitory effect on the activation of Ca-dependent protein kinase which plays an important role in the generation of bursting activity.

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PENTYLENETETRAZOLE-INDUCED BURSTING ACTIVITY AND CELLULAR PROTEIN PHOSPHORYLATION IN SNAIL GANGLIA.
IMAI, S., ONOZUKA, M., KISHII, K. AND SUGAYA, E., Dept. of Physiol., Kanagawa Dental College, Inaoka-cho, Yokosuka

To elucidate the intracellular biochemical mechanism of seizure discharge, phosphorylation of cellular proteins during pentylenetetrazole (PTZ)-induced bursting activity (BA) in snail neurons was investigated. Snail ganglia were incubated in the medium containing $[^{32}P]Pi$, and protein phosphorylation of the ganglia was analyzed by means of SDS-gel electrophoresis and autoradiography. PTZ induced a marked increase in phosphorylation of 50,000 and 34,000 molecular weight protein bands. Dibutyryl cAMP showed no effect on the phosphorylation pattern, whereas the effect of calcium ionophore A23187 was almost identical with that of PTZ. Addition of calcium to the reaction medium containing $[\gamma\text{-}^{32}P]ATP$ and protein from the snail ganglia as substrate also induced phosphorylation pattern similar to that observed in PTZ-treated ganglia with $[^{32}P]Pi$. Phosphorylation of 50,000 molecular weight protein band was especially inhibited by the addition of EGTA or chlorpromazine. Calcium ionophore A23187 induced BA followed by a prolonged hyperpolarization. Chlorpromazine completely blocked PTZ-induced BA.

These findings suggest that PTZ-induced BA in snail neurons is probably mediated via calcium-dependent protein phosphorylation system.

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COMPARATIVE STUDY OF CALMODULIN IN THE MOUSE L-929 FIBROBLASTS AND ITS SPONTANEOUSLY TRANSFORMED ONES. TOKUDA, M., DOI, A., ITANO, T., OHMURA, Y., HATASE, O., AND NISIDA, I. Dept. of Physiol., Kagawa Medical School, Mikicho, Kagawa 761-07

We established a cell line(l-N), spontaneously transformed from the original L-929 cells(L-O), and the doubling time of L-N was shorter than that of L-O, 20hr and 23hr, respectively. Calmodulin(CaM), an ubiquitous and multifunctional Ca^{2+}-binding ptotein, plays critical regulatory roles in cell growth especially in DNA synthesis and cell division, and in transformation processes. We purified CaMs from L-O and L-N, and investigated those properties. The contents of CaM during cell cycle were also studied in the both cell lines by two synchronization methods. The results were as follows:

1) The purified CaMs from L-O and L-N cells showed the same migration pattern in electrophoresis, and the activation profile of CaM-dependent phosphodiesterase.
2) The L-N cells contained more CaM than the L-O cells, 0.49μg and 0.32μg per 10^6 cells respectively.
3) In the both cell lines, the content of CaM began to increase from late G1 phase, and continued to increase during G1-S transitional phase, and reached to the maximum plateau until mid-S phase. And it was reduced to a level one half of maximum plateau after M phase.
4) The L-N cells contained more CaM than the L-O cells through whole cell cycle.

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THE EFFECTS OF TRYPTOPHAN ON MYELINATION IN RAT BRAIN DEVELOPMENT NOMURA,M., OHTSUJI,M. and NAGATA,Y. Dept. of Physiol. School of Med. Fujita-Gakuen Univ. Toyoake, Aichi 470-11, JAPAN.

The serotonergic nervous system is closely related to the physiological behavior of animals.Changes of tryptophan content in the diet regulate the activity of the serotonergic nervos system. Two groups of rats were fed , (a) on a tryptophan deficient diet only or, (b) on the same diet plus a 0.1% tryptophan supplement (semideficient diet) for three months after birth. In daily learning sessions, the rats fed on the tryptophan deficient diet showed lower scores, while those on the semideficient diet sowed the same scores as control rats on normal food. So the scores of learning ability depend on the tryptophan content in the diet. The activity of a marker enzyme of myelin, was found to be decreased in the tryptophan deficiet rats. Furthermore, total contents of myelin protein was shown to be lower than those of tryptophan semideficient rats, especially the small and large protein as well as the intermediate protein fractions in myelin on disc electrophoresis were reduced already at this early stage of development, as measured at two to twelve weeks.

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EFFECTS OF NEONATAL INJECTION OF TUNICAMYCIN ON SYNAPTOGENESIS IN RAT CEREBELLUM. MITA, K., KOHSAKA, S., AND TSUKADA, Y. Department of Physiology, Keio University School of Medicine, Shinjuku, Tokyo 160.

The role of glycoprotein in the development of rat cerebellum has been examined using the inhibitor of protein glycosylation, Tunicamycin (TM). TM (0.2 μg) was injected into the newborn rat brain. The gain in the cerebellar weight was strongly affected by the injection, while the body weight was affected to a small extent. The labelling of protein with ^{3}H-glucosamine was decreased by 35% in TM injected rats. However, the incorporation of ^{14}C-leucine into protein was reduced only by 10%. A reduced capacity to bind concanavalin A was observed in histochemical studies. Light microscopic studies have demonstrated that the arrangement of purkinje cells were slightly deteriorated. Abnormal patterns of purkinje cell dendrites were observed by Golgi staining. The concentrations of aspartate, glutamate and glycine were not changed by the TM treatment. However, the concentration of GABA and the activity of glutamate decarboxylase were significantly decreased. In contrast, the activity of choline acetyltransferase and norepinephrine concentration were increased by 30% and 90%, respectively. These results might indicate that the inhibition of glycoprotein synthesis affects the synaptic formation in the cerebellum.

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ACTIONS OF ATP AND ADENOSINE ON MYENTERIC NEURONS OF THE GUINEA-PIG SMALL INTESTINE. MORITA, K., KATAYAMA, Y. AND HIRAI, K. Dept. Autonom. Physiol., Med. Res. Inst., Tokyo Med. and Dent. Univ., Tokyo 101.

Intracellular recordings were made from the guinea-pig myenteric neurons in vitro to study the actions of ATP and adenosine. In majority cells, ATP(10 nM - 100 μM) produced concentration-dependent hyperpolarization up to about 30 mV. The hyperpolarization caused by ATP was always associated with a fall in input resistance and reversed in polarity at about -100 mV, where the afterhyperpolarization of the action potential was also reversed in polarity. Furthermore, the ATP induced-hyperpolarization was observed in calcium-free or TTX containing solutions. Quinine(100 nM - 10 μM) reversibly inhibited the hyperpolarization by ATP. ATP also reversibly reduced the amplitude of all the synaptic potentials such as the fast e.p.s.p, the slow e.p.s.p and the slow i.p.s.p even when ATP caused no appreciable change in the membrane potential and in the sensitivity to ACh and substance P. The actions of adenosine(10 nM - 100 μM) on the membrane potential or the synaptic potentials were essentially the same to those of ATP.

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Depolarizing Effects of S-Adenosyl-L-Methionine upon the Membrane Potentials of the Isolated Spinal Cord. TONOHIRO, T. and IWATA, N. Biol. Res. Labs., Sankyo Co., Shinagawa-ku, Tokyo 140.

The effects of S-adenosyl-L-methionine(SAM) on the membrane potentials were studied, using isolated spinal cords of bullfrogs and newborn rats. FO-1561(FO, Fuji Chemical Ind.) containing SAM-sulfate-tosylate (100 mg) and mannitol(120 mg) in one vial was used because of its high stability. FO(1-3mM as SAM) depolarized ventral(VR) and dorsal roots (DR) of frogs in normal medium and also in tetrodotoxin(TTX, 10^{-7}M) containing medium. FO(1-4mM) also depolarized VR of newborn rats. After blocking synaptic transmission by treating with TTX medium or low-Ca medium, there remained considerably large responses to FO. Mannitol as much as contained in FO(3mM), depolarized VR to the lesser degree, but did not cause any effect (90 sec of perfusion time) in low-Ca medium. Therefore, at least in low-Ca medium, the effect of FO seemed mainly due to SAM. S-adenosyl-L-homocystein, shown to diminish the inhibitory effect of SAM on the Na^+,K^+-ATPase activity in rat brain microsome, also depolarized VR and did not influence FO(1mM) response at 2 mM in low-Ca mdium, so the effect of SAM seems not due to inhibition of the enzyme.

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EFFECT OF A SPIDER TOXIN (JSTX) ON THE SQUID GIANT SYNAPSE. KAWAI, N., SAITO, M.*, MIWA, A., YAMAGISHI, S.**, AND FURUYA, K.** Dept. Neurobiol., Tokyo Metropolitan Inst. for Neurosci., 2-6, Musashidai, Fuchu-City, Tokyo 183, *Dept. Physiol., Japan Dental Univ., 1-9-20 Fujimi Chiyoda, Tokyo 102, **Dept. Biol. Memb., National Inst. for Physiol. Sci., Myodaiji, Okazaki-City, Aichi 444, Japan.

In order to determine the neurotransmitter at the giant synapse of the squid stellate ganglion, neurotoxin (JSTX) -specific blocker of glutamate recetor- derived from a spider (Nephila clavata) was used. JSTX blocked the excitatory postsynaptic potential (EPSP) without affecting the presynaptic action potential or the antidromic action potential. Time for block of EPSP was decreased by raising the toxin concentration. After blockade, no recovery of EPSP was found by washing the preparation for hours. Iontophoretically or bath applied L-glutamate depolarized the postsynaptic membrane but the depolarization was blocked in the presence of JSTX. The results strongly suggest that glutamate is the transmitter at the giant synapse of squid.

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EFFECTS OF ARGININE DERIVATIVES ON EEG ACTIVITY OF RAT. TSURUTA, K.*, TOMA, J.* AND YOKOI, I. Dept. of Neurochem., Inst. for Neurobiol., Okayama Univ. Med. Sch., Shikata-Cho, Okayama 700

We have reported that homoarginine (HArg) increased in the blood of rats administered ethanol chronically[1]. Rythmical high voltage spikes were recorded on EEG about 15 minitues after HArg application on pia matter of rat topically (10 µl of 0.32 M HArg·HCl), whereas arginine (Arg) had no effect. δ-Guanidinovaleric acid (DGVA), was synthesized from O-methyl-isourea sulfate and δ-aminovaleric acid according to Weiss and Krommer[2] with some modification by us and was confirmed by GC/MS, induced spike discharges 5 to 10 minutes after topical application (5 µl of 10 µg/ml solution of DGVA). HArg has one more carbon in its chain than Arg, and α-amino-DGVA is Arg. γ-Guanidinobutyric acid (amidino-GABA) has a convulsive action[3], and has one less carbon in its chain than DGVA. So HArg and DGVA might act on a Arg system, and/or GVA might act on a GABA system.

1) I. Yokoi, J. Toma and A. Mori, Neurosciences (Kobe) 9, 177 (1983)
2) S. Weiss and H. Krommer, Chemiker-Zeitung, 98, 617 (1974)
3) D. Jinnai, A. Sawai and A. Mori, Nature 212, 617 (1966)

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THE ROLE OF CYTOSKELETON IN PARTICLE TRANSPORT IN SAPONIZED AXON.

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We permeabilized single fin nerves of the squid with saponin, and observed the particle movement along their axons by dark-field optics. The particle movement was reactivated specifically by ATP(5 mM), not by GTP nor by ITP. Depolymerizers of microtubules, colchicine(1 mM) and vinblastine(0.1 mM) blocked a directional movement. A local oscillatory movement, however, continued after such disruption of microtubules. Actin depolymerizers, cytochalasin B and 88K protein isolated from porcine brain also blocked the directional particle movement. Actinogelin, a gelating protein of actin filaments appeared to hinder the smooth movement of particles and slowed their velocities. The present results suggest that microtubules are tracks along which the particles travel and actin filaments play a structural role in the particle transport rather than a role in transport force generation. The force seems to be generated from a dynein-like ATPase.

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CELL LINEAGE ANALYSIS OF SCHWANN CELLS IN THE PNS DETERMINED BY SHIVERER-NORMAL MOUSE CHIMERAS.

MIKOSHIBA, K., YOKOYAMA, M., TAKAMATSU, K., OKANO, H., NOMURA, T. TSUKADA, Y. Dept. Physiol. Keio Univ. Sch. Med. Shinjuku-ku, Tokyo 160, Central Inst. Exp. Animals, Miyamae-ku, Kawasaki 213

The myelin of the peripheral nervous system from the shiverer mutant mice is characterized by the absence of myelin basic protein, while the other myelin protein components are present at normal levels. Myelin lamella formation is normal in the shiverer mutant. Therefore, by using antiserum against myelin basic protein, we can distinguish the shiverer from the wild type control myelin immunohistochemically. To study cell lineage of Schwann cells, we have produced chimeras by the aggregation of eight-cell embryos from wild type mice and shiverer mice. Using myelin basic protein as a marker, we observed that Schwann cells in the sciatic nerve existed as patches of cells with like-genotype. The patches occurred in a linear array along the axons with some intermingling of Schwann cells. Complete randomization by intermingling of Schwann cells was not observed and clones of Schwann cells may persist as contiguous group throughout peripheral nerve development.

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A CELL POPULATION OF PRIMARY CULTURES DERIVED FROM RAT EMBRYO CEREBRAL HEMISPHERES. IWASAKI,N. KANEMOTO,K. ASOU,H. HIRANO,S. 2nd Dept. of physiology, Toho Univ. Sch of Med., Ohmori,Tokyo 143

A procedure for the cultivation of fetal rat brain cells was developed. Neurons,origodendrocytes and astrocytes showed morphological differences, but these cells were identified by the use of antisera to cells type specific marker. The cells were identified by using anti-GFAP,anti-GM1,anti-CNPase sera,and also we used Nissl,Bodian-technique for astrocytes,neurons and oligodendrocytes,respectively. After 53days cultivation of primary cultured cells,neurons were not only maintained but could still survive in our culture system. An analysis of the extracted gangliosides by HPTLC revealed that gangliosides component did not change between in vivo and this culture system. The activity of CNPase rans parallel to the myelination, and glutamine synthetase activity was incresing in proportion as the deveropment of culture. These result that this primary culture method may afford a good advantageous over the conventional ones.

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DEVELOPMENT OF THE CEREBELLAR CORTICAL STRUCTURE IN THE CHICK EMBRYO —— A STUDY WITH MONOCLONAL ANTIBODIES AND ORGAN CULTURE. OBATA, K. AND FUJITA, S.C. Dept. of Pharmacol., Sch. of Med., Gunma Univ., Maebashi, Gunma 371

Monoclonal antibodies (MAbs) were produced against the optic nerve of the chick embryo. Among them, 13 MAbs selectively stained the cellular components in the developing chick cerebellum too. Thus, immunohistochemical mapping of the cerebellar cortex from embryonic and newly hatched chicks demonstrated the development of external granular, molecular and internal granular layers. MAb 82E10 was shown to recognize neurofilament proteins and stained Purkinje cells, climbing fibers and basket cell axons only at certain developmental stages. MAb 83C2 stained the surface of Purkinje cells and MAbs 96D2 and 81H9 stained Bergmann glia fibers. Early before differentiation of cortical cells, afferet fibers were observed in the primitive cerebellum. These fibers probably originated from vestibular nerve. Tissue slices were prepared from embryonic cerebellum and kept for 5-10 days in organ culture. Dendritic arborization of Purkinje cells developed rapidly during the the several days in culture.

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IMMUNOHISTOCHEMICAL ANALYSIS OF THE DEVELOPMENT OF THE VISUAL SYSTEM OF THE CHICK EMBRYO USING A LIBRARY OF MONOCLONAL ANTIBODIES. HARA, H., SHIRAO, T., FUJITA, S. C. AND OBATA, K. Dept. of Pharmacol., Sch. of Med., Gunma Univ., Maebashi, Gunma 371

About 70 monoclonal antibodies (MAbs) were obtained against homogenates of the optic nerves from 8 day chick embryos. These were tested by the indirect immunofluorescence method on sections of retinas, optic tecta and optic nerves of chick embryos of various developmental stages. A number of MAbs showed characteristic staining of the sublaminae of the inner plexiform layer of the 18 day embryonic retina, indicating that the respective antigens were differentially expressed by certain chemical subclasses of neurons. MAb 82E10 specific to the large and medium subunits of the neurofilament primarily stained the major efferent and afferent fibers of the developing tectum. In the cross sections of optic nerves of 8 day embryos, MAb 82E10 stained primarily the fibers in the central and ventral regions, while MAb 87C7 stained the fibers in the dorsal region most intensely. MAb 87D10-positive fibers were primarily in the central region. This indicated the presence of chemically heterogeneous populations of optic nerve fibers.

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Effects of α-kainic acid and its derivatives on an identifiable giant neurone, sensitive to β-hydroxy-L-glutamic acid, of Achatina fulica Férussac. Takeuchi, H., Watanabe, K. Dept. of Physiol., Gifu Univ., Sch. of Med. Tsukasa-machi, Gifu 500; Nomoto, K., Ohfune, Y. and Takemoto, T., Suntory Inst., Shimamoto-cho, Osaka 618.

Erythro- and threo-β-hydroxy-L-glutamic acid showed excitatory effects at the same intensity (minimum effective concentration (MEC): 10^{-4} M) on RAPN (right anterior pallial nerve). α-Kainic acid and domoic acid showed stronger excitatory effects (MEC: 3×10^{-5} M-10^{-4} M) on the same neurone, indicating that these two substances are agonists of β-hydroxy-L-glutamic acid in RAPN receptors. α-Kainic acid methylketone had also excitatory effects (MEC: 3×10^{-4} -10^{-5} M); whereas dihydro-α-kainic acid had no effect. α-Allo-kainic acid and α-allo-kainic acid methylketone had also no effect. Two derivatives of α-kainic acid and domoic acid, (2S,3S,4S)-2-carboxy-4-(1-methyl-5(R)-hydroxymethyl-1(Z),3(Z)-hexadienyl)pyrrolidine-3-acetic acid and (2S,3S,4S)-2-carboxy-4-(1-methyl- 5(R)-hydroxymethyl-1(Z),3(E)-hexadienyl)pyrrolidine-3-acetic acid, had also excitatory effects.

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Pharmacological features of β-hydroxy-L-glutamic acid sensitive-neurones of Achatina fulica Ferussac. Watanabe, K., Shiratori, Y., Takeuchi, H., Gifu Univ., Sch. of Med., Tsukasa-machi, Gifu 500; Nomoto, K., Ohfune, Y., Takemoto, T., Suntory Inst., Shimamoto-cho, Osaka 618. Nakajima, T., Nihonshoji Co. Ltd., Ibaragi, Osaka 567.

Several identifiable giant neurones, such as FAN, VIN, PON, RAPN and d-RPLN, were found to be sensitive to β-hydroxy-L-glutamic acid (L-BHGA); whereas they were not so markedly affected by L-glutamic acid. The pharmacological features of L-BHGA receptors in the five neurones were studied by testing effects of two stereoisomers (erythro and threo) of L-BHGA and their derivatives.

L-BHGA inhibited FAN, VIN and PON; it excited RAPN and d-RPLN. α-Kainic acid and domoic acid are considered to be agonists of L-BHGA on only L-BHGA-excited neurones.

Threo-L-BHGA was more effective than erythro on FAN; threo- and erythro-L-BHGA similarly affected RAPN; erythro-L-BHGA was more effective on VIN, PON and d-RPLN. Strong effects of quisqualic acid were noted only on the markedly threo-L-BHGA-sensitive FAN and RAPN.

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Pharmacological features of catecholamine- and monophenolamine-sensitive giant neurones identified in the ganglia of Achatina fulica Ferussac. Ku, B. S. and Takeuchi, H. Dept. of Physiol., Gifu Univ., Sch. of Med., Tsukasa-machi, Gifu 500.

Of the substances examined, dopamine (DA) was the most excitatory on PON (periodically oscillating neurone) (effective potency range (EPR) : 1.0); the following three subatances were also excitatory: epinine (EP) (EPR: 0.3), L-norepinephrine (L-NE) (EPR: 0.1) and L-epinephrine (L-E) (EPR: 0.1). EP was the most inhibitory (EPR: 1.0) on both TAN (tonically autoactive neurone) and l-VMN (left-visceral multiple spike neurone); DA was also inhibitory (EPR: 0.3), and L-NE and L-E had slight effects (EPR: 0.1 or less). The following four catecholamines showed almost the same inhibitory effects on VIN (visceral intermittent firing neurone): DA, EP, L-NE amd L-E. On the other hand, octopamine was the most inhibitory (EPR: 1.0) on both FAN (frequently autoactive neurone) and d-LCDN (dorsal-left cerebral distinct neurone); synephrine was also inhibitory (EPR: 0.3).

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AMINO ACID TRANSMITTERS DURING THE PRE- AND POST-NATAL DEVELOPMENTAL STAGE OF RAT BRAIN. OKADA, Y., SE, S., YONEDA, K. and ATOJI, Y.* Dept. Physiology, Sch. Medicine, Kobe University, Chuo-ku, Kobe, 650 and Dept. Veterinary Med. Sch. Agriculture, Gifu University, Gifu*, Japan

Microassy methods to measure GABA(γ-aminobutyric acid),aspartate(Asp), and glutamate(Glu)($x10^{-13}$mol) were estabilised. The concentrations of these amino acids were determined in the procencephalon, mesen-rhombencephalon and cerebellum in the developmental stages from embryonic(E) 15 day to postnatal(P) 30 day. Concentraion of Glu(80-100 mmol/kg protein) did show no considerable change in the 3 regions all through the development. Asp(28-30mmol/kg at E15)slightly decreased at E19-P1 but did not show considerable change thereafter. GABA(7-10 mmol/kg protein at E15)did not alter upto P10 and thereafter increased to 20-35 mmol/kg protein)in all 3 regions. Concerning GABA distribution, layer analysis was performed in the cerebellum. It is interesting to note that the dramatic increase of GABA concentration in each layer(especially in Purkinje layer) occurred after P10 day when the synaptic organizations of inhibitory neurons such as basket and stellate cells were started to establish(GABA:upto 7-10 m mol/kg dry),although GABA(1-3 mmol/kg)was found even at E15-P10 stage.

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RELATIONSHIP BETWEEN INTRACELLULAR Ca^{2+} CONCENTRATION AND RELEASE OF ^{3}H-NOREPINEPHLINE. SUGINO, H., TAKAHASHI, M., AND KUDO, Y. Dept. Neurosci., Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194, Japan.

Using clonal rat pheochromocytoma cells (PC12h), effects of high K^+ and monensin were examined on intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and on release of ^{3}H-norepinephline (NE). By means of Quin 2, a fluorescent Ca^{2+}-indicator, $[Ca^{2+}]_i$ was estimated. To a conventional depolarizing stimulus with high K^+, PC12h cells responded dose-dependently with a good correlation of increases in $[Ca^{2+}]_i$ and in releasing ability. Both of the K^+-evoked responses were abolished in the absence of external Ca^{2+}, and suppressed by organic Ca^{2+}-antagonists. Monensin (3×10^{-5} M), a sodium ionophore, induced profound release of radioactivity from PC12h cells, but $[Ca^{2+}]_i$ was raised only slightly by the drug. The radioactivity released with monensin was found to be metabolites of ^{3}H-NE in main, while that with high K^+ was ^{3}H-NE itself in most. The monensin-induced release was diminished by pargyline, a monoamine oxidase (MAO) inhibitor. Thereby, it is likely that monensin expels NE from storage vesicles to cytosol, and then metabolites of NE by MAO are released from PC12h cells in a non-exocytotic manner.

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PEPTIDE ANALYSIS IN BOVINE SPINAL CORD BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY. SAKAMOTO,Y.*, TAKANO,H.* AND UYEMURA,K. Dept. of Physiol., Saitama Medical School, Irumagun, Saitama 350-04

We attempt to establish the sensitive and rapid method for analysis of peptides in nervous system by high performance liquid chromatography(HPLC) and gas chromatography(GC)- or fast atom bombardment(FAB)- mass spectrometry (MASS). Acidic acetone extracts of bovine spinal cord were fractionated by Bio-Gel P2 column chromatography. Then peptides were isolated by HPLC with 3013N ion-exchange resin (Hitachi). Two acidic small peptide fractions, containing Asp and Glu, were permethylated and analyzed by GC-MASS. N-acetyl-Asp-Glu, a possible inhibitor of the glutamic receptor, and PyroGlu-Asn, a new peptide, were determined. Several bioactive peptides were also obtained by diphenyl reverse phase HPLC and analyzed by FAB-MASS. One of them showed molecular weight of 1347, which corresponded with that of Substance P. Two other peptides, which showed molecular weights of 1238 and 1308 respectively, were possible to be new bioactive peptides. Further biochemical analysis of those peptides are in progress.

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BRAIN STEM CULTURES OF RATS: MODULATORY EFFECTS OF SOMATOSTATIN ON THE NORADRENALIN RESPONSES

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Using 21-45-day-old cultures of dissociated neurons from the lower brain stem, we examined the effects of somatostatin on the neuronal responses to noradrenalin (NA). Following the application of NA to the surface of the soma, unit discharges of single cultured neurons showed inhibition (46%), facilitation (26%) and no effect (28%). When the subthreshold dose of somatostatin (10-30 nA) was applied preliminally, the above-mentioned NA responses were changed. An inhibition of the inhibitory NA response, which was similar to an atenolol inhibition, was seen in all types of neurons (multipolar, pyramidal, polygonal, fusiform and oval neurons), but a facilitation was seen only in multipolar and pyramidal neurons. By an analysis based on a Michaelis-Menten type kinetics, it has been proved that somatostatin acts on the sites other than the β_1 receptor. By contrast an inhibition of the facilitory NA response, which was similar to a prazosin inhibition, was seen in almost all types of neurons except oval ones. These inhibitory interaction effects were maintained in the medium which was added 8 mM Mg^{++}, indicating these effects occurred postsynaptically. The details of this mechanisms must be cleared by the further experiment.

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EFFECT OF METHIONINE-ENKEPHALIN ON THE SPONTANEOUS ACTIVITY OF THE RAT PORTAL VEIN.

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Concentrations of methionine-enkephalin (Met-enkephalin) up to 10^{-6}M were applied to the longitudinal smooth muscle of the isolated rat portal vein. With concentrations more than 10^{-8}M, Met-enkephalin increased the spontaneous contraction dose-dependently. When the membrane activity was recorded using a microelectrode, Met-enkephalin had no influence at concentrations up to 10^{-7}M. When the concentration was increased to 10^{-6}M, however, it enhanced the spike burst activity but without any effect on the resting membrane potential. 10^{-5}M of either naloxone, phentolamine or atropine, and pre-treatment of reserpine (7mg/kg intra-peritoneally, 41-49 hr prior to killing the rat) did not inhibit the excitatory effect of Met-enkephalin on the spontaneous contraction at any concentration examined. These results suggest that the excitatory effect of Met-enkephalin on the mechanisms involved in the automaticity may be a direct action on smooth muscle or relate to presynaptic action on a non-adrenergic, non-cholinergic system.

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FACILITATION OF ACH RELEASE IN BULLFROG SYMPATHETIC GANGLIA BY LOW CONCENTRATIONS OF [MET^5]ENKEPHALIN AND NALOXONE.

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Fast-EPSPs evoked by preganglionic nerve stimulations (0.2 Hz) were recorded in a low Ca/high Mg Ringer solution by using an intracellular microelectrode method. Quantal contents were calculated for successive sixty fast-EPSPs by the variance and the failure method. Electrophysiological properties and sensitivity to ACh of ganglion cells were not affected by [met^5]enkephalin (10^{-13}- 10^{-4}M). The mean amplitude and mean quantal content of fast-EPSPs were reversively increased by low concentrations of [met^5]enkephalin (10^{-10}- 10^{-8}M) and were decreased by high concentrations (10^{-6}- 10^{-4}M), whereas the mean quantal size was not affected by [met^5]enkephalin (10^{-13}- 10^{-4}M). The inhibitory effect of [met^5]enkephalin in high concentrations was reversively antagonized by naloxone (10^{-5}M), while the facilitating action in low concentrations was not antagonized by naloxone (10^{-8}M). Naloxone in these concentrations (10^{-8}-10^{-5}M) itself has no effect on the evoked release of ACh. The much lower concentration of naloxone (10^{-10}M), however, slightly but significantly increased the mean amplitude and mean quantal content of fast-EPSPs, without affecting the mean quantal size.

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PRODUCTION OF VIP- AND PHM-LIKE IMMUNOREACTIVITIES IN A HUMAN NEUROBLASTOMA CELL LINE. HOSHINO, M., SATO, H., SUZUKI, T., YANAIHARA, N., OGINO*, K. and YANAIHARA*, C. Laboratory of Cellular Metabolism, National Institute for Physiological Sciences, Myodaiji, Okazaki 444, and *Laboratory of Bioorganic Chemistry, Shizuoka College of Pharmacy, Oshika, Shizuoka 422.

The 3K-dalton VIP-like immunoreactive component produced in human neuroblastoma NB-OK-1 cells was isolated and structurally identified with porcine VIP-28. The cells were also shown to produce, simultaneously with VIP-28, a PHI/PHM-like immunoreactive (IR-PHI/PHM) component which co-eluted with synthetic PHM-27, not with synthetic PHI-27, in reverse phase HPLC. In addition to the PHM-27-like component, another IR-PHI/PHM component was detected in the cell extract, which eluted in HPLC at a retention time distinctly different from that of synthetic PHM-27 and crossreacted with PHI-27 amino-terminal specific antiserum but neither with PHI-27 central-portion specific antiserum nor with PHM-27 carboxyl-terminal specific antiserum. The result suggested possible alternative(s) of post-translational processing of the VIP precursor in the cells in terms of the production of PHM-27-related peptides.

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ISOLATION OF SLEEP SUBSTANCE. NAGASAKI, H., KOMODA, Y., UCHIZONO, K., IRIKI, M., INOUE, S., HONDA, K., HIGASHI, A., ISHIKAWA, M. Dept. Physiol. Yamanashi Med. College Ntnl. Inst. Physiol. Sci., Inst. Med. Den. Engin. Tokyo Med. Den. Univ. Tamaho,Nakakoma,Yamanashi 409-38., Myodaiji, Okazaki 444., Surugadai, Kanda, Chiyoda-ku, Tokyo101

In order to determine the chemical form of the sleep substance, we had tried to purify the brainstem extract of rats which were deprived of sleep by electric foot shock. One of the isolated substance has been found to be uridine. Through purification procedure we selected an active fraction by means of neural activity on sponaneous discharges of abdominal ganglion of crayfish, on locomotor activity and a daily length of paradoxical sleep in mice. These different types of activity were used for screening of the samples, but the active samples were not all coincided among each activity. Thus the final selection was dependent on the daily length of paradoxical sleep in mice. One of the isolated substance was identified to be uridine by means of a retention time on high performance chromatography and neclear magnetic resonance.

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REM SLEEP AND ENDOGENOUS BROMINE COMPOUND. OKUDAIRA, N., KOIKE, H.*, INUBUSHI, S., ABE, A.*, YAMANE, M.*, YANAGISAWA, I.* AND TORII, S. Dept. of Physiology, Toho University School of Medicine, Ohta-ku, Tokyo 143, and Dept. of Biochemistry, Tokyo Medical College.

1-methylheptyl γ-bromoacetoacetate (MHBAA), included endogenously in human cerebrospinal fluid (CSF)(Yanagisawa & Yoshikawa,1973), was shown to induce rapid eye movement (REM) sleep in cats (Torii et al.,1973). Recently, it was found to be a competitive inhibitor of the acetylcholinesterases (Yamane & Yanagisawa,1984). To determine the physiological action of MHBAA, 2 male and 20 female cats weighing 1.9-5.5 kg were sacrificed for the micro-determination of MHBAA in CSF and plasma by the high performance liquid chromatography (Yamane & Yanagisawa,1982). Before the experiments, all cats were bred under diurnal lighting conditions (LD 12:12) with lights on from 0600 to 1800 h, more than 4 weeks. Three of them were deprived of REM sleep for 3 days under continuous monitoring of EEG, neck EMG, EOG and ECG. Within 10 minutes of completion of Nembutal anesthesia (50 mg/kg,i.p.) 1 ml of CSF was withdrawn from the cisterna magna and 5 ml of blood was collected from the femoral artery. Collection time were 0400, 1000, 1600, &2200.

MHBAA level of CSF, very similar to plasma, was 0.03-1.88 n mol/ml and showed a circadian fluctuation, highest at 2200 and lowest at 1000 h. There was a positive correlation between CSF and plasma MHBAA. After the 72 hours REM sleep deprivation, it tended to increase the MHBAA level compared to control group at 1000 h.

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METABOLISM OF CATECHOLAMINES AND INDOLEAMINE IN THE BRAIN OF THE MUSCULAR DYSTROPHIC MOUSE. OHTANI, S., SUGIURA, H. AND HORI, S. Dept. Neurochem., Tokyo Metropolitan Institute for Neurosciences, Fuchu-ciry, Tokyo 183

The ability of NGF-synthesis in the muscular dystrophic mouse is low at the earlier stages of the development before 4 weeks old. To study the role of nerve growth factor (NGF) on the development of the catecholamine neurons and the serotonin neuron in the brain, the metabolic rates of catecholamines and serotonin were determined in the brain of muscular dystrophic mouse. The metabolic rates of dopamine, NE and serotonin in the brain were no significant difference between the control and the dystrophic mouse of 12 weeks old. This result may be explained by the fact that the ability of NGF-synthesis of the dystrophic mouse improves after 5 or 6 weeks old and reaches to the normal level at 12 weeks old. In this paper, the improved method was developed for the determination of tryptophan, 5-HTP, 5-HT, 5-HIAA, tyrosine, dopa, dopamine, NE, E, dopac and HVA in the small amount of tissue-extract, that is, 5 mg wet weight of brain tissue. This method included the high-performance liquid chromatography and the valve system. The serial separation of the materials in the single-loaded sample (50 µl) could be performed by this valve system with two columns which were equilibrated with two different elution buffers, those are, 20 mM citrate-0.2 M $NaClO_4$-0.003% SDS-3% propranol, pH 2.4 and 20 mM citrate-0.2 M $NaClO_4$-3 % propranol, pH 4.0, respectively.

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EFFECTS OF FORPHENICINOL ADMINISTRATION ON AN EARLY STAGE OF MUSCULAR DYSTROPHY IN MICE. TSUJI, S., KUSUMOTO, S., DOI, Y. and MATSUSHITA, H. Dept. Physiol., Wakayama Medical College, 9-bancho, Wakayama-shi, Wakayama 640

Forphenicinol, an immunomodifier produced by actinomycetes, is known to acts on macrophages and increases the number of antibody forming cells. We have found that forphenicinol when continuously administered to dystrophic(dy) mice at an early stage of the disease prevents muscular dystrophy. A complete restoration from clinical symptoms of hind leg paralysis and a normal body weight gain appeared in about one third members of the forphenicinol treated dy mice. In addition, a marked increase of activities of certain enzymes which are so called as markers for the disease, in muscle of the forphenicinol treated dy mice coupled with a significant decrease of these enzyme activities in serum was observed. Moreover, certain proteinase activities which are involved in the degeneration process of dystrophic muscle, decreased extremely in the forphenicinol treated dy mice. Creatine concentration and creatine to creatinine ratio in urine also restored to normal in the forphenicinol treated dy mice. The durability tests for leg muscles shows that the forphenicinol treated dy mice almost regained normal physical ability.

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) STUDIES ON ALBUMIN IN HUMAN AQUEOUS HUMOUR. TAKAHASHI, K., KAWAI, K., HAYANO, S., KUWATA*, K., ERA*, S., NAGAOKA*, S., SOGAMI*, M. Dept. of Ophathalmol., *Dept. of Physiol., Sch. of Med., Gifu Univ., Gifu 500

Samples of human aqueous humour with corresponding samples of blood serum were obtained from 20 patients with cataract. HPLC analyses on PGP 2000 (Asahi Pak GS-520H, 0.75 X 200 cm) with an UV-monitor or on JASCO Fine Pak 102F (0.72 X 100 cm) with a multi-channel detector at neutral pH. HPLC profile of human aqueous humour from 102F column (only molecular sieve) showed albumin (66K) and lower mol. weight components with λ_{max} of 260~265 nm. In the HPLC profile of serum, the later component was not observed. HPLC analysis of serum on PGP 2000 column resolved serum albumin into two components, human mercaptalbumin (HMA) and nonmercaptalbumin (HNA). Fraction of HMA in samples of blood serum of 20 patients (50~80 years) was 0.7~0.5. However, major part of albumin in human aqueous humour was found to be HNA. HNA in chamber aqueous might be produced by an intermolecular SH S-S exchange reaction between HMA and oxidized sulfa-containing amino acids, such as cystine, oxidized glutathione.

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PHOTORESPONSIVENESS OF THE LENS CELLS IN DORSAL EYE OF ONCHIDIUM. KATAGIRI,Y., *KATAGIRI,N., AND **FUJIMOTO,K. Dept. of Physiol., *Dept. of Anat., Tokyo Women's Med. College, Shinjuku-ku, Tokyo 162, **Senshu Univ., Chiyoda-ku, Tokyo 101

Based on the morphological and electrophysiological studies, lens cells, constituents of the lens in the dorsal eye of Onchidium, were identified as a kind of rhabdemeric photoreceptor cells. They have a massive bundle of microvilli at the distal part and generate a depolarizing responses to light stimuli. The composition of the lens was investigated by serial section of the eyes and intracellular injection of HRP to the lens cells. Only 11% of lens cell (n=347 in 51 dorsal eyes) have a process arizing from the proximal part to the optic nerve layer, though its terminal and synaptic structure are obscure. A lens cell isolated solitarily from the dorsal eye can generate the depolarizing response to photic stimulus like as an intact lens cell in dorsal eye. Although the signal transmission from the lens cells is not clearly demonstrated, these cells appear to provide a useful preparation because of the larger cell size (130 μm in maximal diameter) for the study of the membrane properties which generate the receptor potential.

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COMPARISON OF LIGHT RESPONSES OF PINEAL PHOTORECEPTORS IN LARVAL AND ADULT LAMPREYS. TAMOTSU, S. AND MORITA, Y. 1. Dept. Physiol., Hamamatsu Univ. Sch. Med., Hamamatsu 431-31.

Electrophysiological and morphological investigations were carried out in order to compare the properties of pineal photoreceptors in the larval and the adult stages of the liver lamprey, Lampetra japonica. The skin and tissues covering the pineal organ of larva are transparent and the incident light must be more effective than in adult. The ratio of the diameter of the pineal to the body-length was higher in larva. Electronmicroscopical examinations revealed well-developed pineal photoreceptors also in larval lampreys.

The curves calculated from the Naka-Rushton equation, $V/Vmax=I^n/(I^n+\sigma^n)$, were compared between both groups. The mean value of n was 1.23±0.26(s.d.) for larva and 0.94±0.91 for adult. The mean value of σ in larva was -2.95±0.38 in log I (W/m^2). This value shifted about 0.88 log unit toward lower intensity in adult. The peak of the spectral sensitivity curve was 505nm in larvae and 525nm in adults. This shift of the peak suggests that the pigment interconversion occurs between the larval stage and upstream migrants.

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THE INFLUENCE OF THE BLOOD SUGAR OF A FROG ON FMN IN THE RETINA.
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In the present study, the influence of FAD contained in a frog liver to the contents of FMN and rhodopsin in the retinas of the frog was discussed.

The content of FMN per 1g of retina "FMN" and optical density of rhodopsin per 1g of retina "O.D. of rhodopsin" are expressed as follows;

$$\text{"FMN"} = 0.0541 \times F^{1/3} \times G^{1/3} - 0.324$$

and

$$\text{"O.D.of rhodopsin"} = Fat \times G^{1/2}(0.0686 \times FAD^{1/3} \times G^{1/3} - 0.227)$$

where, F, FAD, Fat and G are riboflavin content in 1g of liver, FAD in a total liver and blood suger respectively.

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DIURNAL CHANGES OF THE ANGULAR SENSITIVITY OF THE RETINULA CELLS IN THE COMPOUND EYE OF THE ISOPOD, *LIGIA EXOTICA*.
HARIYAMA,T and TSUKAHARA,Y. Research Center for Applied Information Sciences, Tohoku University, Katahira, Sendai 980

Both the structure of the retinula cells and the ERG amplitude have been known to change diurnally in *Ligia exotica*. Irregular arrangement of microvilli and the increase in the ERG amplitude were observed at night. Intracellular recordings from the retinula cells showed no difference in the slope of V-log I curve but the cells showed higher sensitivity to the light from on axis. The iso- two dimensional angular sensitivity (A.S.) curves recorded at night were apparently irregular which seems to correspond the irregular arrangement of microvilli. It was observed both during the day and night that some retinula cells had only one peak and others had two or more peaks of A.S. The angles between the two peaks were usually 1.5-2°. Morphological measurement of the retina gave the interommatidial angles of ca. 1.5-2° and the interrhabdomeric angles were estimated to be ca.4-8°. Lucifer injection to the retinula cells has marked a single cell, intra- or interommatidially coupled cells. These results suggest that multiple peaks of the two dimensional A.S. are caused by the interommatidial couplings.

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THE RETINAL EFFERENT FIBERS AND CIRCADIAN RHYTHM OF RETINAL ACTIVITY IN FROGS.
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The ERGs were recorded under dark adapted state from unanesthetized, partially restrained frogs (Rana catesbiana and Xenopus laevis) during the period of upto 3 days. The b-wave was found to fluctuate periodically, gradually reaching the maximum amplitude around noon and then slowly decreasing in amplitude with the minimum in the early morning. A decrease in b-wave was also observed by an electric stimulation of the optic nerve. Next, the effect of optic nerve cutting was studied in daytime when the retina responded with large amplitude b-wave, but there was no effect if the optic nerve was cut at night when the b-wave was already reduced in amplitude. The b-wave increasing effect of optic nerve section may probably due to the loss of inhibitory retinal efferent fibers which had been proved to exist by our previous study in Rana catesbiana.

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PHOTOEXCITATION PROCESSES IN THE INSECT PHOTORECEPTORS. MIMURA, K. Fac.Liberal Arts, Nagasaki Univ., Nagasaki 852

Through the bumps produced by a weak illumination to the fly photoreceptors R1-6. I have already demonstrated that two maxima of UV(350nm) and blue-green(480nm) in the spectral sensitivity is due to the excitation at different locations of the photoreceptors which depends on the above two wavelengths(1983). In this report, I have confirmed the above fact by analyzing the ERG responses produced local illumination within the photoreceptor layer and by comparing the M potentials at various depths of the photoreceptor layer produced by strong red flash light to UV or blue-green adapted eyes. Moreover, the microspectrophotometry measured by using the preparations of the photoreceptor layer which were sliced along the long axes of the cell suggested the presence of UV-photopigments at the proximal part of the photoreceptor, while the UV-photopigments have not demonstrated at the distal part.

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MICROSPECTROPHOTOMETRIC MEASUREMENTS OF RHODOPSIN CONTENT IN RHABDOMERES OF LIVING *DROSOPHILA MELANGASTER* REARED ON CAROTENOIDS SUPPLEMENTED MEDIUM. TSUKAHARA, Y. AND ISONO, K. Res. Ctr. appl. Inf. Sci., Tohoku Univ., Sendai, Miyagi 980

Effects of a deprivation and a supplement of carotenoids on the rhodopsin content in rhabdomeres of *Drosophila melanogaster* were studied by microspectrophotometric measurements. The experimental findings are as follows.

1) Changes in the optical density (ΔOD) at 580nm were 0.1 - 0.3 in flies fed on the vitamin A-rich medium and kept on a 12h light-dark cysle. The flies reared on the vitamin A-deficient medium showed no detectable change in ΔOD.

2) supplement of *all-trans* retinal did not cause any synthesis of rhodopsin if the flies were kept in the continuous darkness. An illumination after the supplement induced an increase in rhodopsin content.

3) An action spectrum of the illumination peaked at around 375nm suggesting that the reaction to increase the rhodopsin is lead by a photo-isomerization of *all-trans* retinal.

4) The flies reared on a medium with either xanthophil or β-carotene synthesize a cosiderable amount of rhodopsin and increase the visual sensitivity even in the continuous darkness.

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CHROMOPHORES OF LONG-LIVED PHOTOPRODUCTS OF FROG RHODOPSIN. AZUMA, M. AND *AZUMA, K. Dept. Health Sci., Osaka Kyoiku Univ., Tennoji-ku, Osaka 543 and *Dept. Biol., Osaka Med. Coll., Takatsuki, Osaka 569

Long-lived photoproducts of frog rhodopsin (Rh) in isolated retina and digitonin solution have been investigated by spectroscopy and their chromophores have been analyzed by high-pressure liquid chromatography. By irradiation (>560 nm) at 3°C, metarhodopsin III (MIII) and a photoproduct analogous to MIII are formed, whose chromophores are determined as all-trans and 7-cis retinal respectively. The 7-cis photoproduct decays more slowly ($t_{1/2}$ = 50 min at 22°C) than MIII does. The formation of 7-cis photoproduct is derived from the photoisomerization of lumirhodopsin (L) and metarhodopsin I (MI), but the efficiency of isomerization to 7-cis is better in MI than in L. The λ_{max} of 7-cis photoproduct at -20°C is 455 nm. This absorption band exhibits positive CD ([θ] = 73,000), which is larger than that of Rh ([θ] = 61,000). Examination of absorption spectra of Rh intermediates has shown that λ_{max} of L, MI and MIII are 522 nm, 482 nm and 475 nm respectively.

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CHANGES OF EARLY RECEPTOR POTENTIAL IN FLIES WITH LOW RETINAL RHODOPSIN CONTENT.
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MATSUMOTO, H. AND PAK,W.L. Dept. of Biol. Sci. Purdue Univ., W. Lafayette, IN. 47907 U.S.A.

Several mutations that cause a specific reduction of rhodopsin concentration in the retina have been obtained in Drosophila melanogaster. In one of these, nina C, the microphotometrical measurement of the pigment showed that the concentration was approx. 30 to 40 % of the normal level. M-potential, which can be induced by an intense orange strobo flash and is associated with the net convertion of metarhodopsin M580 to rhodopsin R480, however, was almost normal in spite of the decreased rhodopsin content. Ultrastructural studies revealed that the rhabdomeres of this mutant show a noticeable decrease in size. The decrease in rhabdomeric membrane area seemed to account for the loss of the pigment. In another mutant, nina E, M-potential was almost absent but the rhabdomere structure remained fairly normal. Vitamin A deficient flies showed a similar result to the latter case. From these quantitative observations it was suggested that early receptor potential is dependent on the density of rhodopsin rather than its total amount in the photoreceptor membrane.

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LIGHT- AND DARK-ADAPTATION OF THE PHOSPHODIESTERASE IN GECKO PHOTORECEPTORS
KAWAMURA, S. and MURAKAMI, M. Dept. Physiol., Keio Univ. Sch. Med., Tokyo 160

Intracellular injection of cyclic-GMP into gecko photoreceptors caused a rapid depolarization followed by a slow repolarization. A light flash shortened the duration of the depolarization and accelerated the repolarization kinetics, indicating that the light-activated phosphodiesterase (PDE) increased the hydrolysis rate of the injected cyclic-GMP. The PDE activity in intact gecko photoreceptors was estimated from the duration of the cyclic-GMP-induced depolarization under various light conditions. When an intense light flash was given, the PDE activation was detected with a latency as short as 100 msec. With continuous light, the enzyme exhibited a constant activity, suggesting a steady state of the cyclic-GMP metabolism ("light-adaptation"). After a light flash, the PDE activity recovered the dark level ("dark-adaptation"). The process seemed to coincide with the sensitivity recovery of the photoreceptor potential. The fast activation and the "adaptation" of the PDE observed in this study suggest the involvement of the PDE not only in the generation mechanism of the photoreceptor potential but also in the adaptation process of the gecko photoreceptors.

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THE AUGUMENTATION OF LIGHT RESPONSES BY THE INJECTION OF ACTIVE PHOSPHODIESTERASE IN THE ROD OF THE TOAD RETINA.
SHIMODA, Y. Dept. Physiol.,Kyorin Univ.Sch.Med., Mitaka, Tokyo 181, HURLEY, J. B*, Div.Biol.,California Inst.Technology & MILLER, W. H*, Dept. Ophthal.& Visual Sci., Yale Univ. Sch. Med.

Previous biochemical and physiological studies have shown that light activates the rod phosphodiesterase(PDE) which, in turn, hydrolyze cyclic-GMP into GMP, and they also proposed few hypotheses on the mechanisms of phototransduction in the rod outer segment (ROS). We have further investigated the role of PDE in this system by injecting PDE with pressure application into the ROS of the retina of the toad, Bufo marinus. Injected PDE is extracted from bovine retinas and it is activated by treating with trypsin which can remove the inhibitory subunit. When PDE is injected in the darkness, no immediate detectable change is observed, as measured by ROS membrane potential. The effect by the injection of PDE becomes evident only after illumination. The light response is augumented; the recovery phase of light response is prolonged. We conclude that the prolongation of light response is resulted from hydrolysis of endogenous cyclic-GMP by the injected active PDE. These results provide evidence that PDE affects light-dependent channels in the ROS membrane but do not specify whether the effects act on the initial phase of the receptor potential or recovery phase.

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EFFECT OF OSMOLARITY ELEVATION OF THE PERFUSING AND RINSING MEDIA ON THE OCCURRENCE FREQUENCY OF THE SPONTANEOUS NP-WAVE RECORDED FROM THE ISOLATED CHICK RETINA. SATO, T., YONEYAMA*, T., TAIRA, K., MATSUMOTO, N., SOMEI, K., OKUDA, K. AND SUZUKI, T.A. Dept. Oral Physiol., Sch. Dent., *Dept. Ophthal., Sch. Med., Iwate Med. Univ., Morioka, Iwate 020.

The retina isolated from the anesthetized chick generates the standing potential (SP) and the action potential to light stimuli in the perfusing chamber. The SP, however, varies with occurrence of the spontaneous slow negative and positive deflection (NP-wave). In the course of the NP-wave, a- & b-waves of the ERG are augmented in amplitude and it prevents obtaining reproducible intensity-response curves. So, it has required finding a stable condition in which the NP-wave rarely appears.

When the preparation generated the NP-waves frequently, it was noticed that the retina had been swollen remarkably. Thus, we tried to elevate osmotic pressure of the perfusing and rinsing media, and investigated occurrence frequency of the NP-wave. It was found (1)that the frequency was minimum around 480 mOsmol, of which level was markedly higher than that of the body fluid (350 mOsmol), and (2)that in such osmolarity level well reproducible data of the intensity-response curves were obtained.

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POTASSIUM DIFFUSION AND SLOW PIII RESPONSE IN ISOLATED FROG RETINA. MATSUURA, T. Dept. of Physiol., Kinki Univ. Sch. of Med., Sayama, Osaka 589

A close correlation in time course between the intraretinal slow PIII response and light-induced $[K^+]_0$ decrease around inner segments supports the idea that the slow PIII response is produced by the Müller cells in response to $[K^+]_0$ change. The transretinally recorded slow PIII response, however, differs in shape and time course with that of $[K^+]_0$ decrease in two aspects; the peak time is shorter and the overshooting of potential is frequently observed. To account for the differences, two pairs of electrodes were inserted extracellularly into the aspartate-treated frog retina, one pair was for the separation of fast and slow PIII responses and the other pair of K^+-electrode and its reference was for the depth recordings of the $[K^+]_0$ change and intraretinal PIII response. Toward the inner segmental region the K^+ diffusion both from distal and proximal portions of the retina was detected with the diffusion coefficient one order smaller in the distal portion. It is suggested that this delayed K^+ diffusion from the distal portion evokes a potential which is opposite in sign with the intraretinal slow PIII response.

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THE PHOTORECEPTOR SENSITIVITY AS A FUNCTION OF RHODOPSIN CONTENT IN THE ISOLATED BULLFROG RETINA. TOBA, Y. AND HANAWA, I. Dept. of Physiol., Kobe Univ. Sch. of Med., Kobe, 650

By using the isolated bullfrog retina treated with both aspartate and Ba^{2+}, the relation between the threshold of fast PIII response(I) and the rhodopsin content was examined. We have demonstrated that log threshold increase from the original dark-adapted level(I_o) is not in linear proportion to the fraction of rhodopsin present, but the general tendency of the threshold curve is depicted as being sigmoidal. When more than about 30% of rhodopsin, the relation is described by the Michaelis-Menten equation. Immersing the isolated retina in the 0.01 mM Ca^{2+} or 0.1 mM papaverine solution, the threshold curve significantly shift to the left along the logI_o/I axis, indicating that the threshold change becomes larger than those in the normal condition. But 15 μM IBMX (isobutyl methylxanthin) produces only a small change in the curve. In all cases, there is no significant difference among threshold changes measured with three kinds of stimulating light (430,500,580-nm). The present results indicate that papaverine acts to visual cells in a different manner with IBMX.

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MICROELECTRODE STUDY ON THE B-WAVE OF THE ISOLATED BULLFROG RETINA IN THE PRESENCE OF 0.05 mM BARIUM ION. KATSUDA, S. AND HANAWA, I.
Dept. of Physiol., Kobe Univ., Sch. of Med., Kobe 650.

We reported previously that the slow subcomponent of the b-wave in the dark-adapted bullfrog retina was suppressed by the application of 0.05 mM Ba^{2+} on the vitreous side. In the present study, intraretinal recording of the b-wave was undertaken to estimate the location generating the slow subcomponent. In the presence of 0.05 mM Ba^{2+} on the vitreous side, the b-wave potential change to the receptor side was suppressed and that the vitreous one disappeared when the electrode was located between the inner nuclear layer and the vitreous side. These results suggest that 0.05 mM Ba^{2+} exert on the K^+ conductance of the proximal Müller cell membrane or on the proximal neuronal network responsible for light-evoked extracellular K^+ increase, resulting in decrease in the b-wave current flow in the proximal retina.

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EFFECTS OF EXTERNAL POTASSIUM CONCENTRATION ON THE PIII RESPONSE AND THE TRANSRETINAL POTENTIAL OF THE FROG RETINA. ANDO,H. AND HANAWA, I. Dept. of Physiol., Kobe Univ. School of Med., Kobe 650

When $[K^+]_o$ is altered, changes in both the transretinal potential (TRP) and the amplitude of the PIII response are observed. An application of Ba^{2+} is known to reduce the K^+ conductance. In the present experiment, we studied the effects of rapid $[K^+]_o$ change in the presence or absence of Ba^{2+}. While monitering the TRP and the PIII response to a 500-nm weak light flash delivered at 1 min interval, the bathing solution of the receptor side of an isolated bullfrog retina was replaced by a test solution containing 0, 5 or 10 mM K^+. Reducing $[K^+]_o$, the response amplitude was decreased gradually, and the application of 0.5 mM Ba^{2+} suppressed this effect. Increasing $[K^+]_o$, the response amplitude was also decreased but the application of Ba^{2+} enhanced this effect. Differences between the effectiveness of the fast and slow PIII response to Ba^{2+} may account for these results. When $[K^+]_o$ was altered, the TRP changed in a manner like an out put signal from a high-cut filter having a time constant of about 2.5 min. The presence of Ba^{2+} had little effect on the time course, but significantly reduced the magnitude of the TRP change. The application of 10 μM ouabain markedly shortened the rate of the TRP change, suggesting that Na^+/K^+ pumps would be responsible for tardy change in the TRP.

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ANALYSIS OF OSCILLATORY POTENTIALS IN FROG RODS. TAKAHASHI, K.-I., MIYACHI, E.-I., AND MURAKAMI, M., Dept. Physiol., Keio Univ. Sch. Med., Tokyo.

In amphibian rods, potential oscillations have been reported to occur when the retinas were exposed to TEA, Ba, and low Cl media. In rods of the bullfrog retina perfused with normal Ringer, we also observed the oscillation evoked by transretinal current stimulation. Perfusion experiments with solutions containing various agents or lacking of some ion species revealed that, no matter what the triggering mechanisms were, these potential oscillations so far reported were fundamentally identical in respect that Ca conductance increase was involved. When a current pulse was intracellularly applied, a depolarizing current failed to evoke an apparent Ca response. Instead, a Ca response was observed when a hyperpolarizing current was cut off. The amplitude of the response was graded depending on the intensity of hyperpolarizing current. These results suggest that Ca channel is kept partially inactivated when the rod membrane is depolarized in the dark, and that the anode break triggers Ca entry. In this respect, the membrane properties of the rod seem to be not different from those of such other cells which generate Ca responses as molluscan neurons, cultured neuroblastoma cells, etc.

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TWO CHROMATIC TYPES OF CONE PHOTORECEPTORS CONTAINING COLORLESS OIL DROPLETS IN THE TURTLE RETINA. OHTSUKA, T. Dept. of Information Physiol., Natl Inst. Physiol. Sci., Okazaki

It has been believed that cone photoreceptors containing colorless oil droplets in the retina of the red-eared turtle, Pseudemys scripta elegans, are blue-sensitive. A fluorescence microscopy demonstrated that two thirds of these colorless oil droplets in the Pseudemys retina fluoresced when excited by a near ultraviolet light (330-380 nm), while the remains did not. Intracellular recording and intracellular staining with Lucifer yellow revealed two chromatic types of cones containing fluorescent or nonfluorescent colorless oil droplet: Cones with fluorescent colorless oil droplets (n=7) were red-sensitive (λ max, 620 nm), while cones with nonfluorescent colorless oil droplets (n=3) were blue-sensitive (460 nm). These two types of colorless oil droplets were also found in the retina of the Reeves' turtle, Geoclemys reevesii: Cones with fluorescent colorless oil droplets (n=12) were red-sensitive (620 nm), and those with nonfluorescent colorless oil droplets (n=4) were blue-sensitive (460 nm). Furthermore similar fluorescent oil droplets were also found in the avian retina. These oil droplets pertained to the cone photoreceptors, which contain yellow-absorbing visual pigments (λ max, about 570 nm).

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EFFECTS OF TRANSMITTER CANDIDATES ON THE OCCURRENCE OF SPREADING DEPRESSION (SD). FUJIMOTO, M. and TOMITA, T. Dept. of Physiol., St. Marianna Univ. Sch. of Med., Kawasaki 213

In the retina immersed in low Cl^- Conway's, SDs are evoked spontaneously and periodically in darkness. SD is related to an abnormal synaptic activities in the IPL where many types of synaptic transmitters are known to be involved. The effect of the transmitter candidates and their antagonists on the occurrence of SD was investigated using spreading depression potential (SDP) as an index. The frequency of SDP (FSD) was increased by glutamate, Ach, noradrenaline and dopamine. Overdoses of these substances (1-2 mM), however, frequently abolished SDP. Atropine and curare abolished SDP, though curare left behind small immature SDPs. FSD was decreased by phentolamine of 1-2 mM in concentration but increased in lower concentrations, though propranolol always decreased it. FSD was also increased by 10-50 μM serotonine but decreased by 0.2-2 mM serotonine and by methysergide. These might suggest the presence of adrenergic inhibitory feedback mechanisms and/or the adrenergic presynaptic receptors on the adrenergic neurons themselves, and the presence of an serotonergic inhibitory feedback mechanisms.

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A VOLTAGE-CLAMP ANALYSIS OF GLUTAMATE EXCITATION IN SOLITARY HORIZONTAL CELLS ISOLATED FROM GOLDFISH RETINA. TACHIBANA, M. Dept. Information Physiol., Natl. Inst. Physiol. Sci. Okazaki, Aichi 444.

L-Glutamate, a candidate for the transmitter released by photoreceptors, depolarizes solitary horizontal cells dissociated from goldfish retina (Ishida, Kaneko & Tachibana, 1984; J. Physiol. 348). The glutamate-induced responses in these cells were examined under voltage-clamp conditions using a "Giga-seal" suction pipette in the whole-cell recording configuration. L-Glutamate (>10 μM) induced an inward current at the resting membrane potential (ca. -55 mV). Membrane depolarization reduced the amplitude of the induced inward current and reversed its polarity into outward beyond approximately 0 mV. Membrane hyperpolarization decreased the induced inward current in amplitude, but this phenomenon was prevented when the K current through the anomaouls rectifier was blocked by Cs or Ba ions: in the presence of Cs or Ba, hyperpolarization increased the glutamate-induced current in amplitude. It is concluded that L-glutamate has two effects on solitary horizontal cells; 1) a conductance increase to cations (Na > K, Ca), and 2) a conductance decrease to K ions through the anomalous rectifier. The photo-response of horizontal cells can be interpreted by the combination of the glutamate-induced current and the voltage-dependent currents in solitary horizontal cells.

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INCREMENTAL AND DECREMENTAL RESPONSES OF HORIZONTAL CELL DURING BACKGROUND ILLUMINATION IN THE CARP, CYPRINUS CARPIO. UMINO, O. AND WATANABE, K. Dept. of Physiol., Tokyo Women's Medical College, Kawada-cho, Shinjuku-ku, Tokyo 162.

Intracellular responses of L-cell to a step light of dim or moderate intensity are usually composed of an initial transient and a following plateau. With very bright light, however, after a maximum hyperpolarization a slow depolarizing phase takes place before reaching a steady level. When 500 msec test flashes either brighter or dimmer (from 200% to 20%) than the background were superimposed on it, the incremental and decremental responses showed almost linear relation to the test lights in the steady-state condition of the responses. In the white-noise experiments in which both incremental and decremental responses were involved, the amplitude distribution of fluctuating components was almost symmetry in the steady-state under a strong light adaptation.

During the non-stationary condition of the progressive light adaptation and 70% of L-cells of the steady-state under moderate light adaptation, asymmetry in the amplitude distribution was observed. In such a case, the peak of hyperpolarizing component of the fluctuating response was often scraped unexpectedly if the signal would passively pass through the logarithmic element. These findings provide evidence that L-cell response does not fluctuate on the local slope of the Weber-Fechner relationship under background illumination.

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STEADY SURROUND ENHANCES INCREMENTAL SENSITIVITY OF SPOT RESPONSES IN TURTLE AND CATFISH HORIZONTAL CELLS. NAKA, K.-I. AND KAWASAKI, M. National Institute for Basic Biology, Okazaki 444

A samll spot of light superposed on a luminous background looks dimmer (psychophysically) and produces a smaller response (physiologically). Presence of a steady background decreases incremental sensitivity for a small test spot: this is the Weber-Fechner law. This was not the case with a test spot modulated in white-noise fashion. In turtle and catfish horizontal cells, a steady surround increased the incremental sensitivity for a white-noise modulated small spot. The enhancemenet had three facests: 1) increase in the incremental sensitivity as indicated by larger kernels, 2) faster temporal response as indicated by shorter peak response times, and 3) differentiating (bandpass) response as indicated by biphasic kernels. In both turtle and catfish steady surround desaturated the horizontal cell response by a small spot of light to increase the cell's incremental sensitivity.

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NEURAL CONNECTIONS OF AXON TERMINALS OF CARP HORIZONTAL CELLS. KOUYAMA, N., WATANABE, K. AND SHIMATANI, Y. Dept. of Physiol., Tokyo Women's Medical College, Kawada-cho, Shinjuku-ku, Tokyo 162.

The neural connectivity of axon terminal of horizontal cell in carp retina was studied with electron microscopy and Lucifer Yellow dye injection. In some cases, dye-coupled axon terminals were found at the mid portion of axon. This suggests that the axon terminal makes gap junctional contact not only with other axon terminals but also with the axons. An axon terminal was marked with HRP, and examined along its whole length with electron microscope. This axon terminal had mean diameter of 3.3 μm and 49+19 components connected with gap junctions. The connected components can be divided by their diameter into two groups (Thick: ϕ=3.3 μm, and thin: ϕ=0.8 μm). The former would coincides with the axon terminals and the latter the axons. When dividing them at the diameter of 1.0 μm, this axon terminal had the gap junctional contact with 5+3 axons and 44+16 axon terminals. They were evenly distributed along the length of the axon terminal. No chemical synapse was observed.

This suggests that each axon terminal has inputs not only from its own axon, but also from the adjoining axons via the gap junctions. Thus, the functional distance between the cell body and the axon terminal could be shorter than that has been supposed.

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SIGNAL TRANSMISSION BETWEEN HORIZONTAL CELLS IN THE CARP RETINA. TAKABAYASHI, A. MITARAI, G. and NEGISHI, K. Dept.Aerospace Physiol., Inst. Environ. Med.,Nagoya Univ. Nagoya & Dept.Neurophysiol.,Neuroinform. Inst., Kanazawa Univ., Sch. Med., Kanazawa.

It has been proved that the external horizontal cells(EHC) in the retina receive a feed-back effect from the dopaminergic cells(IPC), and the dopamine(DA) blocks the dye-coupling between these cells. To elucidate its functional significance, the DA effect on the inter-EHC coupling was analysed by means of electrical spread in the perfused isolated carp retina. Current pulse was injected into one HC and the induced potential was recorded from the neighbouring HC(C2). With applying DA into the perfusate, the response to centered spot light increased while those to annular light decreased as already reported, and the current spread to C2 reduced distinctly. With these changes, the input resistance(Ri) of the EHC markedly increased in spite of depolarization of membrane potential(MP). The effects of Co^{++},Ba^{++} and Sr^{++} were also observed. Co^{++} induced hyperpolarization of MP with increase of Ri and reduction of the coupling ratio. Ba^{++}(5mM), in contrast, induced depolarization of MP with decrease in Ri and increase in coupling ratio. Sr^{++} hyperpolarized the MP with increase in Ri but with no change in the coupling ratio. Comparing with these results, DA-induced increase of Ri was specially noticed. It should be reflecting the increase of resistance in the gap-junction, indicating that DA, i.e. the IPC, controls directly the junctional connection.

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SPATIAL PROPERTIES OF HORIZONTAL CELL RESPONSE TO A BRIGHT LIGHT IN THE CARP RETINA. YAGI,T., USUI,S.*, TAKABAYASHI,A. and MITARAI,G. Dept. of Aerospace Physiol., Res. Inst. Environ. Med., Nagoya Univ., Chikusa-ku, Nagoya 464, *Dept. of Information and Computer Sciences, Toyohashi Univ. of Technology, Toyohashi 440

Spatial properties of L-type horizontal cell(soma and axon-terminal:AT) response to a slit-shaped flash were studied in the carp retina in terms of active cable property. The peak amplitude of the hyperpolarizing response of soma and AT decay exponentially along a distance up to 0.8mm with average space constants of 0.33mm and 0.71mm respectively and then become slower as distance increase. When the retina is illuminated with brighter intensity, a hump in the depolarizing phase can be observed at the center cell and apparent space constants increase with changing the waveform as distance increase. The decay of hyperpolarizing phase shows a passive cable property, that is, the change is almost linear-fashion. On the other hand, that of depolarizing phase shows a active cable property, that is, the hump faded away as distance increase. The wide spatial distribution of the AT response may be due to a double convolusion with the individual (soma and AT) spreading function. These observations suggest that the junctional activity and/or the voltage-dependent conductance change in the membrane modulate the lateral spread of horizontal cell response.

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SPATIO-TEMPORAL PROPERTIES OF THE L-TYPE HORIZONTAL CELL RESPONSE IN THE RETINA AND ITS POSSIBLE MECHANISM. USUI, S., Physiol. Eng. Lab., Toyohashi Univ. of Tech., Toyohashi 440, YAGI, T. and MITARAI, G., Res. Inst. of Environ. Med., Nagoya Univ., Nagoya 464

It is known that the horizontal cell in the retina exhibits tremendous wide spatial summation over a few mm due to the electrical coupling through the gap junctions. In the photopic L-type horizontal cell of the carp retina, the hyperpolarizing response waveform to a flash shows the noticeable dynamic nonlinearities clearly (Usui et al: the 7th nuero-science meeting in Japan, 1984 and the 56th ARVO spring meeting, 1984). These experimental evidences are possibly due to the nonlinear reducing mechanism through the neighboring cells (Usui et al: Vis. Res., 23, 1983). A discrete 2-dimensional horizontal cell network model has been constructed, introducing voltage dependent nonlinear gap junction conductance and tested the physiological evidences on the gap junction by Bennett et al (1981). The model response clearly exhibits not only the area and intensity nonlinear dynamical effects but also the spatial properties by slit experiment, including their dynamic waveforms. The possible mechanism for the wide spatial summation might be in the nature of the nonlinear dynamic gap junction property.

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EFFECTS OF DOPAMINE ON ELECTRICAL AND DYE COUPLING BETWEEN HORIZONTAL CELLS IN CARP RETINA. TERANISHI, T., NEGISHI, K. AND KATO, S. Dept. Neurophysiol., Neuroinformation Res. Inst., Sch. Med. Univ. Kanazawa, Ishikawa 920

Three types of light-induced response (L-, RG- and YRB-type S-potentials) recorded from isolated retinas of the carp were identified by their spectral response and later by morphological localization of the recording sites marked with an intracellular Lucifer yellow (LY). Horizontal cells in a given layer, generating one of the above response types, are electrically coupled via gap junctions, so that the injected LY normally diffused to several neighboring cells. The half decay distance of response amplitude with spot displacement was shorter in the order of L-, RG- and YRB-type responses, and correspondingly the dye diffusion area was narrower in the same order of cells. Dopamine (DA; 10-20 μM) increased the amplitude of responses to central spots, decreased that of those to distant spots, and restricted the intracellular LY to single injected cells. In contrast, in retinas from which DA cells had been deprived by prior destruction with 6-hydroxydopamine, the amplitude of responses became smaller, the half decay distance was longer and the dye diffusion area became wider. The results indicate that DA regulates the spatial properties of all types of horizontal cells and that there is an apparent correlation between the degrees of electrical and dye coupling among horizontal cells in a given layer.

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EFFECTS OF EXCITATORY AMINO ACID AGONISTS ON RELEASE OF Ca^{2+}-INDEPENDENT [^{3}H]-γ-AMINOBUTYRIC ACID FROM THE FISH RETINA. KATO, S., NEGISHI, K. AND TERANISHI, T. Dept. Neurophysiol., Neuroinformation Res. Inst. (NIRI), Sch. Med. Univ. Kanazawa, Ishikawa 920

External horizontal (H1) cells have been shown as GABA-containing neurons, the release being Ca^{2+}-independent (Marc et al., 1978; Yazulla & Kleinschmidt, 1982; Lam et al., 1983). To search involvement of 3 receptor subclasses for glutamate-aspartate which are highly probable transmitter candidates of cones, the Ca^{2+}-independent release of ^{3}H-GABA from carp retinas was evoked by 3 excitatory amino acid agonists; kainate (KA), quisqualate (QA) and N-methyl-D-aspartate (NMDA). A retinal particulate fraction was incubated with 0.2 μCi of ^{3}H-GABA and then the release was measured by a superfusion method. KA was found to be most potent; the GABA release was dose-dependent, the minimum effective concentration being about 3 μM. QA showed a comparable potency to that of KA, while NMDA was ineffective on the GABA release with higher concentrations up to 2.0 mM. In similar and parallel experiments, NMDA with low concentrations but neither KA nor QA was found to stimulate ^{3}H-dopamine release by 2-fold; dopamine is a transmitter of interplexiform cells. These data suggest that an amino acid receptor on the H1 cell membrane is preferential to glutamate.

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SYNAPTIC CONNECTIONS BETWEEN PHOTORECEPTORS AND BIPOLAR CELLS: GOLGI-AND HRP-METHODS. SAITO, T., KUJIRAOKA, T. AND YONAHA, T. Dept. of Physiol. St. Marianna, Univ. Sch. of Med. 2095, Sugao, Miyamae-ku, Kawasaki

We identified various types of the carp bipolar cells by both Golgi-impregnation and intracellular injection of HRP, and analysed the synaptic connections between photoreceptors and bipolar cells by electron microscopy. All bipolar cells studied send their dendrites into both rods and cones. They were classified into four types on the basis of the synaptic contact patterns with cones. Type I on-center cells, whose axon terminals were characterized by a big, single swelling, did not send dendritic processes to the synaptic ribbons. Type II on-center cells were characterized by broadly ramifying axon terminals in the sublamina b. Dendritic processes of the cell approached the synaptic ribbons, but did not occupy the central position of the triads. Type I off-center cells included the cells having a big, single swelling or a large ramification of the axon terminals on the upper region of the sublamina a. They send processes to a position opposite the synaptic ribbons as the central element of the triads. Type II off-center cells, whose axon terminals ramified broadly over the thickness of the sublamina a, did not send processes to the synaptic ribbons.

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VOLTAGE-DEPENDENT MEMBRANE CURRENTS IN SOLITARY BIPOLAR CELLS OF THE GOLDFISH RETINA. KANEKO, A. AND TACHIBANA, M. Natl. Inst. Physiol. Sci., Okazaki, Aichi 444.

Membrane properties of solitary bipolar cells, dissociated from the enzym-treated goldfish retina, were studied under current- and voltage-clamp conditions with 'Giga-seal' suction pipettes (pipette solution 138 mM K). The resting potential was ca. -30 mV. The membrane currents consisted of four components: ICa, IK(Ca), IK(V), and Ih. ICa was activated by membrane depolarization (>-30 mV), was maximum at +10 mV and became smaller with further depolarization. ICa was enhanced by Ba, and was blocked by Co. IK(Ca) was seen at >-10 mV, was maximum at ca. +40 mV, and was reduced to zero at >+70 mV. IK(Ca) was suppressed by Co, Ba, TEA or quinine. IK(V) was activated at >-60 mV, and had a slower kinetics than that of IK(Ca). The reversal potential of its tail current was close to EK. Ih was activated at <-60 mV, and showed a time-dependent increase. Ih seems to be carried by a mixture of Na and K ions, since (1) its voltage dependency was not directly related to the driving force of K ions, (2) in low Na solution, Ih became small in amplitude, and (3) the polarity of its tail current reversed at the membrane potential between EK and ENa. Ih was blocked by Cs, but was resistant to Ba. The resting potential and voltage responses of solitary bipolar cells can be interpreted by the combination of above 4 currents.

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RESPONSE DYNAMICS OF TYPE N CELLS IN CATFISH RETINA. SAKURANAGA, M. AND NAKA, K-I.*, Dept. of Physiol., Nippon Medical School, Bunkyo-ku, Tokyo 113, *National Institute for Basic Biology, Okazaki 444.

Type N cells in the retina of channel catfish, Ictalurus punctatus, are stimulated by temporally-modulated inputs and resulting responses are recorded. The cells are known as the sustained amacrine cell in other retina. Stimulus used is either a field of light or current injected into horizontal cell and the time course is modulated in a white-noise fashion. Both light and current stimuli produce the characteristic responses from the cell. The DC potentials which are prominent in the outer layer are lost in the cell and the linear and nonlinear components have comparable share in the dynamic responses. The linear component shows power spectra similar to those from the horizontal and bipolar cells with a roll-off frequency of about 10 Hz. The nonlinear components are the sharp depolarizing transients and small oscillations. Both nonlinear components are reproduced by the second and third order kernels and is apparently introduced when signals are transmitted from the outer to the inner retina. The peaks and valleys in the second order kernel indicates that the cell's signal processing is on the timing of incoming signals.

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MULTIPLE INPUTS TO THE ON-OFF-TYPE AMACRINE CELL IN THE CARP RETINA. WATANABE, S.-I. AND MURAKAMI, M., Dept. Physiol., Keio Univ. Sch. Med., Tokyo.

Chromatic responses of the ON-OFF-type amacrine cells of the carp retina consisted of transient ON- and OFF-depolarizations and sustained portion in between. When the retina was illuminated by 680 nm (red) light, the sustained portion stayed at depolarized level, being accompanied by membrane conductance increase. On the other hand, when 480 nm (blue) light was given, it was at hyperpolarized level associated with conductance decrease. This type of color opponency was observed in about 90 % of the cells examined. The transient ON- and OFF-depolarizations to red light appeared with a short latency, and showed conductance increase. On the contrary, those elicited by blue light had a long latency and were accompanied by conductance decrease. These observations indicate multiple inputs to the ON-OFF-type amacrine cell. In addition, when a red stimulus was superimposed on a blue stimulus with a time delay comparable to the latency difference, the amplitude of the ON-depolarization was not additive, but was greatly reduced. Since a postsynaptic summation of depolarizations with opposite conductance changes should result in an augmentation of the amplitude, it is suggested that some inhibitory interaction between inputs of color coding occurs probably at a level presysnaptic to the ON-OFF-type amacrine cell.

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IDENTIFICATION OF CATECHOLAMINERGIC CELLS IN THE RETINA OF JAPANESE DACE BY DOUBLE LABELING METHOD. HASHIMOTO, Y. AND CHINO, Y.* Dept. of Physiol., Tokyo Women's Med. College, Shinjuku-ku, Tokyo 162 and Div. Basic Sci., Illinois College of Optometry, Chicago, Illinois 60616

Intracellular responses were obtained from the cells in the inner retina of Japanese dace. The cells were classified according to their responses to a small spot and an annulus of either white light or monochromatic light. Following the classification and the investigation of response properties, ethidium bromide (a red fluorescing dye) was injected into the cell for morphological identification. We have stained a total of 102 cells: 38 On-Sustained, 17 Off-Sustained, 6 On-Transient, 2 Off-Transient, and 38 On-Off types. Only two of these units were classified as interplexiform cells; the remaining 100 were amacrine cells. The double labeling procedure (Aghajanian and Vandermaelen, 1982) showed that 17 (12 sustained and 5 transient) out of the 102 cells were identified as catecholaminergic (may be dopaminergic) neurons including the two interplexiform cells. This result is much different from the previous findings in the fish retina which showed that the only dopaminergic cells were interplexiform cells (Dowling and Ehinger, 1978; Negishi et al., 1981).

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EFFECTS OF PUTATIVE TRANSMITTERS ON AMACRINE CELLS OF CARP RETINA. KONDO, H. AND TOYODA, J. Dept. Physiol., St. Marianna Univ. Sch. of Med., Miyamae-ku, Kawasaki 213

The effects of iontophoretically applied L-glutamate, L-aspartate, acetylcholine(Ach), GABA and glycine on amacrine cells were studied in the isolated retina of the carp. Glutamate, aspartate and Ach could depolarize all three types ('on-off', 'on' and 'off') of amacrine cells. However, they had different effectiveness on the cell types. Generally, glutamate gave more potent effect on 'on-off' cells but weaker effect on 'on' and 'off' cells than Ach. Glutamate was effective on 25 of 31 'on-off' cells tested, but only 3 of 34 'on' cells and 8 of 42 'off' cells. Ach had effect on 11 of 18 'on-off' cells, 12 of 24 'on' cells and 13 of 34 'off' cells. Aspartate had similar, but relatively weak, effect to glutamate.

On the other hand, GABA and glycine could hyperpolarize the membrane potential or suppress the light response in the three types of cells. GABA was potent but glycine was very weak. The correlation between these chemicals and the cell types was not apparent in this work.

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FUNCTIONAL ROLE OF RECEPTIVE FIELD SURROUND OF GANGLION CELLS IN CATFISH RETINA. HIDA, E. AND NAKA, K.-I. National Institute for Basic Biology, Okazaki, 444

Ganglion cells of teleost retina form center-surround receptive field. To clarify the functional role of receptive field surround on its center response dynamics, temporal dynamics of off-center ganglion cells of catfish (Ictalurus punctatus) retina was analyzed by using microcomputer system. Receptive field center and surround were stimulated by spatio-temporal random noise and steady uniform light respectively. Increase of the intensity of surround illumination resulted in the increase of the incremental sensitivity of center response in addition to the improvement of temporal dynamics. Illumination of surround by spatio-temporal random noise of the same mean intensity as steady uniform light brought decrement of the incremental sensitivity of center response without changing temporal dynamics. These results showed that in ganglion cells response properties of receptive field center depended on mean intensity of stimulus light and on its fluctuating component.

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A COMPARATIVE STUDY ON RETINAL DOPAMINE CELLS IN SAME-AGED BUT DIFFERENT-SIZED CARP.
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Research has shown that an increase in the size of fish retina with growth is accompanied by a reduction in the density of retinal monoamine cells along with an increase in their cell number per retina. The age of fish, however, has not been considered in the above relation. In the present study, juvenile carp, hatched 2 months priorily, were reared for 10 months under 2 different conditions; one group was kept in an aquarium at room temperature (RT) and the other was kept in an aquarium in which the water temperature was constant (CT). The left eye of some fish was intravitreally injected with 5,7-dihydroxytryptamine. The fish grew faster in the CT than in the RT aquarium, resulting in a large variation in size among fish of the same age. In fluorescent retinal flatmounts, the density of dopamine (DA) cells was found to be lower but the cell number per retina to be greater in the CT than RT group; these differences were statistically significant ($p < 0.001$). The data suggest that the above parameters depend on the size of carp but not on their age. A comparison of the areal size of the original retina with that of the grown retina revealed that circumferential addition accounts for about 23% of the total increase in area while the remaining 77% is due to hypertrophy of the retina.

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DATA BASE CLASSIFICATION OF CATFISH RETINAL GANGLION CELLS.
HOSOKAWA, Y.* and NAKA. K.-I. National Institute for Basic Biology, Okazaki 444

Spike discharges were recorded from catfish retinal ganglion cells. Dynamic stimulus patterns were produced on a CRT by the INNIS-FREE display control. Response parameters were measured from PST histograms for 10 stimulus patterns. The measured parameters from 800 cells were organized into a data base, on which analyses were made. Most of the cells (83%) were clustered into on- and off-center types in the jointed into the horizontal cells to produce discharges. We did not find any directionally selective cells but some cells produce stronger response to one sweep direction. With drifting sinusoidal grating stimulus, 92% of cells responded only to temporal changes in illuminance in the receptive field irrespective of the direction or spatial frequency of the grating.

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Diameter and conduction velocity of ganglion cell axons in the retina of cats and monkeys.
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Axon diameters of ganglion cells were measured electron microscopically in the cat's optic nerve fiber bundle, cross-sectioned at 1.5 mm from the optic disc in nasal retina. A distribution histogram of the diameters revealed two clear modes at 0.5-0.75 and 1.75-2.25 μm and a small tail extending from 3 to 7 μm. This enabled us to segregate the histogram into three parts, corresponding to axons of W, X, and Y cells. Mean diameters of the three classes were 0.83, 1.96 and 4.9 μm. Mean axonal conduction velocities were determined electrophysiologically for W, X and Y cells as 0.6, 2.5 and 5.0 m/sec, respectively. From these measurements, a correlation between axon diameter (D) and the conduction velocity (V) was obtained as follows: $V=3.85\sqrt{D}-2.3$. Similar measurements were made of axon diameters of ganglion cells in the Japanese monkey retinas. The diameter distribution revealed a single mode at 0.45-0.5 μm with a range from 0.2 to 2.4 μm. In view of Perry et al.'s work (1984) the sharp mode was interpreted as corresponding to axons of Pβ or X-like cells while small number of large axons were to those of Pα or Y-like cells. Electrophysiologically determined mean conduction velocities of these two groups of axons were 0.72 and 1.2 m/sec. There was a suggestion of the third group with conduction velocity of 0.93 m/sec, corresponding to a small shoulder around 0.7-1.0 μm in the diameter histogram.

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CHROMATIC PROPERTIES OF VISUAL INTERNEURONS IN THE SWALLOWTAIL BUTTERFLY LARVA.
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The larval butterfly Papilio xuthus has three types of color receptors (UV, Blue and Green), and it has two optic neuropils: lamina and medulla. The medulla neurons recorded respond with an increase or decrease in spike activity associated with a slow depolarization or hyperpolarization of the membrane. 18 types of medulla neurons are divided into three major classes according to the number of dominant input color channels: monochromatic, dichromatic and trichromatic neurons. The latter two are divided into two subclasses: color-opponent and non-color-opponent neurons.

(a) Monochromatic neurons: B+, B-, G+, G- (+ excitatory; - inhibitory)
(b) Dichromatic neurons: color-opponent; UV-G+, B-G+
non-color-opponent; UV+B+, UV+G+, B+G+, UV-G-
(c) Trichromatic neurons: non-color-opponent; UV+B+G+, UV-B-G-
color-opponent; UV+B-G-, UV-B+G+, B+UV-G-, B-UV+G+, G+UV-B-, G-UV+B+

8 types of color-opponent neurons found suggest that color antagonism is a basic strategy for color integration, employed in the larval visual system as in vertebrate visual systems.

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ANATOMY AND PHYSIOLOGY OF THE THIRD ORDER NEURONS OF COCKROACH OCELLUS.
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Nine types of the third order neurons of cockroach ocellus have been identified anatomically and physiologically. All of them receive synaptic potentials from the large second-order ocellar neurons(L-neurons) monosynaptically.

The third order neurons are classified into three classes as follows.

1. Four types of neurons with terminal arborizations in the posterior slope neuropiles of the protocerebrum. Three out of them are non-spiking neurons. The light and current stimuli failed to induce spike activities in these neurons. The other one generates a few spikes at light-off.
2. Two types of neurons with terminal arborizations in the lobula and medulla. They have spontaneous spike activities, and the spike frequency decreases as the illumination to the ocellus increases.
3. Three types of the descending neurons. Two of them have no spontaneous spike activities, and show a few spikes at light-off. The other one is a multimodal neuron. It responds with a burst of spikes to wind stimulation to the antenna and to a moving spot applied to the compound eye. It receives EPSPs from L-neurons at light-off.

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X-TYPE DORSAL LATERAL GENICULATE CELLS RESPONDING TO STIMULATION OF THE PRETECTUM AND/OR SUPERIOR COLLICULUS.
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Whether X-type retinal ganglion cell axons project to both the dorsal lateral geniculate nucleus(LGD) and pretectum(PT) or superior colliculus(SC) was studied in the cat anesthetized with 70% N_2O/30% O_2 by examining responsiveness to stimulation of PT and/or SC for LGD cells. The X-cells in the LGD were identified by spike response latency to stimulation of the optic chiasm(OX), conduction velocity of the optic tract (OT) fibers innervating them and receptive field(RF) property. The X-cells responding monosynaptically to stimulation of the PT and/or SC, i.e., receiving OT fibers branching to the PT and/or SC were distributed as follows: branching to PT; 39/136(29%): branching to SC; 36/139(26%): branching to both PT and SC; 5/114(4%). For X-cells activated from the PT and/or SC, the PT or SC latency tended to be a little longer than OX latency. A close correlation between OX and PT or SC latencies was also found. These X-cells were driven from either the contra- or ipsilateral eye and their RFs consisted of both ON- and OFF-types. The RF positions of the X-cells were located within the RF of multiple-units from the SC of which stimulation could activate the related X-cells. The present results demonstrate that some of X-type LGD cells receive X-type retinal ganglion cell axons branching to the PT and/or SC.

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CHANGES OF UNITARY DISCHARGES TO MOVING SLIT IN THE VISUAL CORTEX OF THE CAT IN APPLICATION OF DRUGS VARYING GABAergic ACTIVITIES.
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When a dose of 300mg/kg body weight of sodium valproate(VPA) was given intravenously, the frequency of spike discharges derived from the dominant eye stimulated with the moving slit, reached about 90% reduction of its initial value at 12 minutes and returned to its initial level after 70-90 minutes. In case of lower doses of VPA(200-100mg/kg), the magnitude of the frequency decrease was smaller and the recovery time was shorter. There was a linear relationship between the frequency decrease and the dose of VPA. However, the frequency of spike discharges resumed after 70-90 minutes did not depend on the temporal change of the concentration of VPA in the blood serum.

Responses of binocular cells to a dose of 300mg/kg of VPA were different from cell to cell; responses derived from either eye were decreased together, or responses from the dominant eye were decreased while those from the nondominant eye were increased. However, binocular interaction mechanism related to the receptive field disparity was well preserved.

The notable changes in spike frequency were not shown over 2 hours following the injection of 12.5mg/kg of thiosemicarbazide caused 38% reduction of pyridoxal phosphate in the blood serum after 15 minutes.

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EFFECTS OF AN EXCITATORY AMINO ACIDS ANTAGONIST ON VISUAL RESPONSES OF STRIATE CORTICAL NEURONS IN THE CAT.
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Glutamate and aspartate are supposed to be excitatory transmitters in the cat visual cortex. To test this possibility and to elucidate their roles in information processing in the cortex, we studied effects of iontophoretic application of kynurenic acid (KYNA), which was reported to be an antagonist of the amino acids, on activities of cortical neurons. In almost all the cells tested, KYNA antagonized excitations induced by application of glutamate but did not depress those by a control excitant, acetylcholine. Effectiveness of KYNA was related to types of visual responses. In 19 of the 28 simple cells tested, KYNA completely depressed visual responses and spontaneous activities, while such a complete block was seen in only 6 of the 22 complex cells. In terms of laminar locations, the great majority of the cells in layers IVab, IVc and the upper part of layer VI were completely depressed by KYNA whereas most of the cells in the other layers were only weakly or not depressed at all. These findings suggest that glutamate/aspratate may be excitatory transmitters from geniculate afferents to first-order neurons in the visual cortex.

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ELECTROPHYSIOLOGICAL AND MORPHOLOGICAL STUDIES OF DEVELOPMENT OF INTRACORTICAL CONNECTIONS IN CAT'S VISUAL CORTEX USING SLICE PREPARATION.
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Current-source density (CSD) analysis of the field potentials evoked in the visual cortex of whole cat preparation anaesthetized with N_2O and Alphadione demonstrated that excitatory transmission across the geniculo-cortical synapses is effectively working at birth , but not polysynaptic transmission across the intracortical synapses. A slice preparation was made from the brains used for CSD analyses, and HRP was injected into white matter. Among the efferent cells retrogradely labelled by HRP, spiny stellate (SS) cells in layer IV and star-pyramids (SP) in layer II were selected as possible candidates for source and target cells in the intracortical transmission. At birth dendritic arbors in the SP cells were short with few spines, while those in the SS cells were relatively well-developed with numerous spines. The increase in the number of the spines during the postnatal period was much more remarkable in the SP cells than in the SS cells. By contrast, axonal arborization of the SS cells was very poor at birth. Axonal projection to the supragranular layers reached the adult level a few months after the birth.

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RECEPTIVE FIELD PROPERTIES OF CELLS IN THE CAT AREA 19 AS STUDIED BY SINUSOIDAL GRATINGS. TANAKA, K., RAMOA, A. S.* AND OHZAWA, I.* NHK Broadcast. Sci. Res. Labs., Kinuta, Setagaya, Tokyo and School of Opt., Univ. of Calif., Berkeley, California

The receptive fields of area 19 cells were explored by using drifting sinusoidal gratings. The extent of area 19 was determined by a systematic mapping of receptive field, and cells were sampled so that their eccentricity evenly distributed from 0 to 10°. Two-thirds of the cells showed strong end-stop inhibition. The properties of their central excitatory fields were determined by presenting a grating 1-3.5° long. 1) Three quarters of the cells showed mainly unmodulated responses, and the remainder modulated ones; 2) the best spatial frequency distributed around 0.4 c/deg; 3) the spatial tuning band width widely distributed from 0.7 to 3.3 octaves; 4) the temporal frequency tuning tended to be broad with a peak around 2-4 Hz. The properties of the peripheral suppressive fields which caused the end-stop inhibition were then determined by presenting an additional grating to the outside of the central excitatory field. This second grating suppressed the excitatory responses evoked by the first one. 1) The phase relation between the two stimuli did not affect the strength of the suppression; 2) the largest suppression was obtained by surround stimuli of 0.4-0.56 c/deg; 3) the spatial band width of the suppression was 1.5 times larger than that of the excitation.

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INHIBITORY EFFECTS OF PASSIVE EYE MOVEMENT ON THE CAT'S VISUAL CORTICAL NEURONS. ENOMOTO, H. AND ICHIHASHI, K. Dept. of Physiol., Kawasaki Med. Sch., Kurashiki 701-01

The effects of passive eye movement on neuron discharges in the primary visual cortex were investigated in adult cats anesthetized with nitrous oxide. About 60% of transient cells, the transient responses evoked by visual stimulation were inhibited if the passive eye movement was applied simultaneously. The inhibitory effect was observed after a latent period of about 40-60 msec. Following the transient inhibition, neuronal discharges were argumented for 80-150 msec, which would be post-inhibitory rebound. On the other hand, none of sustained cells showed inhibition of visual responses when concurrent stimulation was given by passive eye movement. The initial inhibitory and secondary excitatory effects were seen when passive eye movement was applied to the same direction which the neuron showed the orientation selectivity. It is suggested that this effect will play an important role in visual recognition during and after saccadic eye movement.

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CORTICOFUGAL PROJECTION OF CONTRALATERAL MONOCULAR MOVEMENT IN THE CAT. TAMAI, Y., FUJII, T.*, NAKAI, M., KOMAI, N.* and TSUJIMOTO, T. Dep. of Physiology and Dep. of Neurosurgery*, Wakayama Medical College, Wakayama 640

We reported that the electrical stimulation of the internal cortex of the coronal sulcus in the cat elicited contralateral monocular movement and that the movement of contralateral eye was impaired by the lesion of this cortex. Then, the direct pathway from the internal cortex of the coronal sulcus to the ventral region of the pontine reticular formation was shown by using the electrophysiological method and the retrograde labeling method with HRP. HRP labeled neurons were detected in the internal cortex of the coronal sulcus following HRP injection into the pontine reticular formation where the contralateral eye movement was induced. The latency of 17msec, with which the contralateral eye movement was induced by the stimulation of the pontine reticular formation, suggested the polysynaptic connection to the contralateral dorsal mesencephalic reticular formation adjacent to the oculomotor nucleus where ipsilateral eye movement was evoked with latency of 10 msec.

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Pupilloconstrictor areas in the parietooccipital cortex in the cat.
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A pupilloconstrictor area in the posterior medial lateral suprasylvian (PMLS) area was related to another pupilloconstrictor area in the cat posterolateral occipital cortex (Barris, 1936) by using local lesion and local cooling of the posterolateral cortex.

Cats were anesthetized by alpha-chloralose and urethane. The area of the left pupil was monitored by an infrared pupillometer and an infrared video system. PMLS was stimulated by a bipolar acupuncture electrode. For local lesion, an acupuncture needle was inserted into the posterolateral cortex, and the region was explored from which pupillary constriction was evoked. By passing dc-current, lesions were made in the posterolateral cortex until PMLS-evoked pupillary response reduced clearly.

By local lesion, the pupillary responses evoked by stimulating PMLS of either side were reduced to about 50% of the original responses. The site of lesion was confirmed in the histological sections, which was in the area 20 and adjacent white matter. By local cooling, PMLS-evoked pupillary response was reversibly reduced to about 50%.

From these results, it is concluded that area 20 plays an important role in the PMLS-evoked pupillary constriction.

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PARIETAL VISUAL NEURONS RESPONDING TO ROTARY MOVEMENTS OF THE VISUAL SIMULUS.
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In the posterolateral part of area 7a of alert behaving monkeys, we found a group of neurons (N=22) which responded specifically to rotary movements of the visual stimulus. Eight neurons responded optimally to the rotation of a slit around the fixation point on the tangential screen in one direction, either clockewise or counterclockwise, and were inhibited by the rotation in opposite direction. They responded similarly to the same direction of rotation regardless of orientation or shape of the stimulus, and responded even to a pair of spots rotated around the middle point. Most of them responded to the same rotating stimulus presented anywhere within the range of several tens of degrees from the fixation point. We also found six neurons responding to the rotation in the saggital plane, four neurons preferring the horizontal plane and four others preferring the diagonal plane. Five of these rotation-sensitive neurons enhanced their response when the rotating stimulus was combined with the tracking eye movement. Three neurons were found to respond to the rotation of the monkey chair in the dark with the same directionality as the visual rotation, suggesting the convergence of visual and vestibular inputs.

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TASK-RELATED RESPONSES AND VISUAL RESPONSES OF MONKEY VISUAL CORTICAL NEURONS .
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In the previous work, we found that many visual cortical cells of monkey showed task-related responses while the animal was performing visual fixation task. To reveal the functional significance of these task-modulation, we examined visual responses at various phases of the task . The stimuli was full screen (C.R.T. 22 × 15 deg. in visual angle) of random dots with spatial frequency selected variously and was lighted for 500 msec in flip-flop manner (3.5 ∼ 28 Hz) .

Among 56 visual responsive neurons from 311 total samples, 58.9 % showed special response type ; the response during fixation period was weaker than that of inter trial interval , whithin which pre-task (about 1 sec before task start) response was stronger than that of post-task I.T.I. (about 1 sec after task end). The ratio of cells showing these visual response modulation was 38.1 % in prestriate cortex , 75.0 % in inferior temporal cortex and 57.1 % in S.T.S. polysensory area. This tendency is comparable to that of task-related response.

These results may suggest that non-sensory inputs observed as task-related activity also modulate the neuronal response to the sensory information .

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MORPHOLOGY OF THE TECTORIUM AND THE SENSING MEMBRANE IN THE FROG AMPHIBIAN PAPILLA AND PROBABLE CORRELATION WITH THE TONOTOPIC ORGANIZATION. YANO, J., OOYAMA, H. and SUGAI, T. Dept. of Physiol., Kanazawa Med. Univ., Uchinada, Ishikawa

In the bullfrog amphibian papilla(AP), the sensory macula resides on the ceiling of the AP and consists of 2 patches, rostral triangular and caudal S-shaped ones. The tectorium(TCT) hangs from the macula. The thin sensing membrane(SM) extends dorsoventrally from the TCT to the floor of the papilla and from the posterior edge of the rostral macula anteromedially to the column of the AP nerve branch posterolaterally. These sound-conducting apparatus were reconstructed with the serial sections of the AP. The orientation of the hair cells(HCs) on the macula was related to the microstructure of the TCT and the SM. In the middle portion of the S-shaped macula, the orientation of the HC seems to be at right angle to the SM. HCs of the triangular macula and the anterior curvature of the S-shaped macula are polarized along axes parallel to the respective maculae. In these regions the medial TCT connects to the anteromedial extremity of the SM, whereas lateral TCT to the middle SM. When the tonotopic organization of the maculae is considered, this observation suggests the frequency selectivity on the SM, that is, anteromedial and posterolateral SM corresponding to low and high frequencies, respectively.

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IMMUNOHISTOCHEMICAL LOCALIZATION OF TRIPHOSPHOINOSITIDE IN THE COCHLEAR HAIR CELLS. HORIKOSHI, T., YANAGISAWA, K. AND YOSHIOKA, T.* Dept. Physiol., Sch. Dent. Med., Tsurumi Univ., Yokohama 230, *Dept. Physiol., Sch. Med., Yokohama City Univ., Yokohama 232

Aminoglycoside antibiotics are known as ototoxic drugs. Schacht (1976) studied the neomycin toxicity to the inner ear and suggested that polyphosphoinositide (PPI) serves as a receptor for neomycin. In this study, we examined the localization of triphosphoinositide (TPI; one of the PPI's) in the cochlear hair cells by immunohistochemical technique. For production of the antibody to TPI, rabbits were immunized with TPI-hapten suspensions (Kataoka & Nojima, 1970). The characteristics of the antisera were examined by enzyme-linked immunosorbent assay (ELISA) and radioimmunoassay (RIA). The obtained antiserum highly reacted to TPI, but its cross reactivities to other phospholipids were low. For immunohistochemical detection of TPI, the antiserum was applied to the organ of Corti fixed with 3% paraformaldehyde and 1% glutaraldehyde mixture. Then the specimens were stained with the PAP method. The positive staining was observed at the stereocilia of both inner and outer hair cells. The rabbit serum which was not reacted to phospholipids was applied to the similar specimens, and no specific staining was observed. After the antiserum to TPI was absorbed by TPI, the reactivity to TPI was decreased to about 30% (by RIA) and the staining of the stereocilia was decreased. These results suggest that TPI is localized at the stereocilia in the cochlear hair cells.

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VOLTAGE-DEPENDENT IONIC CURRENTS IN HAIR CELLS OF GOLDFISH. SUGIHARA,I., AND FURUKAWA,T. Dept. of Physiol., Sch. of Med., Tokyo Med. & Dent. Univ. Yushima, Bunkyo-ku, Tokyo 113

Patch clamp method was applied to hair cells isolated with papain from the saccule, lagena and utricule of goldfish. When tested with electrodes filled with isotonic KCl under the whole cell clamp mode, the input impedance and zero-current potential in 10 cells were 0.5-1.2 Gohm and 40-70 mV respectively. The outward current observed during depolarizing pulses seemed to be consisted mostly of Ca dependent K current. On the other hand, the inward current during hyperpolarizing pulses, which seemed to represent anomalous rectifier K current, varied in magnitude among hair cells. When CsCl-filled electrodes were used, a prominent inward tail current was recorded upon the cessation of depolarizing pulses. Presumably, it represented Ca current, but it was not certain whether it was the same current as the one that functioned to trigger the transmitter release. In five cases, outward single channel currents were recorded with KCl-filled electrodes under the cell attached condition. The single channel conductance of this channel was about 170 pS. This channel seemed to be the Ca dependent K channel through which the outward current flowed under the whole cell clamp mode.

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MECHANOELECTRICAL TRANSDUCTION IN THE ISOLATED VESTIBULAR HAIR CELL OF THE CHICK.
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Properties of the mechanically gated channel were studied in the papain (7-8 μU/ml) dissociated hair cells of the chick vestibular organ. In response to mechanical stimuli applied to the hair bundle by a glass rod, hair cells generated currents which basically followed a waveform of the mechanical stimuli with occasional steps and spiky appearances. The current was blocked by streptomycin in a voltage dependent manner. The unitary step had a conductance of 50 pS. Current steps with conductance of the integer multiples of 50 pS were frequently observed, suggesting concerted activities of elementary mechanosensitive channels. The mechanoelectrical transduction (MET) channel was permeable to Li, Na, K, Rb, Cs, choline, Tetramethylammonium, and to Tetraethylammonium in the order listed here, and the reversal potential changed from +10.7 (Li) to -25.3 (TEA) (mV) when intracellular CsCl-EGTA solution was used. Ca, Sr, Ba, Mn and Mg were also permeable in this order through the MET channel, and the reversal potential was +28.2 (Ca) and +18.0 (Mg) (mV) when CsCl-EGTA was used intracellularly. The MET current was stably generated in salines with $[Ca]_o$ down to 20 μM. Sr could replace Ca and maintained the MET activity, however, Ba, Mg and Mn could not. A presence of 50-200 μM Ca in isotonic Ba saline could maintain the MET activity.

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PERIPHERAL BRANCHING PATTERN OF AUDITORY FIBERS OF GOLDFISH AS REVEALED BY INTRA-AXONAL INJECTION OF LUCIFER YELLOW
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This study, an extension of a previous one made with an injection of Procion Yellow (Furukawa, 1978), aimed at elucidating, by the use of a more efficient fluorescent dye, Lucifer Yellow, fine terminal branching patterns of auditory fibers. Successful observations were made on 31 large and 23 small saccular fibers (S1 and S2 fibers respectively). As a finding common to S1 and S2 fibers, it was observed that fibers mostly made one, or rarely two, bifurcations before they entered the saccular macula, although some fibers made no branching outside the saccular macula. In S1 fibers that distributed over a limited area on the rostral part of the saccular macula, each axon that entered the macula was found to send fine dendritic processes to on the average ten hair cells that were located within the area of 25 to 50 μm across. On the other hand, each S2 fibers that entered the caudal part of the macula sent fine branchings to about 20 hair cells that were located within the area of 50 to 150 μm across.

Another interesting finding was that the dye spread into the hair cells in about 1/4 of S1 fibers studied. This finding poses an interesting question as to the route of the dye spread.

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ELECTRICAL ACTIVITY OF AFFERENT FIBERS IN THE ISOLATED SACCULUS-SACCULAR NERVE PREPARATION OF GOLDFISH.
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A novel *in vitro* method for studying electrophysiological property of hair cells and auditory nerve fibers of goldfish was developed. The sacculus and saccular nerve was isolated from goldfish and perfused with a physiological salt solution. Electrical activity of auditory nerve fibers was recorded either extracellularly by a capillary suction electrode or intracellularly by glass microelectrodes. To apply mechanical stimulation to the hair cells, a glass probe of a piezoelectric vibrator was made contact with the saccular otolith. When a square voltage pulse was delivered to the vibrator, an extracellular potential change positive in sign (delay 0.5-2.0ms, duration about 10ms) was observed. This potential was markedly depressed by applying tetrodotoxin (0.003mM), dihydrostreptomycin (0.3mM), acetylcholine (3mM), kainic acid (0.3mM) or high concentration of Mg^{2+} (5.5mM). These results were consistent with the idea that the electrical activity of the saccular nerve was generated through the process of transduction in the hair cells and the synaptic transmission in the afferent synapses. It was suggested that the present preparation was suited for studying the mechanism of the synaptic transmission between hair cells and auditory nerve fibers.

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SUPPRESSIVE EFFECT OF 5-HT ON THE AMPULLARY ELECTRORECEPTOR OF PLOTOSUS.

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The afferent discharges of isolated electroreceptors of Plotosus are reversibly suppressed by bath-applied 5-HT. Since the receptor has only afferent synapses, the afferent suppression has been further examined in terms of pre- and post-synaptic responses. PSP was externally recorded in the presence of TTX. The sigmoidal input-output relation in terms of PSP was dose-dependently shifted to right along I axis, in such a way that the "resting" PSP was gradedly reduced. PSP suppression to 50 and 100 % occured at 1.3 ± 0.21 and 4.9 ± 1.0 mM (mean ± S.D., n=6), respectively. Structural analogues to 5-HT, such as 5-methoxy tryptamine and melatonin, also induced the PSP suppression at similar doses. Cathecholamines, such as NorAd or dopamine, were also effective at higher doses, though often irreversible. The "resting" DC level in the ampulla was hyperpolarized by 5-HT. The presynaptic activity, i.e. the receptor cell activity, could be estimated from the ampullary responses. This measure was similarly shifted to right along I axis. Thus, the effect of 5-HT is clearly presynaptic, in that suppression of the graded Ca activity of receptor cells reduces the release of the transmitter to result in the afferent suppression.

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CIS-PLATINUM EFFECTS ON COCHLEAR POTENTIALS

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Cis-Platinum has ototoxic side effects. In this study,effects of cis-Platinum on cochlear potentials were investigated. Cis-Platinum was administered intraperitoneally every consecutive days. When Preyer-reflex was disappeared, potentiols in the scala media were recorded. After pysiological experiments, animals were sacrificed and then the cochleae were removed and perfused with histochemical reaction solution containing nitro-blue tetrazolium (NBT) and phosphate buffer. Reaction with NBT was photographed by surface preparation technique. There is a good agreement between the disappearance of Preyer-reflex and the deficit of NBT reaction on outer hair cell.
Main results were: 1) almost no effect on endcochlear potentials 2) deficit of NBT reaction was observed on outer hair cells in all animals but not on inner hair cells 3) the extent of deficit of NBT reaction was the greatest in first row and the least in third row, and was greater in lower turns of the cochlea 4) truncation of input-output curve of CM was observed 5) good correlation between N_1 amplitude and the extent of deficit of outer hair cells.

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THE RESPONSES OF THE AMPHIBIAN PAPILLA NERVE FIBERS TO TONE BURSTS FOLLOWING THE ELECTRICAL STIMULATION OF THE EIGHTH NERVE.

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Single unit discharges were recorded from the amphibian papilla nerve branch of the anesthetized toad. Electrical condition stimuli, in trains of 3-20 pulses of 0.5-12V at 500-600/s, were delivered to the eighth nerve trunk about 80msec before tone bursts test stimuli. The thresholds to the tone bursts increased by the condition stimuli, in particular at the characteristic frequency(CF) of the unit,and the tuning curve was broadened. The threshold elevations at the CF's ranged from 8 to 24dB. Discharges to the CF tone bursts, 10dB above the threshold of the units,decreased to null near the beginning of the tone bursts by the condition stimuli. Recovery to the control level required about 200msec.

Both the increase of the thresholds and decrease of the discharges might be caused by the activity of the efferent fibers in the eighth nerve. These effects were observed more often in the low CF units than in the high CF units, which is in accordance with the anatomically known efferent innervation to the papilla.

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TIME COURSE OF CHANGE IN FIRING PROBABILITY DENSITY IN NERVE FIBERS OF CAT COCHLEA. ITO, S., HORIKAWA, J., MINAMI, S*. AND MURATA, K. Dept. of Neurophysiology, Med. Res. Inst., Tokyo Medical and Dental Univ., Kanda-surugadai, Tokyo, 101

Impulse trains to continuous CF tone stimuli were recorded from cochlear nerve fibers, and the time course of change in the firing probability density after a previous discharge (hazard function) was estimated. The value of the hazard function without intentional stimulation (standard hazard function) began to increase after an absolute refractory period (t-ref) and asymptotically approached a plateau (p-max) with a time constant (t-const). In high CF fibers, the stimulation increased p-max, but t-ref and t-const showed no great change. So the hazard functions under the stimuli can be expressed as the multiplication of the standard hazard function and a factor of the input process which depends on stimulus intensity. In low CF fibers, the stimulation caused a periodic oscillation on the hazard function due to phase locking. Estimation by assuming that this oscillation is due to a periodic fluctuation of the input factor gave a smoothed hazard function which seems to have the same t-ref and t-const as the standard hazard function.

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A MACROSCOPIC LAW OF THE JUNCTIONS BETWEEN HAIR CELLS, A SPIRAL GANGLION AND COCHLEAR NUCLEI. OONUKI, M., Dept. of Physiol., Sch. of Med., Kitasato Univ., Sagamihara, Kanagawa 228

A macroscopic law for the transmission in neurons(1982,J.theor.Biol.) was applied to the analysis of the junctions in the title, using the experimental data of the latency and the firing rate of cat(Kites et al, 1978,J.Neurophysiol.). A developmental law for the junctions was obtained: $1/G1\sqrt{m1}+1/G2\sqrt{m2}$=constant value: G1 stands for the average of the time constants at the hair cell-spiral neuron junctions:G2, the average of the time constants of the dendrites of a cochlear nucleus neuron: m1,m2 are the transmission probabilities defined as the ratio of the firing rate of a presynaptic cell to that of a postsynaptic one. G1,G2,m1,m2 are varied with the sound pressure level and the frequency, but the sum above descrived is kept constant. This result shows that some developmental law controls the junctions between neurons and sensory receptors, and the equation above stated is the electrophysiological face of that law.

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COMPARISON OF ACOUSTIC MIDDLE EAR MUSCLE REFLEX BETWEEN RATS AND SOME OTHER ANIMALS. MURATA, K., ITO, S. AND HORIKAWA, J., Dept. Neurophysiol., Med. Res. Inst., Tokyo Med. and Dent. Univ., Kanda-surugadai, Tokyo, 101

The acoustic reflex in albino rats was studied on its sensitivity, the middle ear transmission loss due to the reflex and the transfer characteristics of the reflex by change in CM and by the motor unit activity of the tympanic muscle for comparison with the reflex of guinea pigs, rabbits, cats and humans. The sound frequency that could elicit the reflex was below 12kHz with the best frequency at about 3kHz, and the transmission of sounds was suppressed by the reflex up to more than 20kHz, i.e., there occured a large transmission loss at the best frequency. The best frequency and upper frequency limit of reflex-elicitation were lower than those of the other animals in which transmission loss is known to be below 1-2kHz, i.e., there occured no transmission loss at the best frequency. The reflex was faster and damped better in rats than in some of the animals above. This stability of the reflex of rats may be due to the fact that, unlike the other animals, periodic change in the discharge rate of motor units of the tympanic muscle during the reflex was not synchronous between units.

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PITCH-SYNCHRONOUS RESPONSES OF MEDIAL GENICULATE NEURONS TO FORMANT ANALOG. HASHIMOTO, T. Inst. Med. & Dent. Eng., Tokyo Med. & Dent. Univ., Surugadai, Kanda, Chiyoda-ku, Tokyo 101.

In unanesthetized guinea pigs, neuronal activity was recorded in response to formant analogs and sinusoidal click trains. For most units responding to these stimuli, the impulse discharges were synchronous with the pitch period of the stimulus. In previous reports, the present author has demonstrated that the pitch-synchronous response to vowels is preserved in the auditory system from the cochlear nerve up to the medial geniculate body. The present study described the functional properties of the pitch-synchronous response in the neuronal representation of speech sounds and natural animal calls. The repetitive element of an utterance could be represented up to the repetition rate of 400 Hz in the neuronal activity of the medial geniculate body. The pitch-synchronous property was not always monotonic to the increase or decrease of the pitch of the formant analog or the repetition rate of the click train. A minor selectivity to the stimulus pitch was observed at the integral multiple of 40 Hz or 60 Hz.

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RESPONSES OF BAND OF NOISE UNITS. MARUYAMA, N., SAITOH, K., KUDOH, M. AND FURUKAWARA, M. Dept. Neurophysiol., Brain Res. Inst., Niigata Univ., Niigata 951

As reported previously, about half of the cat auditory cortex (AI) units were band of noise (BN) units which were divided into 3 types based upon the best bandwidth. These units responded to saw-tooth AM tones (STAMs). Spectrum envelopes of the most effective BN and STAM were very similar for each unit. The discharges of most BN units were phase-locked to the modulation saw-tooth, when the pitch frequency of STAM was lower than about 200 Hz. The response of some BN units to STAM had several local maxima in terms of STAM pitch frequency. When the pitch frequency was lower than 200 Hz, such units exhibited phase-locked responses at the local maxima. But the responses were not phase-locked at the local maxima in the higher pitch than 200 Hz. We investigated also the response of the BN unit to the combination of 2 BNs. The combined BNs were not more effective than single BNs for all neurons studied.

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ULTRASOUNDS PERCEPTION IN THE RODENTS. TSUTOMU KAMADA AND KAZUO KAMEDA Dept. Oral Physiol., Hokkaido Univ. School of Dent., Sapporo 060

Pups of Mongolian gerbils (Meriones unguiculatis) emit the ultrasounds like other rodents. To investigate further the role of ultrasound in rodent communication we examined the sensitivity of single units to ultrasounds in the inferior colliculus (IC) of the gerbil. A few hours after surgery under Nembutal, a glass micropipette filled with 3 M KCl was penetrated to exposed IC dorso-ventrally. Acoustic signals were presented to animal in open field from condenser speaker with 5.5 cm diameter. 25 Units out of 82 responded to the sounds above 20 kHz. Best frequency ranged from 1.15 to 52.5 kHz. Minimum threshold of units was 4.5 dB SPL at 14 kHz. Threshold of the unit with best frequency of 52.5 kHz was 76.5 dB SPL, while that of the unit with best frequency of about 20 kHz was 18 dB SPL at minimum. This finding corresponds to the result that the threshold curve of the evoked potential of IC has the second deep peak at about 40 kHz (Brown 1973). The tuning curves of units were rather broad: Q_{10} dB being 8.25 at average (1.16 -53.89). Latencies of units were 3.4 - 8.6 msec. Dorso-ventral tonotopicity was recognized.

The result indicates the possibility to use the ultrasound as a specific communication signal in this animal.

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WHY HAVE NOCTURNAL OWLS A ROUND AND LARGE FACE ? YOSHIDA, A. Dept. of Physiology, Sch. of Med., Gifu Univ., Gifu 500

Some nocturnal owls can accurately locate a sound source in total darkness. The ratio of the size of the owl's head to that of the body is much larger than eagles' and hawks'. A barn owl has a large face with a radius of about 6 cm. By using this large face, it can effectively collect sounds. As the result, the owl can get much greater intensity difference between two years. In the case of the barn owl, when the radius decreases into 2/3 of it, it may no longer be able to locate sounds. here we explain that what an extent it is for the owl to be able to distinguish the minimum intensity difference between two years and why it is necessary for nocturnal owls to have a large and round face.

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MULTIPLE REPRESENTATION OF TARGET DISTANCE IN AUDITORY CORTEX OF THE MUSTACHED BAT. HORIKAWA, J. AND SUGA, N*. Dept. of Neurophysiol., Medical Research Inst., Tokyo Medical and Dental Univ., Kanda, Tokyo 101, Japan. *Dept. of Biology, Washington Univ., St. Louis, Mo 63130, U.S.A.

Two areas of neurons sensitive to target distance were found in the dorsal part of the auditory cortex of the mustached bat (Pteronotus parnellii parnellii): one was FM-FM area, the other was dorsal fringe (DF) area. Neurons in these two areas responded to the combinations of two from four simulated harmonics of orientation sounds (OS), especially to the frequency modulated (FM) part of OS, and each of the neurons was tuned to a particular deley (best delay, BD) of FM_n, the n-th harmonic, relative to FM_1. The BD shortened toward the rostral part of the auditory cortex. The neurons were also tuned to a particular echo amplitude (BA) within a range of 20 to 80 dB SPL in FM-FM area and of 28 to 80 dB SPL in DF area. Iso-impulse-count contour lines as to the delay and amplitude of the echo revealed two types of the neurons: a) distance sensitive type with the delay independent of the amplitude, and b) target-persuit type with the delay whose value to give the maximum response was decreased with increase of the amplitude.

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Electrical activity of vocalization nuclei, robustus archistriatalis (RA), in canary brain slice preparations. HIRONOBU SAKAGUCHI and NOZOMU SAITO, Dept. of Physiol., Dokkyo Univ. Med. Sch., Mibu, Tochigi 321-02

Using slice techniques, we succeeded in the recordings of electrical activity from vocalization nuclei, robustus archistriatalis (RA), in adult canary. A slice was made by sagittal section. The thickness of the slice was 0.5-0.8 mm. The canary brain slice was maintained in the same manner as the mammalian brain slice was usually made. Under low power magnification, two telencephalic vocal control nuclei, RA and hyperstriatum ventrale pars caudalis (HVc), were clearly visible in the same plane of slice preparation. Evoked potentials were recorded by stimulating dense fiber bundles from brain stem or several separate fibers from HVc. These potentials consist of the initial negative deflection and the late components which were blocked by a solution with Mg^{2+} and lowered Ca^{2+} concentration. The initial deflection was viable for more than 7 hours, while the late components did not survive the initial component and were abolished within 2.5 hours. This seems to permit the late component to be of synaptic nature.

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MOVABILITY OF MECHANOSENSORY SETAE AND THE RECEPTIVE FIELDS OF THE INNERVATING NEURONS.
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Mechanosensory hairs on the crayfish (Procambus clarkii) antennas were classified into three types, L, S and F. The movability in relation to directionality measured with step mechanical stimuli showed the needed force of 0.35 and 0.49 x 10^{-4} N per 10 degrees for down- and upward movement. The value obtained by this method showed a rather larger value because of the inertia of the hair-transducer system. To avoid this difficulties, ramp mechanical stimuli were used for displacement. In the S type hairs both on the inner flagellum of the first antenna and on the second antenna the force needed for 10 degrees movement ranged from 0.6 - 1.3 and 0.05 - 0.3 X 10^{-6} N for up- and downward shifts. The smallness of the values than those obtained by ramp pulses is resulted by devoiding of the transient phase at the begining of the stimulation. The U/D ratio was ranged from 6 to 8 in the S type hair. The stress contour for movement was a skewed circle with a strong decrease in downward direction. These directional differences are relating to the shape of hair pits, and directionalities of the neuronal receptive field is relating partly to these structures.

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RESPONSE CHARACTERISTICS OF IMMATURE PACINIAN CORPUSCLES IN THE KITTEN'S MESENTERIES TO MECHANICAL STIMULATION.
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An investigation of the postnatal developments of the Pacinian corpuscles in the mesenteries of 20 cats aged from 19 days to 3 months was performed. The Pacinian corpuscles of a one-month kitten among these corpusles were strikingly immature.

An electrophysiological investigation was made on the response to mechanical stimulation of a single Pacinian corpuscle separated from the above mentioned kitten mesentery. These results showed that the Pacinian corpuscles observed could be divided into two kinds, the rapidly adapting type and slowly adapting type. The number of the slowly adapting type,however, appeared to be fewer in adalt cats. No difference between the electron microscopic findings of the two types could be detected.

On-off responses and a static response were observed in the response of the slowly adapting type to pulse mechanical stimulation. The former was a response to vibratory stimulation of 30 - 600Hz, while the latter was a response to 0.01 - 0.1 Hz. The room temperature was 28 - 32° C.

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ULTRASTRUCTURAL ASPECTS OF INNER-CAPSULAR CELLS OF FROG MUSCLE SPINDLE.
FUJITSUKA, N., KIM, N., YOSHIMURA, A. and ITO, F. Dept. Physiol., Nagoya Univ. Sch. Med., Nagoya 466

In muscle spindles isolated from semitendinosus muscles of frogs (Rana escrenta), the ionic concentration in the inner-capsular space is different from those of the body fluid. For example, calcium concentration measured by a X-ray micro-analyser was 3 X 10^{-5} M/Kg wwt. Ultra-thin transections or freeze fracture replicas showed that the inner-capsular cells contain many presecretory vesicles, pinocytotic vacuoles, mitochondria and filamentous materials. These results suggest that the ionic concentration in the inner-capsular space is due to a selective permeation or secretion of the inner-capsular cells.

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EFFECTS OF RETARDATION OF Na^+ INACTIVATION ON THE RESPONSES OF FROG MUSCLE SPINDLE. ITO, F., FUJITSUKA, N., KIM, N. and YOSHIMURA, A. Dept. Physiol., Nagoya Univ. Sch. Med., Nagoya 466.

The present study on muscle spindles isolated from semitendinosus muscles in Rana nigromaculata demonstrated that the combined application of two kinds of K^+ channel blockers makes it possible to reversibly change the individual terminal spikes with short duration into those with long duration, in addition with reversible change from the sustained and irregular discharges into non-sustained regular discharges. Similar parallel changes in the duration of individual spikes and the pattern of discharges were observed during perfusion with $NaIO_3$ or N-bromoacetamide or during depolarization of the sensoey terminal which have been known to retardate the inactivation of Na^+ channel.

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REGIONAL ACCUMULATION OF MITOCHONDRIA IN THE SENSORY NERVE TERMINAL OF FROG MUSCLE SPINDLE. KIM, N., FUJITSUKA, N. and ITO, F. Dept.Physiol. Nagoya Univ. Sch. Med., Nagoya 466.

Transected areas of mitochondria in comparison with those of non-myelinated axon were measured electron-microscopically in ultra-thin sections of muscle spindles isolated from semitendinosus muscles of frogs (Rana escrenta). The transected area of the bulbal regions along the non-myelinated axon was occupied by 15 - 30 % with mitochondria, while tubules by those of less than 10 %. As mitochondria is known to be a main candidate for controlling the intracellular concentration of calcium ions, the results suggest that Ca^{2+} spikes are elicited in the bulbar regions.

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RELATION BETWEEN THE MAGNITUDE OF TAIL FLICK RESPONSE AND NOXIOUS CUTANEOUS STIMULATION IN THE RAT. I. HEAT STIMULATION TSURUOKA, M. and MATSUI, A. Dept. of Physiology, Showa University School of Dentistry, Shinagawa-ku, Tokyo 142

The effects of tail heat stimulation and electroacupuncture on the tail flick response were examined. The electromyogram(EMG) of the tail flick muscle(m. extensor caudae medialis) evoked by heat stimulation was recorded and integrated with an integrator to obtain the magnitude of muscle activity. Relation between the stimulus intensity and the magnitude of the EMG followed the power function with exponents of about 3.2 in a range of higher intensities and about 8.4 over a range of lower intensities. When electroacupuncture stimulation(EAS) was given to Zusanli point with pulses of 5 ms duration at 50 Hz for 30 min, the magnitude of EMG was reduced to 44 % of the control during EAS. After cessation of EAS, the suppressed EMG recovered to the control after about 60 min. The temperature for producing the tail flick response and the tail flick latency did not changed during and after EAS. These results indicate that the magnitude of EMG of the tail flick muscle was a possible index of pain induced by noxious heat stimulation.

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RELATION BETWEEN THE MAGNITUDE OF TAIL FLICK RESPONSE AND NOXIOUS CUTANEOUS STIMULATION IN THE RAT. II. ELECTRICAL STIMULATION MATSUI, A. and TSURUOKA, M. Dept. of Physiology, Showa University School of Dentistry, Shinagawa-ku, Tokyo 142

The rat tail was stimulated electrically by a bipolar stimulating electrode inserted subcutaneously with rectangular pulses of 0.1 ms duration at 1 Hz at various intensities. The electromyogram(EMG) of one of the tail flick muscles, ECM(m. extensor caudae medialis) was recorded with a bipolar hook electrode and was integrated with an integrator to obtain the magnitude of muscle activity. Relation between the stimulus intensity and the magnitude of the EMG followed the power function with an exponent of about 3.9 over a range of lower stimulus intensities. When electroacupuncture stimulation(EAS) was applied to Zusanli point with pulses of 5 ms duration at 50 Hz for 30 min, the magnitude of EMG was reduced to about 50 % of the control value. After cessation of EAS, the suppression of EMG remained for some time and the EMG recovered slowly to the control level after about 60 min. It was suggested that the magnitude of EMG of the tail flick muscle evoked by electrical stimulation could be used as an index of pain and analgesia.

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SENSITIVITY OF THE OUTERMOST LAYER OF DENTIN.
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The sensitivity of the tooth, especially in the outermost layer of dentin, was investigated in anesthetized cats. Recordings were made from single fibers dissected from the inferior alveolar nerve during stimulation of the ipsilateral canine tooth. Mechnical stimulation such as probing and drilling the tooth was applied through a window of a chamber fixed to the tooth crown.

Fourteen out of 24 units responded to drilling and all of them gave more vigorous response in the inner layer than in the outer layer of dentin. Of 16 units tested with probing the dentin surface, 10 units responded to the probing and all of them responded only after etching the drilled surface with 30% orthophosphoric acid for 30 seconds except when the surface was situated near the tooth pulp. In such cases, some units responded to probing both before and after etching. It is thought that the sensitivity of the tooth is more remarkable to drilling in the inner dentin layer and that the sensitivity of the drilled dentin surface depends mainly on whether dentinal tubules are open or closed.

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THE EFFECT OF LONG-TERM MECHANICAL STIMULATION ON THE ACTIVITY OF PERIODONTAL MECHANORECEPTORS IN THE CAT. KARITA, K. and TABATA, T. Dept. of Physiol., Tohoku Univ. Sch. Dent., Sendai 980

To clarify the adapting mechanism of periodontal mechanoreceptor activity, the effects of long-term mechanical stimulation on single unit response recorded from a superior alveolar nerve were investigated in the cat. Response of the periodontal mechanoreceptors to mechanical stimulation was found to be affected by the duration and direction of conditioning stimulus. Intense long-term conditioning stimulation also fluctuated the response to test stimulation. The unit which responded as a slowly-adapting type to stimulation in the preferred direction often changed into a relatively rapidly-adapting type when stimulation was applied to the different directions. With stronger stimulation, however, the unit changed into the slowly adapting type with small change of the sensitivity. This finding, when coupled with Cash & Linden's report(1982) that the response pattern of periodontal mechanoreceptor depends on the position of receptors located around the tooth, suggests that the temporary dislocation of mechanoreceptors elicited by tooth mechanical stimulation would be attributable to the change of their response properties.

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THE EFFECT OF TOOTH TEMPERATURE ON THE ACTIVITY OF THE PERIODONTAL MECHANORECEPTOR IN THE CAT. TABATA,T. and KARITA,K. Dept. of Physiol., Tohoku Univ. Sch. of Dent., Sendai 980

The anterior superior alveolar nerve was torn into a single fiber, and its unitary discharge elicited by mechanical stimulation of the canine tooth was recorded. The dental pulp cavity of the canine tooth was perfused with 0.9% NaCl solution at temperatures ranging from 8 to 45 °C to change the temperature of the periodontal ligament. In total, 43 periodontal mechanosensitive fibers were examined. They could be classified into three groups from a view-point of the optimal temperature level in which the maximum discharge is produced. These three groups were called as "high temperature type" (49% of the fibers detected), "intermediate temperature type" (37%) and "low temperature type" (14%). Their optimal temperatures were in ranges of 35-45 °C, 20-35 °C and 8-20 °C, respectively. The present finding that there are three types in the thermal property of the periodontal mechanosensitive fiber suggests that mechanical sensation from periodontal ligament would be influenced by the teeth temperature at the primary afferent level.

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SUPPRESSIVE EFFECTS OF FOCAL APPLICATION OF CHEMICAL ALGESICS TO THE DIFFERENT TISSUES AND BODY REGIONS ON THE JAW-OPENING REFLEX IN RATS. KAWAKITA, K. AND FUNAKOSHI, M. Dept. of Oral Physiology, Gifu College of Dentistry, Hozumi, Motosu, Gifu 501-02

Suppressive effects of focal application of chemical algesics to the different tissues and body regions on the pulpal evoked jaw-opening reflex (JOR) were examined in lightly anesthetized rats. Intra-dermal injection of algesics (5μg bradykinin, 50μl 120mM KCl) induced a clear suppression of JOR and it continued for 2-3 min. The whole body tested were effective on JOR suppression, and the face of homo-lateral side to pulpal stimulation was more effective than that of contra-lateral side. On the other hand, intra-muscular injection of algesics induced a more potent and rapid suppression of JOR. The face and limbs were effective but abdomen was almost ineffective. In the hind legs, contra-lateral side was more effective than that of homo-lateral side.

These results show that chemical stimulations of the cutaneous or muscular polymodal receptors by focal injection of algesics induced potent analgesic effects and their effectivenesses were different with the injected tissues and body regions.

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EFFECTS OF CALCIUM DEPLETION ON ACTIVITIES OF THE POLYMODAL RECEPTOR. [2]MIZUMURA, K., [1]SATO, J* AND [1]KUMAZAWA, T. [1]Dept. Nervous & Sensory Functions, Res. Inst. Environ. Med., [2]Dept. Physiol., Nagoya Univ. Sch. Med., Nagoya Univ., Chikusa-ku, Nagoya 464

In some sensory receptors importance of Ca^{2+} in transduction was reported, but nothing is known about the polymodal receptor. In this report effects of Ca^{2+} depletion was studied on chemical responses of the testicular polymodal receptor using in vitro testis-superior spermatic nerve preparations excised from anesthetized dogs. Responses of single polymodal receptor units to hypertonic saline (615 mM) (Na) and to 60 mM K^+ solution (K) augmented by depleting Ca^{2+} (+EGTA 1 mM) (Ca(-)), while response to bradykinin (9×10^{-8} M) (BK) diminished to almost zero. After replenishment of Ca^{2+}, responses to all of three chemicals returned to levels before Ca(-). One of possibilities of difference of effects of Ca(-) between BK, and Na and K was supposed to arise from intermediary steps of action of BK such as prostaglandin (PG) production through activation of Ca^{2+} dependent phospholipase A_2 and PG effects on BK response. Externally added PG-E_2 (1.4 or 2.8×10^{-7} M) still augmented BK responses, though very weak, in about half of the units tested after more than 30 min of Ca(-), but not in another half. This result suggests multiple sites of Ca^{2+} action are involved in BK response.

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STIMULUS-RESPONSE CHARACTERISTICS OF RAT TRIGEMINAL UNITS ACTIVATED FROM THE TOOTH PULP. SATOH, T. AND IKEDA, N.* Dept. Physiol. and Oro-Maxillo-Facial Surg.(II)*, Sch. Dent. Med., Aich-Gakuin Univ., Nagoya 464

Electrical stimulation of the rat tooth pulp evokes a negative slow mass potential(SMP) in the trigeminal spinal nucleus. We have isolated single units in the SMP field and examined their stimulus-response relationship. The response magnitude as expressed by the spike number or the SMP amplitude was plotted against the stimulus intensity on linear and log scales. The SMPs showed a relatively good fitting to both linear and power functions, while single units tended to be better fitting to either of the two functions. The gradient in linear functions and the exponent in power functions were in most cases definitely greater in single units than SMPs. Most of the single units having a short latency and a narrow response duration showed a better fitting to power function, whereas a majority of units having a longer latency and a wider response duration was better fitted to linear function.

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INHIBITORY ACTIONS OF NORADRENALINE ON THE DESCENDING ANTINOCICEPTIVE SYSTEM THROUGH RAT RAPHE MAGNUS. TODA, K. Dept. Physiol., Fac. Dent., Tokyo Med. Dent. Univ., Bunkyo-ku, Tokyo 113.

Extracellular recording was made from single cells in the nucleus raphe magnus (NRM) which received excitatory connections from the periaqueductal gray matter (PAG) and projected to the spinal cord. The spontaneous firing rate of about 70% cells increased after acupuncture stimulation of Ho-Ku and the acupuncture-induced excitation was antagonized by naloxone injected into PAG, suggesting that these NRM cells were concerned with the descending analgesic system. When 1 M-noradrenaline bitartrate (NA) was iontophoretically applied to the acupuncture-excited NRM cells, the spontaneous activity and the PAG-induced orthodromic spikes were strongly suppressed in 60% of the cells. In these cells, firing index of the antidromic excitation produced by stimulation of the spinal cord was decreased during iontophoretical application of NA. These results suggest that NA inhibits NRM cells with hyperpolarizing effect which relay the descending analgesic system through PAG to spinal cord activated by acupuncture stimulation. Therefore,in case of acupuncture analgesia, it may be assumed that NA actions could be depressed by acupuncture stimulation in the supraspinal structures.

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Involvement of serotonergic system in acupuncture effects on the neuronal activity of the rat hypothalamic arcuate nucleus. HAMBA, M. and TODA, K. Dept. Physiol., Sch. Dent., Showa Univ., Tokyo 142 and Dept. Physiol., Fac. Dent., Tokyo Med.& Dent. Univ., Tokyo 113

Electroacupuncture (EA) effects were investigated both on the unit activities of the arcuate nucleus of the hypothalamus (ARH) and on the magnitude of the digastric reflex in rats. Each rat was applied EA stimuli (5 ms, 300-500 μA) at Ho-Ku for 15 min at 3, 45 and 100 Hz, respectively. The mean firing rate (MFR) of the spontaneous discharge either increased (Type I), decreased (Type II) or did not change (Type III). In normal rats, after EA at higher frequency 1) the number of Type II neurons was highly observed, 2) MFR and responses to noxious tail pinch were remarkably suppressed in Type II group. Long-lasting suppression of the digastric reflex was induced with the same degree at any frequencies. In serotonin-depleted rats by pretreatment with PCPA, however, the inhibitory influence on the digastric reflex, the increase of Type II, and the remarkable suppression of MFR and responses to noxious inputs were scarcely induced by EA at higher frequency.

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INHIBITED REGION BY ANALGESIA INHIBITORY SYSTEM IN ACUPUNCTURE NON-POINT STIMULATION-PRODUCED ANALGESIA. LUO,C.P., HISHIDA,H. AND TAKESHIGE,C. Dept. of Physiology, School of Medicine, Showa Univ., Tokyo 142

We reported that the central pathway(R_2) of acupuncture point stimulation-produced analgesia(AA) is different anatomically as well as pharmacologically from R_1 of the appearing after lesion of the analgesia inhibitory system, the lateral centromedian nucleus of the thalamus(lCM). For example, R_2 and R_1 pathways passed through the dorsal periaqueductal central gray(dPAG) and lateral PAG(lPAG) respectively. Since acupuncture non-point stimulation-produced analgesia(NAA) is produced by lesion of the lCM, the effect of lesion and stimulation of the lCM on dPAG and lPAG stimulation-produced analgesia(dPAG-SPA), and stimulation effect of lCM on evoked potentials and neuron activities in the lPAG caused by acupuncture point and non-point stimulation have been investigated. The lCM lesion had no effect on the dPAG-SPA, while augmenting lPAG-SPA completely during stimulation. The lPAG evoked potentials were completely inhibited by lCM stimulation. Neuron activities in the lPAG produced by acupuncture non-point stimulation were completely inhibited by lCM stimulation. No regional change in lPAG histological examination could be found between the stimulated region and the recorded region in lPAG evoked potentials in the above experiment. It was concluded that analgesia inhibitory system exerted its inhibitory effect on the lPAG.

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THE ANALGESIA PRODUCING CENTRAL PATHWAY OF ELECTRIC STRESS INDUCED ANALGESIA. USAMI,S. AND TAKESHIGE,C. Dept. of Physiology, School of Medicine, Showa Univ., Tokyo 142

Stress induced analgesia(SIA) producing central pathway was explored by comparing it with that of so called acupuncture analgesia(AA) caused by acupuncture point stimulation and that of acupuncture non-point stimulation produced analgesia(NAA), which appeared after lesion of the analgesia inhibitory system, the lateral centromedian nucleus of thalamus(lCM). Electric stress shock of 1 sec duration was applied between back and upper thigh of the rats at 1/5 sec. SIA reached a maximum at 30 min after onset of stimulation and then decreased gradually. Analgesia remained after stimulation off(after effect). Such SIA was abolished by hypophysectomy, naloxone(1mg/kg i.p.) or dexamethasone(0.4mg/kg 24hr. before, and 0.2mg/kg 1hr. before). Individual variation was observed in SIA, but this variation has no correlation with that of AA. SIA was not influenced by lesion of the dorsal periaqueductal central gray(dPAG), which is the site of the afferent analgesia-producing pathway in AA from acupuncture point to the hypophysis, or by intrathecally applied naloxone(0.2μg), which abolished AA. Four days were necessary to recover normal SIA. SIA had no effect on AA and NAA for four days after SIA. Like NAA, SIA was abolished by lesion of the lateral PAG, however unlike NAA, lCM lesion had no effect on SIA. It was concluded that the central analgesia producing pathway for SIA is different from that of AA or NAA.

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INHIBITORY ACTION ON ANALGESIC INHIBITORY SYSTEM AND AUGMENTING ACTION ON NALOXONE REVERSAL ANALGESIA OF D-PHENYLALANINE. MURAI,M., TANAKA,M. AND TAKESHIGE,C. Dept. of Physiology, School of Medicine, Showa Univ., Tokyo 142

The relationship between D-phenylalanine(DPA) action and lesion effect of the lateral centromedian nucleus of the thalamus(lCM) and the effect of DPA on naloxone reversal AA has been studied. After lesion of the lateral septum(lSp), which is the site from the acupuncture point to the hypophysis, AA disappeared. Under these conditions, treatment with DPA(250 mg/kg intraperitoneally) caused analgesia. After lesion of lCM, DPA had no effect on analgesia caused by acupuncture non-point stimulation, with or without lesion of lSp. Therefore, it was shown that DPA action is equivalent to that of lCM lesion. After excluding DPA action on the lCM by lesion of the lCM, DPA augmented naloxone reversal analgesia. Augmentation was more marked in the presence of AA-producing afferent pathway(R_2) than in the absence of R_2. Individual variations in effectiveness of such analgesia disappeared after DPA treatment. It was concluded that 1) DPA inhibited the analgesic inhibitory system and that 2) DPA abolished the individual variation in effectiveness in naloxone reversal analgesia.

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CORTICAL SOMATOSENSORY POTENTIALS RESPONSIBLE FOR EVALUATION OF PAIN.

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The right index finger in man was stimulated electrically with a strong(S) and a weak(W) stimulus intensity for producing, with the probability of about 70%, painful(Y) and nonpainful(N) sensation, respectively. Four psychophysical response categories among the stimulus intensities and subjective sensations were obtained: S-Y(hits), W-Y(false alarms), S-N(misses), and W-N(correct rejections). While stimulating the finger with the 2 stimulus intensities, the somatosensory potentials(EPs) of 9 subjects were also recorded from the scalp, at C_3, and were classified according to the 4 psychophysical categories. It was found that the late components of an EP, P190, N220 and P270, belonging to either S-Y or W-Y category had significantly greater amplitude than those belonging to the remaining S-N and W-N. In addition, the amplitude of thses late components was found to increase as the magnitude of subjective sensation increased. The results indicate that the amplitudes of the above 3 EP components may concern pain evaluating system in the brain.

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EFFECTS OF MONOAMINE ON SELF-MUTILATION OF RATS INDUCED BY PERIPHERAL NERVE LESIONS

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After chronic section of peripheral nerves of the fore- or hindpaw, the rat self-mutilates not only the ex-innervated areas but the adjacent ones in several days to two weeks. Such mutilation is thought to relate to phantom limb pain or phantom sensation. The sympathetic nervous system is supposed to participate significantly in those processes, showing that guanethidine suppresses the mutilation. In this experiment, effects of monoamine on the mutilation were examined. The main nerves including the ulnar, radial and median nerves were sectioned unilaterally in the rat. Pargyline (10-20mg/kg), monoamine oxydase inhibitor, was injected daily during two weeks after the section. Pargyline significantly facilitated the mutilation. The facilitation was marked about a week after the section. Since then the mutilation advanced rapidly until the forepaw was totally mutilated. Except for self-mutilation, the rats showed no marked behavioral changes caused by pargyline injection.

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NEUROBLASTOMA CELL AS MODEL FOR TASTE CELL: MECHANISM OF DEPOLARIZATION IN RESPONSE TO VARIOUS BITTER SUBSTANCES.

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The mouse neuroblastoma cell (N-18 clone) was used as a model for a taste cell. The N-18 cell was found to be depolarized reversibly by various species of bitter substances such as strychnine, quinine, papaverine, sucrose octaacetate, lincomycin, caffeine and L-leucine. The minimum concentrations of bitter substances which induced depolarization (threshold concentration) varied greatly with species of bitter substances. There was a good correlation between the order of the threshold concentrations for various bitter substances in the N-18 cell and that in human. Replacement of Na and Cl with impermeable ions had practically no effect on the magnitude of the depolarization in response to bitter substances and reduction of Ca concentration from 1.8 mM to 0.2 mM led to a little increase in the responses. The input membrane resistance was little changed during the depolarization induced by bitter substances. It was suggested that the depolarization of the N-18 cell by bitter substances is induced by changes in the phase-boundary potential at the outer surface of the cell.

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MECHANISM OF THE WATER RESPONSE IN CARP GUSTATORY RECEPTORS.
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The carp gustatory responses to various salts and those to distilled water after adaptation of the receptors to the salts were recorded from the palatine nerve under varying conditions. (a) As far as $Na_4Fe(CN)_6$ solution prepared freshly was used, any peak in the dose-response curve was not observed, while the solution stored overnight induced a large peak response at the low concentration region as Konishi reported. The magnitude of the water response after adaptation to the stored solution was practically equal to that after adaptation to the fresh solution. (b) The responses to salts depended largely on the species of both cations and anions of the salts. In contrast, the response to distilled water was practically independent of the species of both monovalent cations and anions of the salts used for the adaptaption. (c) It was concluded that the receptor site for the water response is different from that for the salts. The mechaism how distilled water leads to depolarization of the taste cell was explained in terms of the electric double-layer potential.

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POSSIBLE TASTE RECEPTOR FOR AMINO ACIDS IN THE CARP. MARUI, T., HARADA, S. AND KASAHARA, Y. Dept. of Oral Physiol., Kagoshima Univ. Dent. Sch., Kagoshima 890

The stimulatory effectiveness of amino acids and analogs on the external gustatory receptors of the carp was investigated with extracellular electrophysiological technique to determine possible taste receptor model. Taste responses to amino acids containing 3 to 4 carbon atoms having unbranched and uncharged side chains are stimulatory. Further, the α-amino group and the α-hydrogen seem to be essential for maximal effectiveness and for some amino acids ionically charged primary carboxyl group was generally unnecessary for a maximal receptor response. Peptides as gustatory stimuli were less effective than the most potent amino acid residue in the molecule; any peptides tested containing L-Ala produced significantly reduced gustatory response as compared to L-Ala itself. However, it is still uncertain to establish a definite model of receptor site for an amino acid, since evidences of relatively independent receptor mechanisms for amino acids were accumulated. From the present results, taste receptor sites are not so simple as models reported in fish olfaction and taste by now, and it is suggested that many subsites participate in appearence of taste stimulatory effectiveness in the carp facial taste system.

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RECEPTOR POTENTIALS OF TASTE CELLS ON THE ORAL FLOOR OF XENOPUS LAEVIS.
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The electrical responses of the taste cells on the oral floor mucosa of the Xenopus laevis to the various chemical stimuli were investigated by intracellular microelectrode method.

The average value of the resting potentials was -23.8±4.3 (S.E. of mean, n=50). Almost taste cells responded to many kinds of chemical stimuli, various salts, HCl, quinine-HCl and amino acids. Multiple sensitivity of individual taste cell was showed as same as other animals, mudpuppy, frog, rat, hamster and mouse. Receptor potentials to many salts, HCl and quinine-HCl increased in magnitude with increasing concentration. The thresholds for salts were about 1/100 M for NaCl, 1/200 M for LiCl, KCl and NH_4Cl, 1/800 M for $CaCl_2$ and $MgCl_2$. The average magnitudes (S.E.) of responses to 1/5 M NaCl, LiCl, KCl, NH_4Cl, $CaCl_2$ and $MgCl_2$ were 21.3±8.1, 25.1±8.8, 18.2±7.6, 18.5±9.5, 50.1±11.9 and 48.2±13.1 respectively. Correlation coefficients between to six salts were found to be high, $r_{NaCl-LiCl} = 0.95$, $r_{CaCl_2-MgCl_2} = 0.90$ especially.

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COMPARISON BETWEEN NaCl- AND WATER-INDUCED RESPONSES OBTAINED FROM LINGUAL EPITHELIUM AND THOSE FROM OTHER EPITHELIA IN FROG

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Frog lingual epithelium adapted to Ringer's solution scarcely developed a trans-epithelial potential between mucosa and serosa, when compared the values of skin or bladder membrane. NaCl or water stimulus to mucosa elicited a potential response on lingual or other tissues. Electrophysiological nature of the responses obtained from lingual epithelium was resembled that of receptor potential in taste cells but did not always resemble that from others. Ouabain had significant effect neither on potential difference in resting state nor NaCl- and water-induced responses obtained from lingual epithelium, while it markedly decayed potential difference and NaCl-induced response obtained from skin or bladder but slightly affected on water-induced response. Effects of local anesthetics was stronger on NaCl- and water-induced responses obtained from lingual epithelium than those from other tissues. The results indicate that NaCl- and water-induced responses obtained from lingual epithelium may be produced by a change of passive transport of some ions and important in the process of taste reception.

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EFFECT OF VARIOUS IONS ON RECEPTOR MEMBRANE SURFACE UPON ACID-INDUCED RECEPTOR POTENTIAL IN FROG TASTE CELL.

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In the preceding report, we mentioned that the generation of the acid-induced receptor potentials in a frog taste cell may be related to both the diffusion potential of Ca^{2+} and Na^{+} ions and the phase boundary potential occurring at the taste receptor membrane. In this experiment, we investigated what kinds of ions can substitute for Ca^{2+} or Na^{+} ions lying on the taste receptor membrane in a generation of receptor potential in frog taste cells in response to acids. We found that K^{+}, Li^{+} and $choline^{+}$ ions can substitute for Na^{+} ions, but NH_4^{+} cannot, and that Sr^{2+} ions can substitute for Ca^{2+} ions, but Mg^{2+} and Co^{2+} ions cannot. We conclude that when the frog tongue surface is adapted to the normal Ringer solution, the acid-induced receptor potential of a taste cell is generated by the diffusion potential of Ca^{2+} and Na^{+} ions, the phase boundary potential and the modulatory action of both ions on the binding process of H^{+} ion stimulus to the receptor site, all of which occur at the taste receptor membrane. The action of these ions is not specific, but is replaced with other ions.

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ROLE OF Ca AND CATECHOLAMINES IN FROG TASTE ORGANS.

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In order to explore the role of catecholamine and Ca in the synaptic transmission from taste cell to sensory nerve terminals, the effects of various agents added to atificial solution perfusing the lingual artery on the frog taste nerve responses were examined. 1) Injection of reserpine or guanetidine, catecholamine-depleting agents led to a great reduction of the frog taste nerve responses. Addition of catecholamines to the perfusing solution did not practically enhance the spontaneous impulse discharges but recovered the response to all the taste stimuli examined. Norepinephrine was most effective and is the most probable candidate for the transmitter. 2) The enhancement of the responses by noreinephrine was suppressed by desipramine, cocaine or imipramine, suggesting that the enhancement was brought about by incorporation of norepinephrine into taste cells. 3) In a previous paper, we showed that the responses to the stimuli of one group depended on Ca, cGMP and cAMP added to the perfusing solution and those to the stimuli of another group did not depend on these agents. After the reserpine injection 3 or addition of reserpine to the lingual artery, which probably modified the permeabilities of the artery, the responses to all the stimuli came to exhibit dependences on these agents.

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CHANGES IN FROG GUSTATORY RESPONSES INDUCED BY TREATMENT OF THE TONGUE WITH PROTEASE AND PROTEIN CHEMICAL MODIFICATION REAGENTS. SUGIMOTO, K. Dept. Physiol., Fac. Dent., Tokyo Med. Dent. Univ., Bunkyo-ku, Tokyo 113

The chemical characteristics of the frog taste receptor membrane was investigated by recording the gustatory nerve and cell responses elicited by water and taste stimuli (quinine, NaCl, HCl and sucrose) before and after treatment of the tongue with protease or chemical modification reagents of protein. Treatment with pronase E greatly suppressed the neural responses to sucrose and significantly reduced the water response, without producing significant changes in responses to the other taste stimuli. Woodward's reagent K, which reacts with carboxyl groups, remarkably inhibited the neural responses to sucrose and those to water at a lesser extent. Sulfhydryl reagent, N-ethylmaleimide (NEM), inhibited the neural responses to sucrose as well as quinine. On the other hand, 2,4,6,-trinitrobenzene sulfonic acid, which reacts with amino groups, exhibited no inhibitory effect on the neural responses to any taste stimulus. In gustatory cell responses, the mean amplitude of receptor potential to sucrose significantly decreased after application of NEM, while those to NaCl and HCl scarcely decreased. These results suggest that (1) sugar and water stimuli may interact with taste receptor molecules composed of protein, and (2) carboxyl and sulfhydryl groups in protein may play an important role in sugar reception and carboxyl groups in water reception.

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INTRACELLULAR TASTE CELL RESPONSES OF MOUSE.
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Intracellular recordings of membrane potentials of taste cells in mouse were using a glass microelectrode filled with procion yellow dye solution. Sucrose produced a depolarization response and the response was accompanied by an increase or no membrane resistance change. NaCl produced a depolarization, hyperpolarization or null response and the response was accompanied by a decrease or no membrane resistance change in all cases. Both sucrose and NaCl produced the depolarization responses, however they produced the opposite characteristic membrane resistance changes in their responses. Q-HCl and HCl produced relatively variety of response profile and the membrane resistance changes were also varied in each taste cells. It is concluded that the relationship between the response profile and the membrane resistance change is complicated. It is suggested that each taste cell produces its own characteristic response profiles and membrane resistance changes for the fundamental four basic taste stimuli.

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DIFFERENCES IN THE RESPONSE CHARACTERISTICS OF THREE TASTE NERVES IN THE MOUSE. SHINGAI, T. AND BEIDLER, L.M.* Dept. of Physiol., Niigata Univ. Sch. of Dentistry, Niigata 951 and Dept. of Biological Science, Florida State Univ., Tallahassee, Florida 32306, U.S.A.

Gustatory responses from three nerves, the chorda tympani, glossopharyngeal and superior laryngeal nerves were recorded in the mouse to compare their response characteristics to chemical stimuli. The three nerves did not respond in a similar way to the same chemical stimuli. The chorda tympani responded strongly to HCl and sucrose, but weakly to quinine-HCl, while the glossopharyngeal responded well to quinine-HCl rather than to either HCl or sucrose. The order of effective stimulation was $NH_4Cl > NaCl > KCl$ for the chorda tympani, whereas $NH_4Cl > KCl > NaCl$ for the glossopharyngeal nerve. The response characteristics of the superior laryngeal nerve were quite different from those of the other nerves. Sensory units in the superior laryngeal nerve behaved like osmosensitive receptors. The results obtained in this study suggest dissimilar contributions of the three nerves to the physiological functions.

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QUALITATIVE DISCRIMINATION OF GUSTATORY STIMULI AFTER SECTION OF THE CHORDA TYMPANI NERVE IN MICE. NINOMIYA, Y., MIZUKOSHI, T., HIGASHI, T. AND FUNAKOSHI, M. Dept. of Oral Physiol., Gifu College of Dentistry, Hozumi, Motosu, Gifu 501-02

Qualitative discriminabilities among various taste stimuli were studied by examining the generalization patterns of a conditioned aversion from single chemicals to the other compounds before and after section of the chorda tympani nerve in mice. Selectivities in generalization patterns among sugars and saccharin Na were largely reduced after section of the chorda tympani nerve, but were only slightly if at all those among salts, HCl and quinine-HCl. The reduction of discriminabilities among sugars was not evident after section of the glossopharyngeal nerve and when mice were familiarized with one of sugars prior to section of the chorda tympani nerve, but when mice were familiarized with it after section of the chorda tympani nerve. These results suggest that the information conveyed by the chorda tympani nerve is very important for differentiating among sugars on the basis of taste quality and acquisitions of a conditioned aversion and learned safety to a particular stimulus among various sweeteners.

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DEPRESSANT EFFECTS OF HEAVY METAL IONS ON GUSTATORY RESPONSES IN MICE. IWASAKI, K., KASAHARA*, T. and SATO, M. Department of Neurophysiology, Tokyo Metropol. Instit. for Neurosci., Fuchu, Tokyo 183

Effects of $MnCl_2$, $FeSO_4$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $ZnCl_2$ and $CdCl_2$ on gustatory responses were studied by recording the integrated nerve discharges from chorda tympani in ddy mice. $CuCl_2$ and $ZnCl_2$ at 0.1 mM specifically depressed resopnses to sugars and Na saccharin without showing little effect on other taste responses. At 1 mM $CdCl_2$ produced a depression of sucrose response, but the effect of the remaining four salts was very small. All the seven salts at 1 mM slightly depressed responses to NaCl and quinine-HCl, but not HCl response. The depressant effectiveness of the above salts on taste responses is in approximate agreement with the complexing ability of transition metal ions to organic ligands. Two-bottle preference tests were carried out with the metal salt solutions. They revealved that mice rejected $CuCl_2$, $ZnCl_2$, $CdCl_2$, and $NiCl_2$ but preferred $CoCl_2$ and $MnCl_2$, and that preferences for sucrose and Na saccharin were reduced by addition of Cu or Zn salt above 0.1 mM.

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ACTIVITIES OF BRAINSTEM NEURONS ELICITED BY STIMULATIONS OF ORAL MUCOSA AND ITS AFFERENT NERVES IN THE FROG. NOMURA, H. AND SUZUKI, H. Dept. Oral Physiol., Matsumoto Dental Coll., Shiojiri, Nagano 399-07

In order to obtain some information on the central pathways of oral reflexes in the frog, evoked responses in efferent nerves and brainstem neurons were studied. By recording the discharges of trigeminal motor neurons elicited by electrical stimulation of the submaxillary nerve and by chemical (tap water) and tactile (a stroke with a brush) stimulations on the palate, it was found that there are both mono- and polysynaptic pathways in the oral reflex arcs of the frog. Relay neurons in the polysynaptic pathways appeared to be present in the medial reticular formation of the lower brainstem, because there were some reticular neurons responding to electrical stimulation of the trigeminal and facial nerves and to chemical and tactile stimulations on the palate in a similar manner to reflex discharges in the peripheral efferent nerves. In addition to these neurons, there were other neurons responding to the stimulations in different manners. Since the polysynaptic oral reflexes were not significantly influenced by lesions of the cerebellum and optic tectum, the central pathways of the reflexes may be restricted within the brainstem.

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PHYSIOLOGICAL PROPERTIES OF THE TASTE UNITS IN THE THALAMIC TASTE AREA OF RATS

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While applying taste stimulation to the whole oral cavity, we recorded a total of 35 taste units from the thalamic taste area (TTA) of SD albino rats anesthetized with amobarbital Na. Averaged response magnitudes of TTA taste units were ca. half as large as those of the parabrachio-thalamic taste relay (P-T) units, and 70 % of the TTA units were excited by at least 3 of the 4 basic taste solutions (NaCl, sucrose, HCl and quinine). Sucrose showed a distinct across neuron pattern from the other 3 basic solutions, while the latter produced rather similar patterns with each other. Most (n=12) of the 16 taste units studied had an RF on the tongue, and the RFs were found bi- (n=8), contra- (n=4), or ipsi-laterally (n=4) in the oral cavity. Locations of the RF were in marked contrast with the P-T units, because most of the latter had an RF on the ipsilateral side of the tongue or the tongue and palate. However, when taste neurons were classified according to the best of the 4 basic taste solutions, taste units of different best stimuli showed the RFs of a different laterality, as expected from the projection patterns of the P-T neurons. Half the TTA units (5/9) examined had a large RF for intense mechanical stimulation. It was concluded that a single TTA unit receives a large convergent input from taste and tactile afferents of ipsi- and contra-lateral origins.

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RESPONSES OF CORTICAL NEURONS DURING LICKING AND CHEWING IN BEHAVING RATS.

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Unitary discharges of cortical neurons were recorded in rats while they were licking liquids from a drinking spout and eating dry pellets. Stimulus liquids consisted of distilled water (room temperature, 5°C, 40°C), and sucorse, NaCl, HCl, quinine and monosodium glutamate solutions. The recording electrodes (Pt-Ir wires, 25 μm in dia.) were chronically implanted so that the tip was positioned in the orofacial somatosensory area (OSA), or the taste area (TA). Of 27 cortical neurons sampled, 22 were situated in the OSA and 5 were in the TA. Eight of these 22 neurons increased or decreased their spontaneous discharges continuously during lickig and chewing behavior, whereas other 14 neurons showed rhythmic phasic activities corresponding to a particular phase of licking or masticatory cycles. Of 5 neurons that were located in the TA, 3 showed excitatory or inhibitory responses to one or two of taste stimuli. Other 2 neurons increased their firing before the start of licking and decreased during licking; these responses might be related to anticipation and/or reward.

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CORTICAL PROJECTIONS OF TEMPERATURE SIGNALS:SUMMATED THALAMIC RESPONSES TO COLD STIMULI IN CAT.

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As a first step to elucidate the cortical mechanism of temperature sensation, we have analyzed the localization of the thalamic relay of temperature signals from a tongue surface. In lightly anesthetized cats, the cold stimuli were applied to the tongue surface and summated thalamic responses were recorded with glass-insulated tungsten microelectrodes using an averaging technique. The primary thalamic responses consisted of three waves(N1,P1,N2) with average onset latencies of 33, 97 and 137 msec, respectively. The N1 wave was considered to be caused by mechanical stimulation of the tongue by pouring a cold solution on the tongue surface. The amplitude of the N2 wave varied depending on the temperature of the cold stimuli. The lower was the temperature of the stimuli, the greater became the amplitude of the N2 wave. Thus, the N2 wave was supposed to be caused by thermal stimulation of the tongue. Such thalamic thermosensitive areas were located in the caudal part of the tongue projection area of the medial ventroposterior nucleus(VPM).

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NEUROBLASTOMA CELL AS A MODEL FOR OLFACTORY CELL: MECHANISM OF ODOR DISCRIMINATION. KASHIWAYANAGI, M.* AND KURIHARA, K. Fac. Pharm. Sci., Hokkaido Univ., Sapporo 060

The mouse neuroblastoma cell (N-18 clone) was used as a model for the olfactory cell to study the mechanism of the odor discrimination. The changes in the membrane potential and the membrane fluidity were measured with fluorescence probes. The results obtained are as follows. 1) Various odorants depolarized the N-18 cell dose-dependently. 2) For respective odorants, the odorant-induced membrane fluidity changes measured with DPH occurred at similar concentrations which changed the membrane potential. 3) Changes in the membrane fluidity in response to odorants were measured with ANS, 7-AS, 12-AS, 12-AO and DPH which reflect the membrane fluidity at different sites in the membrane. The results obtained suggested that the different species of odorants are adsorbed at different sites in the membrane. 4) Exogenous application of oleic acid and cholesterol to the N-18 cell led to an increase in the amplitude of depolarization in response to certain odorants without affecting the depolarization in response to other odorants examined.

The results obtained suggested that quality of odor can be discriminated if the membrane composition of each olfactory cell is different from one cell to another.

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MORPHOLOGICAL CHANGES AND SINGLE UNIT RESPONSES OF NEWT OLFACTORY EPITHERIUM AFTER KEEP IT ON LAND. IIJIMA, M., AMAGAI, Y. *INOUCHI, J. AND *SHIBUYA, T. Department of Oral Physiol. Tohoku Dental University, Koriyama, Fukushima 963 and Institute of Biological Sciense, The University of Tsukuba Niihari-gun Ibaraki 305.

Morphological changes and responses after environment of keeping newt(Cynops Pyrrhogaster Pyrrhogaster) was changed from water to land were investigated. The ratio between olfactory and respiratory epithelium was increased from 1:1 to 4:1(7 days on land). Time to snap at odorized agar which put at the center of cylindorical container(22 cm in diameter) were prolonged after land. L-Glutamine was slightly effective rather than DL-Alanine. Also D-Campher and Cynnamyl alcohol were used for electrophysiological study. Single unit and EOG were recorded from newt in water with these 4 odorants in solution(1/100 mol). EOG amplitude to Cam. and Cyn. were decrease to 1/4 after 2 days keep on land. On the contrary responses to the gasious stimulations of the same odors were increased. Various threshold and response pattern of single unit were recorded. Amino acid solution could elicite response in 12 days on land newt.

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HYPERPOLARIZING POTENTIALS IN THE GANGLION CELLS OF THE NERVUS TERMINALIS IN THE CARP. FUJITA, I., SATOU, M. and UEDA, K. Zool. Inst., Fac. Sci., Univ. Tokyo, Bunkyo-ku, Tokyo 113

In the carp, intracellular potentials were analyzed in the ganglion cells of the nervus terminalis (NT cells) following electrical shocks to the olfactory nerve (ON), medial (MOT) or lateral (LOT) olfactory tract. All the NT cell recordings showed long-lasting hyperpolarizing potentials after MOT shocks, but no response after LOT shocks. ON shocks also elicited long-lasting hyperpolarizing potentials in 76 % of the NT cells. The hyperpolarizing potentials in the NT cells had different properties from IPSPs in mitral cells in the medial part of the olfactory bulb (mMC): 1) the MOT-evoked IPSPs in the mMC had two successive phases, while no such phases were observed in the MOT-evoked hyperpolarizing potentials in the NT cells, 2) repetitive MOT shocks at a frequency higher than 0.5 Hz caused potentiation of the IPSPs in the mMC, while the hyperpolarizing potentials in the NT cells were not potentiated by repetitive MOT shocks, and 3) the onset latencies of the MOT-evoked hyperpolarizing potentials in the NT cells were longer than those of the MOT-evoked IPSPs in the mMC.

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RESPONSES OF PREPIRIFORM CORTEX NEURONS TO ODOR STIMULATION IN THE RABBIT.

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Single unit responses to odor stimulation were recorded in the prepiriform cortex (PC) of the lightly anesthetized rabbit. A polyethylene tube was inserted from the trachea rostrally to the postnasal cavity. A negative pressure pulse was applied to the tracheal tube to produce an artificial air intake (A-I) and to deliver odors on the nasal mucosa. Sixty-four different odors were used. Out of 57 PC units examined, 31 (54.4%) responded to at least one of the odors. Most of them (22 units) responded to 2-6 different odors. Response probabilities of neurons to each odor (the ratio of the number of units responding to each odor to the total number of units examined) varied from 0% to 35%. Sixteen units responded with different temporal firing patterns to different odors. So far, we could not find clear correlation between the position of units with a certain temporal firing pattern and the laminar organization of the PC. The presence of different kinds of temporal firing patterns in the PC suggests that the temporal firing patterns encode important aspects of the olfactory information.

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STEPWISE SARCOMERE SHORTENING IN FROG SKELETAL MUSCLE FIBERS. TORIDE, M. AND SUGI, H. Dept. Physiol., Sch. Med., Teikyo Univ., Itabashi-ku, Tokyo 173

It has been a matter for debate whether sarcomere shortening in striated muscle occurs in a stepwise fashion or not. We studied the time course of sarcomere shortening by locally activating about 10 sarcomeres with a pipette in contact with the fiber surface, and recording the sarcomere shortening with a 16 mm high-speed cine-camera at 3,000 frames/sec. The local contraction took place symmetrically with respect to the pipette axis, so that the time course of shortening could be observed on the same sarcomeres. The spacings of 5-10 sarcomeres were measured with a densitometer on each frame or on the streak pattern produced by projecting the cine records on rapidly moving 35 mm films. It was found that, in many fibers examined, the sarcomere shortening showed distinct steps, though no clear steps were seen in some fibers. In all the fibers, the average velocity of sarcomere shortening over the first 20-40 msec after the stimulus agreed well with the shortening velocity of a muscle fiber under very small load (Buchthal & Kaiser, 1951), indicating that the steps may not be due to irregular friction between the fiber and the pipette. In view of a highly variable nature of the stepwise sarcomere shortening, it seems unlikely that this phenomenon is associated with the fundamental contraction mechanism.

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RADIAL COMPRESSION STUDIES IN RELAXED SKINNED FROG MUSCLE FIBERS IN HIGH CONCENTRATION OF MAGNESIUM ION AND LOW pH. UMAZUME,Y, ONODERA,S. and HIGUCHI,H. Dept. of Physiol., Jikei Univ. Sch. of Med. Minato-ku Tokyo 105

One of the interaction energies between myofilaments is electrostatic repulsive energy which is exerted by negative charges of the filaments. Divalent cations or low pH would decrease the negative charges. From the relation between the osmotic compressing force exhibited by PVP (K-30) and fiber width (D) or lattice spacing (d_{10}), we can determine the radial stiffness (K) which would reflect the electrostatic repulsive energy. We studied K of relaxed skinned frog muscle fibers in high magnesium ion (Mg) and low pH solutions by measuring D under the microscope and/or d_{10} from equatorial reflections of X-ray diffraction patterns at 20°C. When PVP was added, skinned fiber shrinked in D or d_{10}. Above 1 % PVP, the rate of shrinkage of D was highly proportional to that of d_{10}. d_{10}'s were 41.4, 38.2 and 41.1 nm in pH 7, pH 6 and 30 mM Mg solutions respectively. In 6 % PVP, each d_{10} was 32.7, 28.8 and 28.6 nm. Below 35 nm of d_{10}, K showed significantly smaller values in pH 6 and 30 mM Mg. We considered that a change in conformation of HMM can exprain these changes. A provisional model is proposed, in which the effect of Mg or low pH is to decrease the rigidity of the S1-S2 or S2-LMM hinge and bring S1 into closer proximity to the filament shaft by higher osmotic compressing force.

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ROLE OF THE ELASTIC COMPONENT IN SKINNED MUSCLE FIBERS. HIGUCHI,H. AND UNAZUME,Y. Dept. of Physiol., Jikei Univ. Sch. of Med., Minato-ku Tokyo 105

Role of the elastic component for maintaining the ordered structure in relaxing and contracting state of skinned fibers was investigated mainly by optical diffraction and electronmicroscopic methods. The resting tension of skinned fibers decreased very much by dissociation of thick filaments. When the resting tension after dissociation of thick filaments decreased nearly zero at a sarcomere length shorter than 3.0 μm, the intensity of the first ordered difraction line decreased remarkably. It was shown by analysing the data of optical difraction that this decrease of the intensity was not caused by the change of structure in sarcomere, diameter of fibril and arrengement of fibrils, but by the disorder of sarcomere length. This shows that the elastic component bound to thick filaments was important for maintaining the ordered structure in relaxing state. On the other hand, Z-to-M distance measured from electron microgrophs of skinned fiber was 1.41± 0.17 μm (mean ± SD) during contraction. Because the elastic component bound to thick filaments generated tension at Z-to-M distance longer than 1.25 μm, it is suggested that the elastic component bound to thick filaments play a role of maintaining thick filaments at the center of sarcomere during contraction.

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TWO DIFFERENT STIFFNESSES IN OSMOTICALLY COMPRESSED SKINNED MUSCLE FIBERS OF THE FROG. TSUCHIYA, T. AND SUGI, H. Dept. of Physiol., Sch. of Med., Teikyo Univ., Itabashi-ku, Tokyo 173

The instantaneous stiffness and the tension transients as a function of relative fiber width were measured in relaxed, activated and rigor skinned muscle fibers from the frog. Fiber width was reduced by adding PVP (polyvinylpyrrolidone) or dextran to the bathing solution.

The stiffness was detected in a relaxed, reduced-width fiber and it increased with degree of compression. Higher stiffness was observed in activating solution than in relaxing one at the same degree of compression. It is noticeable that the increased stiffness induced by activating solution in addition to that in relaxing one is nearly constant in a compressed fiber in the wide range of polymer concentration. This fact suggests that there are two component stiffnesses, Ca-sensitive and Ca-insensitive, in a compressed fiber and they exist in parallelly arranged structure in a fiber. The observation of the tension transients of a relaxed reduced-width fiber and an activated reduced-width one supports the above view. In a compressed rigor fiber also, it was observed that the stiffness consists of Ca-sensitive and Ca-insensitive components.

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TIME-RESOLVED X-RAY DIFFRACTION OF GLYCERINATED SKELETAL MUSCLE FIBERS DURING FORCE DEVELOPMENT UNDER LOW IONIC STRENGTH. SUGI, H. AND TANAKA, H. Dept. Physiol., Sch. Med., Teikyo Univ., Itabashi-ku, Tokyo 173

When a skeletal muscle fiber, in which the surface membrane was made permeably to small ions and molecules, is placed in distilled water (DW), a slow but marked tension development takes place; on returning to a relaxing solution, the tension relaxes rapidly (Natori and Natori, 1982). To examine the relationships among tension, equatorial reflection intensities and filament-lattice spacing, time-resolved X-ray diffraction studies were performed on glycerinated rabbit psoas muscle fibers during the tension development in response to DW and during the relaxation in the relaxing solution. On DW application, the filament-lattice spacing decreased by about 15% for the first 3 sec, and then started to increase above the initial value, while the tension reached a maximum in about 5 sec with an almost coincident change in the intensity ratio between 1,0 and 1,1 reflections. The DW-induced tension stayed constant in DW, whereas the filament-lattice spacing continued to increase almost linearly with time at least as long as 30 sec. On returning to the relaxing solution, the filament-lattice spacing rapidly decreased to the initial value in a few sec, while the relaxation of DW-induced tension took more than 5 sec.

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BIPHASIC DEVELOPMENT OF CALCIUM-INDUCED TENSION IN GLYCERINATED PSOAS MUSCLE FIBER. TSUJIMOTO, T. AND NONAKA, Y. Dept. of Physiol., Wakayama Med. Coll., Wakayama 640

Simultaneous recording of the tension mechanogram and ATPase activities were made on the glycerinated rabbit psoas muscle fiber in the presence of ATP-regenerating and pyruvate-assaying system. Ca-induced tension development was divided into an initial fast component and a secondary slow component. The conditions necessary for the separation were: ATP concentrations (0.4 to 1.2 mM) at which accompanied with an inhibition of MgATP-induced tension were the favorable concentration. Above which the fast phase became slower and smaller, and finally disappeared at 2.3 mM. Lowering of temperature retarded and reduced the development of the slow phase, but augumented the fast phase. At the high free Ca^{++} concentration, around pCa 5.2, the slow phase fused with the fast phase. Critical concentration of the phase fusion depends on a Ca-sensitivity of the fiber, and the submaximum Ca^{++} concentration is the requisite for the phase separation. Both Sumarin (a potent inhibitor of sarcoplasmic reticulum) and Dantrolene (an established blocker of T-tubule) reduced the initial phase. In contrast, vanadate (an inhibitor of myofibrillar ATPase) reduced the slow phase. The results revealed that the initial fast phase depends on a residual system other than the contractile element, and the secondary phase depends on the contractile element.

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ON THE pCa^{2+}-CONTRACTION DIAGRAM OF Ca^{2+}-INSENSITIVE GLYCERINATED MUSCLE FIRBER.
WATANABE, Y., NAKAYAMA, Y. AND YAMAGUCHI, M. Dept. of Physiol. & Anat.; Kyoritsu Coll. of Pharm., Tokyo 105 and Dept. of Biochem. and Nutri. Juntendo Univ., Narashino 275

Ca^{2+}-insensitive glycerinated rabbit psoas muscle fiber(CaIS-fiber) developed maximum contraction even the threshold of contraction in Ca^{2+}-sensitive fiber(CaS-fiber). This fact is caused by the loss of Ca^{2+} regulatory protein(TN-TM system) in muscle fiber. However, CaIS-fiber recovered Ca^{2+} sensitivity until the near level of the threshold of CaS-fiber when it was incubated in the solution containing TN-complex from rabbit back muscle at 0°C for 2-3 days. We examined the recovery of Ca^{2+} sensitivity between skeletal muscles of some vertebrates. Ca^{2+} concentration of recovery solutions was 10^{-7} - 10^{-8} mol from the pCa-contraction diagram. CaIS-fibers of psoas muscles of rabbit, rat, guinea-pig and bullfrog were incubated in the solutions of TN-complex which extracted from these animals. Consequently, it was found that the recovery ratio was relative high values from 78 to 88 % between rabbit TN-complex and CaIS-fibers of animals, but low values from 8 to 64 % in the TN-complex of other animals.

From these results, it was presumed that the difference of recovery ratio in exchange of molecular will depend upon the animal species and the physicochemical affinity of TN-complex under the some physiological conditions.

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MECHANISM OF Ca^{2+}-INDUCED Ca^{2+} RELEASE FROM SARCOPLASMIC RETICULUM OF SKELETAL MUSCLE.
KOSHITA, M. and HOTTA, K.
Dept. of Physiol., Nagoya City Univ. Med. Sch., Mizuho-ku, Nagoya 467

The effects of Mg^{2+}, adenine nucleotides, caffeine, and procaine on Ca^{2+} efflux by external Ca^{2+} from sarcoplasmic reticulum(SR) isolated from bullfrog skeletal muscle were studied. Active $^{45}Ca^{2+}$ uptake was initiated by adding acetyl phosphate into a reaction solution, and $^{45}Ca^{2+}$ efflux was induced by 20-fold dilution with a solution containing various concentration of free Ca^{2+} and reagents. $^{45}Ca^{2+}$ remaining in SR was measured by Millipore filtration method. Increase of Ca^{2+} up to 10^{-3}M enhanced Ca^{2+} efflux, and further increase of Ca^{2+} inhibited it in the presence of 1 mM $MgCl_2$. The maximum value of Ca^{2+} efflux was reduced in the presence of procaine or millimolar concentration of Mg^{2+}. Adehine and AMP increased the maximum value of Ca^{2+} efflux. ADP, ATP, caffeine, or micromolar concentration of Mg^{2+} increased Ca^{2+} efflux and the maximum value of Ca^{2+} efflux in low Ca^{2+} concentration. Procaine counteracted the effect of caffeine.

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COMPOSITION AND VOLUME OF THE SARCOPLASMIC RETICULUM DURING CAFFEINE CONTRACTURE.
YOSHIOKA, T., NAKANO, S. Dept. Physiol., Tokai Univ., Sch. Med., Isehara, Kanegawa 259-11 AND SOMLYO, A.P.* Pennsylvania Muscle Inst., Univ. Pennsylvania, Sch. Med., Philadelphia, PA 19104, USA

The effects of caffeine on the composition and on the volume of the terminal cisternae (TC) of the sarcoplasmic reticulum (SR) in frog skeletal muscle were determined with rapid freezing, electron microscopy and electron probe analysis. Caffeine (5mM) released approximately 65% of the Ca of the TC in 1 min. and 84% after 3 min. The release of Ca from the TC was associated with a significant increase in its Mg content. There was also a small increase in the K content of the TC at 1 min., although not after 3 min. of caffeine contracture. Based on the increase in Mg content during caffeine contracture and during tetanus (Somlyo et al. (1981) J Cell Biol 90), we suggest that both mechanisms of Ca release are associated with an increase in the Ca and Mg permeability of the SR membrane, the two ions possibly moving through a common channel. Mitochondrial Ca content was significantly increased (at 1 and 3 min.) during caffeine contracture. The TC were not swollen in rapidly frozen, caffeine-treated muscles, in contrast to the swelling of TC observed in conventionally-fixed, caffeine-treated preparations. The fractional volume of the TC in rapidly frozen control (resting) frog semitendinosus muscles (about 2.1%) was less than the volume (about 2.5%) after glutaraldehyde-osmium fixation. [This work was supported by NIH grant HL15835 to the Pennsylvania Muscle Institute]

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THE RELATION BETWEEN LATE AFTER POTENTIAL IN SKELETAL MUSCLE FIBER AND ITS EXCITATIN-CONTRACTION COUPLING. YAMAGUCHI, T. Dept. Biol., CLA, Intern. Christ. Univ. Mitaka, Tokyo, 181

The late after potentials (LAP) of skeletal muscle fibers were examined in several conditions to ascertain them as a manifest of the excitation-contraction coupling. The decay half time of LAP was significantly prolonged on application of 10 μM dantrolene which is known to diminish contraction without any change in membrane properties. In fatigued muscle fiber, during the early period of cessation of repetitive stimulation, LAP disappeared as well as the reduction of the resting membrane potential, whereas both of them recovered to the normal amplitude as the contractility recovered. The potentiator substance, caffeine, which increases the myoplasmic Ca ions, had no effect on LAP. After removal of TTX,which had been applied for 1 hour, LAP was diminished, whereas it increased again as the twitch recovered; showing that the failure of tubular excitation diminishes LAP amplitude. Although any common charactor of LAP on E-C coupling has not yet been obtained, a tentative explanation of decoupling in each case was given.

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EFFECTS OF Ca CURRENT BLOCKERS ON INTRACELLULAR Ca TRANSIENTS IN SINGLE SKELETAL MUSCLE FIBERS OF FROG. KURIHARA, S., KONISHI, M., KAWAMURA, T. AND SAKAI, T. Dept. of Physiol., Jikei Univ. Sch. of Med. Tokyo 105

Effect of Ca current blockers on intracellular Ca transients in twitch, tetanus and rapid cooling contracture was observed using single muscle fibers injected with aequorin. Ca transient and tension in twitch response were not substantially altered by verapamil, nicardipine and D600. But in tetanic stimulation, Ca transient and tension were partially abolished by verapamil, nicardipine and D600, which was due to the lack of action potential. D600 slightly decreased Ca transients in the plateau of tetanic tension only at low temperature. These Ca current blockers applied with low concentration of caffeine further raised intracellular Ca level than caffeine alone, and Ca release by rapid cooling was augmented by these drugs. These results suggest that Ca current blockers might accelerate Ca-induced Ca release mechanism when caffeine exists, and that Ca-induced Ca release mechanism which is considered to be responsible for Ca release by caffeine and in rapid cooling might not be important in physiological Ca release mechanism.

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INHIBITION OF EXCITATION-CONTRACTION (E-C) COUPLING BY PHENYLGLYOXAL(PGO) IN SKELETAL MUSCLE OF FROG, R. JAPONICA. M. FUJINO, Y. SATO, T.ARIMA,T. TAKAI. 1. dept of Physiol., Natl. Def. Med. Coll., Tokorozawa.

1. Necessary and sufficient condition for complete E-C uncoupling is 20 min Ringer-immersion with 3 mM PGO followed by 30 min immersion without PGO in case of single fibers and 40 min immersion with 3 mM PGO followed by 60 min immersion without PGO in case of whole muscles. 2. Several PGO-derivatives (p-hydroxy-, p-nitro-, p-azido-PGO), which also react with guanidyl group, inhibit E-C coupling similarly to PGO, suggesting that guanidyl group or a resembled structure to it takes a part in E-C coupling. 3. Mercaptoethanol-acrolein complex, which removes completely the inhibiting action of acrolein on E-C coupling, removes, though partially, the inhibitory action of both PGO and Dantrolene, suggesting that these inhibiting substanses react with a common chemical site. 4. PGO increases the number of terminal cisternae (tc), where a small less-dense portion occurs within tc. 5. Biochemical analyses done so far indicated that: protein of lower dalton value appears intensely dependently on the grade of E-C uncoupling due to PGO; its isoelectric point is on weak alkaline side; it is PAS-reaction-positive.

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ARSENAZO III Ca-TRANSIENTS IN *XENOPUS* SKELETAL MUSCLE FIBERS DURING SINGLE OR REPETITIVE STIMULATIONS. OCHI, K., MATSUMURA, M., TOYOTA, H. AND MATSUKI, Y. Dept. of Physiol., Kawasaki Med. Sch., Kurashiki 701-01

Arsenazo III (AZ III) signals due to intracellular Ca-transients were recorded at the wavelengths of 651-721 nm in *Xenopus* twitch muscle fibers. The latency of AZ III signals (T_1), the time from the onset to peak (T_2) and the half decay time (T_3) were 1.8, 3.9 and 16.6 msec, respectively, at 19-23°C, and prolonged with falling of the temperature in standard Ringer solution. The falling phase had a Q_{10} of 2.1 over the range 7-29°C. When NaCl in standard Ringer solution was replaced with equimolar $NaNO_3$, the peak of the AZ III signal showed a 30% increase. In NO_3 solution, T_1, T_2 and T_3 were prolonged to 2.1, 6.3 and 22.3 msec, respectively. AZ III signals after adding of 40 μM $ZnCl_2$ or 0.9 mM caffeine were also increased in peak amplitude and prolonged in falling phase. AZ III signals responding to repetitive stimulation with frequency of 10, 20 and 30/sec showed the descending staircase. Obvious summation was observed at the stimulating rate more than 30/sec. The falling phase is prolonged by an increase in stimulating pulses and in stimulating frequency, suggesting that some factors other than the Ca-reuptake by sarcoplasmic reticulum can contribute to the falling phase of the signal.

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INHIBITION OF EXCITATION-CONTRACTION (E-C) COUPLING DUE TO Ca-ANTAGONISTS AND Ca-TRANSIENTS IN SKELETAL MUSCLE FIBERS OF FROG, R. JAPONICA. M. FUJINO, Y. SATO, T. ARIMA. 1. Dept of Physiol., Natl. Def. Med. Coll., Tokorozawa.

Effects of Ca-antagonists on E-C coupling were studied using single fibers. Results: 1. At lower temperatures, E-C coupling was inhibited by Nicardipine(Ncp), of which threshold concentrations were about 5 μM at 6°C. The inhibition disappeared by warming and reappeared by re-cooling. The action was removed by divalent cations (Ca, Mg) and was not by 100 μM Isoproterenol. Ca-transients (arsenazo III) of twitch were inhibited under the above inhibitory conditions. 2.Diltiazem(Dtz) behaved similarly to Ncp in the avove respects, exept for two points: (a) For the appearance of action of Dtz of low concentrations, Dtz must be applicated not only at lower temperatures but also under membrane depolarization; (b) threshold concentration was about 1 μM and 1 mM under depolarized and polarized conditions, respectively. Other conditions to lower the threshod concentrations for Dtz-action is Cl-deficiency, 10 mM Borax at pH 8.0, and longer application. 3. Intensity of the inhibiting action was in the order: Verapamil > Dtz > Prenylamine > Ncp >> Nifedipine.

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THE MECHANISM OF E C DECOUPLING OF SKELETAL MUSCLE.
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In order to analyze the inhibitory effect of manganese ion on the local contraction of skeletal muscle fibres, A.L.Hodgkin & W.A.H. Rushton's method of unmyelinated nerve fibres (1946) was tried to measure the threshold depolarization of local contraction and conductance. B.Katz applied this method to skeletal muscles fibres (1948). The experimental merit of the application of this old method to the measurement of local contraction threshold was discussed.

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ULTRASTRUCTURAL CHANGES IN TRIADS DUE TO DANTROLENE-Na(DaNa) IN SARTORIUS MUSCLE OF FROG, R. JAPONICA. M. FUJINO, Y. SATO. T. ARIMA,H. TAKAI, S. TAKAMATA. 1. Dept. of Physiol., Natl. Def. Med. Coll., Tokorozawa.

An ultrastructural behavior caused by DaNa was found in triads,especially, terminal cisternae (tc). Results: 1. In longitudinal sections of isolated single muscle fibers without DaNa-treatment, no special ultrastructures are almost recognizable within tc, as widely accepted, and only found in tc would be a small electron-less-dense portion (l-portion) near triadic junction in low percent (about 20% of total triads observed). 2. Whereas, 30 μM DaNa increase the percentage of appearance of l-portion to about 50 regardless of time durations of DaNa-immersion (10 sec to 60 min; 10 sec is the shortest time capable of experimenting), suggesting a possibility of immediate appearance after application of DaNa. 60 min after removal of DaNa, the increased percentage decreases somewhat. 3. The increase of percentage appears at 50 nM DaNa for 10 min to a smaller extent and at 100 nM for 10 min to a similar extent to the 30 μM-case. 4. Under these conditions, twitch inhibition increases in proportion to grade of the structural behavior. 5. Depolarization by 110 mM KCl increases the percentage to about 77%.

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SILVER IONS INDUCE TENSION DEVELOPMENT IN SKELETAL MUSCLE OF BULL FROG. OBA, T. AND HOTTA, K. Dept. Physiol., Nagoya City Univ. Med. Sch., Mizuho-ku, Nagoya, 467

In single fibers in a Cl-free MOPS solution containing 1.8 mM Ca, tension developed slowly in the presence of low concentration of Ag. This tension was not blocked by administration of Co or Ni. In fibers pretreated with 0-Ca MOPS solution containing either 2 mM Co or 1 mM Ni for 10 min, a repetitive twitch-like contraction and a subsequent large transient one developed with application of 5 μM Ag. After reversion to the resting tension, the fiber was contracted by adding more than 0.1 mM Ca or 25 mM caffeine. In response to Ag, tension did not develop in fibers in which the T-tubules had been disrupted. Pretreatment of the fiber with Ca-free solution for 30 min followed by Co treatment for 10 min greatly reduced the Ag-induced transient tension. A sulfhydryl reagent did not contract the muscle fiber and there were no response when Ca was added to the medium. These data indicate that enhanced Ca permeability in the T-tubular membrane play a significant role during Ag-induced mechanical activation.

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MECHANISM OF THE INHIBITION INDUCED BY EXTERNAL Ca^{++} REDUCTION OF EACH COMPONENT OF THE BIPHASIC POTASSIUM CONTRACTURES IN FROG SINGLE TWITCH MUSCLE FIBERS. TSUTSU-URA, M., KANAYA, H., TAKAUJI, M. AND NAGAI, T. Dept. of Physiol., Sapporo Med. College., Sapporo

We examined the inhibitory effect of external Ca^{++} reduction in long term (20 - 40 min) on the peak tension of the initial component and the secodanry component of 80 mM K contractures and the effects of Na^{+} and 0.5 - 1 mM caffeine on each component under condition of the Ca^{++} reduction, using single twitch fibers of the frog. The results obtained are as follows:1) The time course of the Ca^{++} reduction effect on the initial component was much slower than that on the secondary component. 2) The inhibition of the initial component induced by the Ca^{++} reduction was removed by Na^{+}, while that of the secondary component was not removed. 3) 0.5 - 1 mM caffeine reversed, by 60 - 90 % of control tension, the peak tension of the initial component which had been abolished by Ca^{++} reduction for 20 - 40 min, but did not reverse the secondary component. These results indicate that the inhibition of the initial component by external Ca^{++} reduction was clearly different from that of the secondary component. It was also demonstrated that the inactivation induced by conditioning depolarization with 20 and 30 mM K in the presence and absence of Ca^{++} were not inhibited by Na^{+} and 0.5 mM caffeine. On the basis of these results, the mechanisms by which the initial and secondary components are inhibited by external Ca^{++} reduction were discussed.

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EFFECT OF MAITOTOXIN ON FROG SKELETAL MUSCLE FIBERS, GOMI, S. AND SUGI, H. Dept. Physiol., Sch. Med., Teikyo Univ., Itabashi-ku, Tokyo 173

Maitotoxin(MTX), obtained from marine dinoflagellates, is reported to cause irreversible contraction in mammalian vascular smooth muscle, possibly by opening Ca channels. We studied the effect of MTX on the membrane systems of frog skeletal muscle fibers, purified MTX being kindly supplied by Prof. K. Yasumoto of Tohoku Univ. MTX (more than 10^{-10}g/ml) caused irreversible contraction in isolated semitendinosus muscle fibers, but the MTX-induced contraction was eliminated by the removal of external Ca or after the treatment of T-tubule disruption. MTX at subthreshold concentrations for eliciting contraction produced a gradual decline of membrane potential tp result on an evetual loss of action potential in both intact and T-tubule disrupted fibers; during the course of the above MTX action, a stage was observed in which the twitch tension was markedly enhanced while the height of action potential decreased appreciably. MTX did not produce tension in skinned muscle fibers loaded with Ca, and did not produce Ca release from the fragmented sarcoplasmic reticulum (SR) also loaded with Ca. These results indicate that MTX may cause an increase in Ca permeability in the T-tubule membrane, but not in the surface membrane and the SR membrane.

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EFFECTS OF PROSTAGLANDINS ON FROG SKELETAL MUSCLE.
Ishizawa,M. Physiology,School of Allied Health Professions,Sapporo Medical College. Sapporo, 060, Japan.

The effects of PGD_2,E_2 and F_{2a} were investigated in frog sartorius and rectus abdominis muscles in vitro.

In the sartorius muscle, PGD_2,E_2 and F_{2a} (1 - 10 μM) augmented and prolonged the twitch responses stimulated directly. The augmentation of twitch responses was enhanced in the presence of caffeine (1 mM) by 43 ± 24 %(10 μM PGE_2) and 58 ± 20 %(10 μM PGF_{2a}), respectively.

In the rectus abdominis muscle, PGD_2,E_2 and F_{2a} (1 - 10 μM) augmented and prolonged the electrically-stimulated contractions with an elevation of the tone of muscle, although these PGs had no effect of their own on the muscle tone. The augmentation of contractions was also enhanced in the presence of caffeine(1 mM).

The results indicate that PGD_2,E_2 and F_{2a} may augment the contractile response in frog skeletal muscle by the maintenance of free Ca ion in the cytoplasma.

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THE SLIDING OF ACTIN AND MYOSIN IS DUE TO HYDROPHOBIC BONDING.
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In previous papers, we have pointed out the importance of hydrophobic bonding in the explanation of various cellular phenomena of particular interest has been the explanation at a molecular level of membrane excitation, when the hydrophobic bonding in the cellular membrane is given due consideration. Application of these concepts concerning hydrophobicity and hydrophilicity appears to allow for a unified explanation of a wide variety of cellular phenomena without contradiction or additional ad hoc hypotheses. In this statement, a molecular mechanism for the sliding phenomenon of actin and myosin is proposed as follows:When Ca^{++} binds to actin fibers, these fibers change their molecular conformation, thus exposing hydrophobic ligands. Primarily, the hydrophobicity of the head regions of the myosin is strong. Due to hydrophobic bonding, the hydrophobic ligands of these molecules face one another and become hidden from the surronding intracellular aqueous solution. This hypothesis predicts the following phenomena: (i) Myosin fibers, like actin fibers, move in an aqueous solution. (ii) A change in cellular volume occurs during contraction.

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THE EFFECT OF CROSS-BRIDGE ORIENTATION ON THE EQUATORIAL X-RAY INTENSITY. TANAKA, H. AND SUGI, H. Dept. of Physiology Sch. of Med., Teikyo Univ. Tokyo 173

Lymn (1978) suggested that different orientation of cross-bridges gave different numerical values for the equatorial X-ray intensities of I(1,0) and I(1,1), assuming the same number of cross-bridges attachment. It is expected that cross-bridges in rigor muscle show different orientation depending on lateral spacing of the myofilament lattice, because cross-bridges attached to thin filaments may be displaced with increase in lattice spacing and rotate to give the 90° orientation. Spacing of the hexagonal lattice of the glycerinated rabbit psoas muscle fiber bundles was controlled within a range from 36 to 52 nm (unit cell) by changing salt concentration and/or PVP concentration of the bathing medium.

With increase in lattice spacing, marked increase in I(1,1) and small increase in I(1,0) were observed. A computational approach was made in which we approximated both filaments as simple cylinders and cross-bridge as a sphere. A possibility arose that no change in cross-bridge orientation was induced with increase in spacing of myofilament lattice, and changes in I(1,0) and I(1,1) could be explained by change in scattering angle.

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HEAT MEASUREMENTS OF FROG SKELETAL MUSCLE DURING ISOMETRIC CONTRACTION WITH AN INFRARED THERMO-DETECTOR. KOBAYASHI, T., KAMIYAMA, A. AND SUGI, H. Dept. Physiol., Sch. Med., Teikyo Univ., Itabashi-ku, Tokyo 173

Though heat production in muscle is generally measured with a thermopile, the time resolution is limited by the time required for heat flow from muscle to the thermopile. On the other hand, the infrared radiometer can measure heat production in a limited area of muscle surface without any thermal contact to give a high time resolution. The radiometers hitherto available, however, required complicated signal processing procedures to yield a number of technical difficulties. We constructed a new infrared thermo-detector system by use of pyroelectric sensors to measure local heat production along the length of frog sartorius muscles. The pyroelectric sensor can detect temperature changes of 1 mdeg with a time resolution of about 0.2 msec. With the above system, we succeeded in measuring heat production localized in various small areas along the length of muscles during an isometric twitch or tetanus at 20-23°C, at which frogs move normally. It was found that there were marked non-uniformities in heat production along the muscle length, and this result was confirmed by the observation of contracting muscles with a thermography.

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ON THE ORIGIN OF THE LABILE MAINTENANCE HEAT IN FROG SKELETAL MUSCLE. KOMETANI, K. AND YAMADA, K. Dept. of Physiol., Medical College of Oita, Oita 879-56

Effects of the sarcomere length, caffeine and temperature on the maintenance heat production were investigated in frog skeletal muscles. Muscles were tetanically stimulated for 4 sec. The total heat output was corrected for heat loss and was divided into two parts, stable and labile, by fitting it with the relationship: $h(t)=h_b t+ ha/a(1-\exp(-at))$ (Aubert, 1956).

The relation between the maintenance heat and the sarcomere length (S.L.) was studied at 0°C using semitendinosus muscles. Mean values for isometric tension at each S.L. decreased linearly with increasing S.L. Mean values for h_b, ha/a, ha and a at each S.L. also decreased linearly with increasing S.L. At long S.L., where no active tension was developed, h_b reduced to about 30% of that at l_o (S.L.=2.29μm), whereas about 70% of ha/a remained.

Effects of caffeine and temperature were studied at l_o using sartorius muscles. Addition of 1 mM caffeine caused an increase both in ha and a. However, h_b and ha/a were scarcely affected. As temperature was increased, h_b, ha and a increased with Q_{10} of about 3, while Q_{10} of ha/a was about 1.

All the results described above are consistent with the idea that a larger part of the labile maintenance heat is produced by calcium cycling.

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Structural changes associated with contraction of chemically skinned smooth muscle cells isolated from taenia coli of the guenia pigs. OOTA, I. and YABU, H. Dept. of Physiol., Sapporo Med. College, Sapporo 060.

Single cells were isolated by the method of Obara (1984). Chemically skinned cells were prepared by treating with saponin (7μg/ml) for 10 min at 35°C. When the free Ca concentration in medium was changed from pCa 8.5 to 6.0, the cells shortened actively to about 50% of their initial length. The cell surface of the contracted cells was completely covered by numerous evaginations, which were similar to those of contracted intact cells. In addition, the characteristic feature of skinned cells in contracted state was the occurrence of many delicate and fibrous sarcoplasmic projections. They were connected the cell body with evagination or between evaginations. Thus, the irregularity of surface profile of these cells more marked than in contracted intact cells. Saponin is known to complexes with cholesterol, which decreases the membrane fluidity, and to remove from plasma membrane. Therefore, it seems likely that the increase of the irregularity in skinned cells is caused by the increase of membrane fluidity.

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RHEOLOGICAL STUDY ON PRESSURE-VOLUME RELATION OF UTERINE CERVIX OF RAT Y.SUZUKI, K. KAWANO, M.UYETAKE*, S.KIKUCHI* and Y.SHINAGAWA, Dept. of Physiology, Nippon Medical School, *Dept. Obst. & Gynec., Nippon Medical School, Tokyo 113

The inner pressure of uterine cervix of rat, which was generated by micrometer-syringe injection of Kreb's solution into cervix lumen, was measured by means of transducer method. The pressure, P, was represented as a function of the inner volume of cervix, V, as follows;

$$P = \alpha (V - \delta - V_0 + \beta e^{-(V-\delta)/V_0})$$

where V_0 is the initial volume. The physiological meaning of the constants, α,β and δ are assigned as elasticity, visco-elasticity and plasticity, respectively. One of the cervix of uterus duplex was cut in order to be free from contraction wave from corpus uterus, at the middle stage of pregnancy. We can compare the P-V curves of the cut cervix with that of non-separated one as was referred to normal maturation.

No significant difference in P-V relation between the separated and the non-separated cervix was observed in the middle stage of pregnancy. At the terminal stage of pregnancy, the plasticity constant, δ, in P-V relation revealed significant difference (non-separated cervix, $\delta = 19.1 \pm 12.8$ μl, cut cervix referred as zero point), of which value increased during maturation.

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RECORDINGS OF INTERNAL PRESSURE AND CONTRACTION WAVE IN GUINEA PIG URETER. TAKEI, N., TSUCHIYA, T. AND SUGI, H. Dept. Physiol., Sch. Med., Teikyo Univ., Itabashi-ku, Tokyo 173

Physiological properties of guinea pig ureter were studied by recording the internal pressure and the contraction wave propagation with a pressure transducer and a video system respectively. When an isolated ureter was stimulated with a brief current pulse through massive electrodes, it showed an all-or-none twitch-like pressure response, the magnitude of which increased with increasing internal pressure. If a part of the ureter was locally stimulated, a contraction wave was initiated at the point of stimulation and propagated along the length of the ureter with a velocity of 20-40 mm/sec. The propagation velocity did not change appreciably irrespective of the direction of the contraction wave. With increasing internal pressure, the velocity of constriction at each elementary part of the ureter decreased while the extent of constriction increased. Both the velocity and extent of constriction at each elementary part did not change markedly according to the direction of propagation. The duration of a constriction-reexpansion cycle was longer at the proximal region than at the distal region. The threshold for producing contraction was lowest at the most proximal region, and highest at the most distal region.

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ELECTRICAL RESPONSE OF THE RAT COLON TO SHORT-CHAIN FATTY ACIDS.
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The effect of luminal chemical stimuli on colonic ion-transport is still unclear. Short-chain fatty acids (SCFA) produced by gut microbes such as acetic, propionic, butyric and valeric acid, are major anions in the colon. In the present study we examined effects of SCFA and their analogs on colonic ion-transport in vitro. The ion-transport was indirectly estimated by changes in transmural potential difference (p.d.) and short-circuit current (SCC) across colonic segments. The application of SCFA (C3-C6) from the mucosal side evoked phasic increase in p.d. (lumen negative) and SCC but not change in tissue resistance. The response started after 3-10 sec and reached the maximum within 60 sec. The SCFA-analogs (unsaturated and halogen, keto, hydroxy or amino organic acids) lacked stimulatory effect. Carboxyl group was essential for stimulation. Colonic segment did not respond to cumulative addition of either the same SCFA or a different SCFA. 3-Cl-propionate competitively inhibited the stimulation of p.d. produced by propionate. Atropine, TTX and procaine partially inhibited the effects of propionate while eserine enhanced it. These results suggest that SCFA stimulated colonic ion-transport, presumably via an enteric reflex involving SCFA-receptive mechanism and cholinergic neuron.

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CANINE STOMACH SMOOTH MUSCLE, NEURAL STRUCTURE AND RESPONSES TO ELECTRICAL FIELD STIMULATION.
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Structure and function of innervation of the lesser curvature of canine gastric corpus circular smooth muscle was investigated. Responses of muscle strips to electrical field stimulation (FS) with 0.5 msec pulses at 0.5-20 Hz, 40 V were studied. In general, FS tended to elicit sequences of varying magnitudes of transient on-contraction, on-relaxation, off-relaxation and off-contraction. Pharmacological assesment suggests that excitatory cholinergic, and both excitatory and inhibitory noncholinergic (NC) and nonadrenergic (NA) innervation exist in this muscle. NC excitation was susceptible to tachyphylaxis by substance P and 5-HT. NA inhibitory response disappeared after scorpion venom treatment. Nerve varicosities, most containing predominantly small agranular vesicles, but some containing large granular vesicles, were located outside muscle bundles. In agreement with functional results, very few nerves containing small granular vesicles, presumably adrenergic, were found.

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EFFECTS OF EXTERNAL SODIUM IONS ON THE NON-ADRENERGIC INHIBITORY POTENTIALS IN THE SMOOTH MUSCLE CELLS OF THE GUINEA-PIG DUODENUM.
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The non-adrenergic inhibitory potentials (i.p.s) in the duodenal smooth muscle cells of the guinea-pig were recorded intracellularly. The effect of drugs affecting the activity of the autonomic nervous system on the i.p. were examined. The amplitude of the i.p.s was decreased by hexamethonium, 5-hydroxytryptamine and nicotine without the changes in the resting membrane potential while procaine slightly increased the amplitude of the i.p. TTX (10^{-7}- 10^{-6}g/ml) completely blocked the i.p.s evoked by single and repeated stimulations without the resting membrane potential change.

In the Li-solution, the i.p.s could be evoked during a long perfusion period but the amplitude of the i.p. was decreased slightly. Similar results were obtained in the choline- and sucrose-solutions. In the low-sodium solution, the removal of external calcium ions abolished the elicited i.p.s. The obtained results suggest that the excitation-secretion coupling in the non-adrenergic inhibitory neurons is mainly dependent on the external calcium ions but not sodium ions. The non-adrenergic inhibitory pathway is discussed based on the drug actions.

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CONTRACTILE RESPONSES IN THE SMOOTH MUSCLE OF GUINEA PIG URETER. ICHIKAWA, C., TSUCHIYA, T. AND SUGI, H. Dept. Physiol., Sch. Med., Teikyo Univ., Itabashi-ku, Tokyo 173

The mode of activation of the contractile mechanism in the smooth muscle of guinea pig ureter was studied on the helical strip preparation by recording its tension responses to electrical stimulation and to high $[K]_o$, while the action potentials were recorded by the sucrose-gap method. The all-or-none twitch-like response associated with the action potential disappeared in the hypertonic solution (two time the normal tonicity), while the action potential remained unaffected. With transverse AC field stimulation (50 Hz, 5 sec), the threshold for the all-or-none response was about 1 V/cm, whereas the graded mechanical response appeared at 5-7 V/cm to reach a maximum at 25-30 V/cm. When $[K]_o$ was increased above 30 mM, the preparation exhibited K-contracture tension development, which reached a maximum at 100 mM-K. The magnitude of contracture tension with 100 mM-K was dependent on $[Ca]_o$, increasing with increasing $[Ca]_o$ up to 20 mM: The K-contractures were eliminated with Mn ions, low pH and Ca antagonists such as verapamil and D-600. Caffeine (5-20 mM) did not produce tension development. These results indicate that, in the smooth muscle of guinea pig ureter, the contractile mechanism is activated by the inward movement of external Ca ions.

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IN VITRO EFFECTS OF OVARIAN HORMONES ON MEMBRANE AND CONTRACTILE ACTIVITIES OF RAT UTERINE MUSCLES. OGASAWARA, T. AND OSA, T. Dept. of Physiol., Yamaguchi Univ. Sch. of Med., Ube 755

In vitro application of estradiol-17β (20μM) and progesterone (20-100μM) caused a contractile depression in the longitudinal muscle of diestrus rat uterus. In late-pregnant uterus, estradiol depressed the membrane and contractile activities of both longitudinal and circular muscles. Progesterone induced a contractile potentiation accompanied with a prolongation of burst discharge in the longitudinal muscle and caused an inhibition of contractions in the circular muscle. In postpartum uterus, estradiol induced a contractile depression, whereas progesterone caused an initial potentiation of contractions followed by a depression in the longitudinal muscle. In the circular muscle, estradiol induced a contractile inhibition in spite of vigorous spike discharge, and progesterone caused a progressive depolarization accompanied with a contractile depression. It was proposed that the effects of ovarian hormones on rat uterine muscle were exerted via changes in membrane activity and via a dissociation of excitation-contraction coupling.

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COUPLING BETWEEN CALCIUM FLUXES AND MECHANICAL ACTIVITY OF SMOOTH MUSCLE. Hotta, K., Yamamoto, Y. & Oba, T. Dept. Physiol., Nagoya City Univ. Med. Sch., Nagoya 467.

Many of the smooth muscles exhibit spontaneous membrane potential change. Calcium which enters the cell in association with the electrical activity triggers contraction, however, role of the intracellularly-stored calcium on the activation of contractile element is unknown. We investigated the correlation between Ca fluxes and movement of the intracellular Ca using the rat portal vein. Upon generation of the action potential, a rapid increase of Ca influx which coincided with the tension development was observed. Relaxation begun much earlier than the extrusion of Ca from the cell. High-K induced contracture of the portal vein consisted of two components: rapid transient and sustained tonic ones. The first component was preceded by the rapid increase of Ca influx. It is suggested that the rapid Ca influx, such as Ca spike, participates rapid activation of contractile system, while Ca-induced Ca release from the intracellular stores may be responsible for the slow phase of contraction. Ca uptake activity of the internal store sites may take an important part for the rapid relaxation of the muscle, together with Ca pump of the cell membrane.

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PROPERTIES OF CARBACHOL-SENSITIVE INTRACELLULAR CALCIUM STORE IN STOMACH SMOOTH MUSCLE OF THE NEWT. KOSAKA, I., Dept. Physiol., Sapporo Med. Col., Sapporo 060 Japan

Carbachol (CCh) contracture in stomach circular muscle (about 100 μm in diameter) of the newt, Cynops pyrrhogaster, was inhibited markedly with 1 mM EGTA or 100 μM verapamil. But little inhibition was observed when the muscle was treated with EGTA or verapamil in normal solution after pre-treatment with EGTA or verapamil under K-depolarization. K contracture was abolished by EGTA or verapamil. CCh contracture was abolished immediately after immersion in high K solution and then gradually recovered to the control level. The rate of this recovery was markedly increased by treatment with EGTA or verapamil. After treatment with Ca-free high K solution containing EGTA, the muscle responded with a contracture which was slightly reduced with K-depolarization time to the addition of 1.8 mM Ca. When this Ca-free high K solution with EGTA was replaced with the same solution without EGTA, CCh contracture was inhibited to about 10 % of control within 30 sec after depolarization. CCh contracture in depolarized muscle was abolished by lowering temperature to 3 - 5°C. This inhibitory effect was reduced by treatment with EGTA or La (0.1 mM). From these results, it is suggested that above-mentioned inhibitory effects on CCh contracture may be due to the reduction of CCh-sensitive intracellular Ca store resulting from the depolarization accompanying Ca entry.

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STUDIES ON THIN-FILAMENT-LINKED CALCIUM REGULATION SYSTEM IN THE ABALONE MUSCLE. ASADA, N. and YAZAWA, Y. Division of Nutrition Phsiology, Hokkaido University of Education, Hokumoncho 9, Asahikawa 070

The calcium regulatory system in abalone muscle was studied. Abalone native actomyosin was Ca-sensitive, and actomyosin reconstituted from abalone myosin and rabbit skeletal actin showed Ca-sensitivity in Mg-ATPase assay. Mg-ATPase activity of the abalone native actomyosin was greatly enhanced by washing with 2mM Tris-HCl (pH 7.6). Crude troponin and tropomyosin fraction prepared from abalone ether powder had inhibitory effect on superprecipitation and Mg-ATPase activity of the reconstituted rabbit skeletal actomyosin. These fractions always inhibited Mg-ATPase of washed abalone actomyosin. Crude troponin fraction was separated into two fractions by CM-cellulose chromatography, one consisted of actin and tropomyosin, and the other consisted of mainly single protein. Molecular weight of this protein was calculated about 27000 dalton. 27000 dalton protein and purified tropomyosin had inhibitory effect on superprecipitation of rabbit actomyosin and Mg-ATPase of abalone actomyosin. Purified tropomyosin had only little effect on these activities therefor, 27000 dalton protein is assumed to play ainhibitory role in the abalone muscle.

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CONTRACTION OF SMOOTH MUSCLES IN Ca-FREE SOLUTION. ASHOORI, F., TAKAI, A. AND TOMITA, T. Dept. Physiol., Nagoya Univ., Nagoya 466

It has been reported that some smooth muscles can produce small tonic contractions repeatedly without decay in Ca-free medium. In the present study, the noradrenaline response in the rat vas deferens, the oxytocin response in the rat myometrium and the carbachol response in the guinea-pig stomach muslce were examined. When the agonist was applied for 4 min at 20 min intervals in Ca-free solution containing 2 mM EGTA, the noradrenaline response of vas deferens was gradually increased during first 2-3 hrs, and then slowly decreased to about a half of the maximum at 47 hr of superfusion. On the other hand, the oxytocin response of myometrium and the carbachol response of stomach increased to 200-300% for first 24 hrs and thereafter the increase became less, but there was no indication of decrease for next 24 hrs. In the vas, caffeine (30 mM) produced a weak tonic contracture and reversibly abolished the response to noradrenaline. Caffeine also blocked the responses in the myometrium and stomach without any increase in resting tension. The response to agonists was potentiated after removal of caffeine.

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ACTION OF ADRENERGIC ALPHA$_1$ BLOCKING AGENT ON RESPONSES OF THE CALCIUM INWARD CURRENT IN VAS DEFERENS SMOOTH MUSCLE TO ATP. INOMATA, H. AND WAKUI, M. Dept. of Applied Physiol. Sch. of Med., Tohoku Univ., Sendai, Miyagi 980

The effects of adrenergic alpha$_1$ blocker on the membrane potential and current in the guinea-pig vas deferens to ATP (1 X 10^{-3}g/ml) was investigated under current- and voltage-clamped conditions. A Krebs medium which 50% Na ions were replaced by equimolar TEA ions was used. In the absence of prazosin (1 X 10^{-5} g/ml), an application of ATP caused a transient depolarization in the membrane potential and a reduction in the amplitude and rate of rise of the action potential, while in the presence of prazosin, the application of ATP did not cause any change in the resting and action potential. Under voltage-clamped condition, the application of ATP decreased the maximum calcium current (I_{Ca}) and shifted appreciably its reversal potential (E_a) towards negative voltage with decreasing the chord conductance ($\bar{g}_{Ca}$), but also increased its inactivation time constant. On the steady-state outward current due to K ions, ATP did not alter the reversal potential or the maximum chord conductance ($\bar{g}_K$). On the other hand, in the presence of prazosin, the application of ATP did not reveal any detectable change in the behavior of the calcium current. These results suggest that even alpha$_1$ blocker such as prazosin may have a competitive action with ATP for purinergic receptors.

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TENSION MAINTENANCE, CALCIUM CONTENT AND ENERGY PRODUCTION OF THE TAENIA OF THE GUINEA-PIG CAECUM UNDER HYPOXIA. ISHIDA, Y. AND TAKAGI, K.* Dept. of Pharmacology, Mitsubishi-Kasei Institute of Life Sciences, Machida, Tokyo 194

When potassium (high K, 45mM) was applied, the isolated taenia developed a sustained tonic tension during aerobic conditions (95% O_2: 5% CO_2). Under hypoxic conditions (95% N_2: 5% CO_2) the taenia lost its ability to respond to high K with sustained tonic contraction. Under hypoxia in the presence of high K, raising a glucose concentration caused development of the tonic contraction, increase in the ATP content and increase in the lactate release of the taenia. Good correlations (γ>0.9) were observed between tension development, the ATP content and lactate reelase of the taenia in the presence of high K under hypoxia when the glucose concentration was varied between 5.5 and 55.5 mM. The cellular calcium content of the taenia, determined by the lanthanum method, was increased in the presence of high K under hypoxic conditions. Raising the glucose concentration in the presence of high K did not affect the cellular calcium content. These results suggest that under hypoxic conditions the high K-induced sustained contraction of the taenia is increased by raising the glucose concentration owing to increased ATP production through the glycolytic pathway, but not through mechanisms increasing the intracellular Ca concentration.

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EFFECTS OF CYCLIC NUCLEOTIDES AND RELATED SUBSTANCES ON THE GUINEA-PIG TRACHEAL SMOOTH MUSCLE. ITOH, M.*, BABA, K.*, SATAKE, T. AND TOMITA, T. 2nd Dept. Int. Med. and Dept. Physiol., Nagoya Univ., Nagoya 466

Suppressing effects of various cyclic nucleotides and the agents which are supposed to modify intracellular cyclic nucleotides were studied in the guinea-pig tracheal smooth muscle which was contracted by readmission of Ca in normal (5.9 mM) or excess (40 mM) K solution. Based on the effects, the agents could be classified into three groups. (i) The agents which markedly suppress contractions both in 5.9 mM K and 40 mM K solution. In the excess K medium, slightly higher concentration was necessary. Isoproterenol, forskolin, dibutyryl cyclic AMP and xanthine derivatives belong to this group. (ii) The agents which produce a strong suppressing effect at 5.9 mM K, but become significantly less effective at 40 mM K. These are nitroprusside, $NaNO_2$, 8Br-cyclic GMP, ATP and GTP. (iii) The agents which have little effect both at 5.9 mM K and 40 mM K. 5'AMP, 2'3'AMP, cyclic AMP, 8Br-cyclic AMP, 5'GMP, 2'3'GMP, cyclic GMP, dibutyryl cyclic GMP are included in this group. The difference in action may be partly related to the permeability of the agents and to the site of action.

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CONTRACTIONS INDUCED BY Ca, Sr AND Ba IN THE GUINEA-PIG TRACHEAL SMOOTH MUSCLE. BABA, K.*, ITOH, M.*, SATAKE, T., KAWANISHI, M. AND TOMITA, T. 2nd Dept. Int. Med., Dept. Anesth., and Dept. Physiol., Nagoya Univ., Nagoya 466

Sr and Ba could produce contraction in the guinea-pig tracheal smooth muscle exposed to Ca-free solution containing EGTA. When application of these divalent cations for 20 min was repeated at 30 min intervals, the size of contraction remained roughly the same at least for 3 hrs, suggesting a possibility that both Sr and Ba are able to activate the contractile machinery. Verapamil (10 μM) had a very weak inhibitory action on the basal tone in normal Krebs solution which is likely to be maintained by prostaglandins (PGs) and also on the contraction produced by carbachol or PG $F_{2\alpha}$ in the presence of Ca. On the other hand, verapamil strongly suppressed Ca-induced contraction in excess K solution and all contractions produced by carbachol, PG $F_{2\alpha}$ as well as excess K in the presence of Sr or Ba. These results may be explained by assuming that the divalent cations and verapamil have different affinities at the voltage-dependent Ca channel and receptor-operated Ca channel.

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METABOLIC SUPPORT OF A Na-PUMP IN THE GUINEA-PIG TAENIA COLI. TAKAI, A. TOKUNO, H. AND TOMITA, T. Dept. Physiol., Nagoya Univ., Nagoya 466

In the glycogen-depleted taenia coli of the guinea-pig, the oxygen consumption under the relaxed condition in Ca-free solution was dependent on the external Na concentration to some degree. This Na-dependent oxygen consumption could be supported not only by glucose but also by β-hydroxybutyrate, and significantly reduced by ouabain (10^{-6} M), suggesting that this is due to a Na-pump activity. Readmission of K after a 10 min treatment with K-free solution produced only 2-4 mV hyperpolarization in glycogen-depleted preparations. On the other hand, the same procedure hyperpolarized the membrane more than 10 mV in the presence of glucose or β-hydroxybutyrate. These changes in membrane potential could easily be demonstrated in the presence of verapamil, without interference from the spike activity. The hyperpolarization caused by K-readmission has been interpreted to be due to activation of an electrogenic Na-pump, because the hyperpolarization was reversed to depolarization in the presence of ouabain. Thus, in the guinea-pig taenia coli, oxidative phosphorylation can support the Na-pump without involvement of glycolysis.

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EFFECTS OF RELAXANTS ON THE GUINEA-PIG TRACHEAL SMOOTH MUSCLE IN Na-FREE SOLUTION. KAWANISHI, M., BABA, K.*, AND TOMITA, T. Dept. Anesth., 2nd Dept. Int. Med., and Dept. Physiol., Nagoya Univ., Nagoya 466

Isolated preparations of the guinea-pig tracheal smooth muscle produce continuous tonic contraction in normal Krebs solution. This contraction is dependent on the external Ca concentration and is suppressed completely by indomethacin (1 μM), suggesting involvement of prostaglandins. Isoprenaline (1 μM), a beta-receptor stimulant; theophylline (0.5 mM), a phosphodiesterase inhibitor; and forskolin (1 μM), an adenylate cyclase activator, also suppressed the tension completely. However, the relaxing effects of these substances were gradually reduced and nearly abolished after more than 1 hr when Na in the external solution was totally replaced with choline. On the other hand, when Na was substituted isosmotically with K or Mg keeping Cl concentration constant by adjusting with sucrose, the relaxing effect remained for more than 1 hr without a significant reduction, even in Na-free solution. It seems that the relaxing action of these agents is not influenced by Na removal and that choline has some deleterious effects on this mechanism.

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EFFECTS OF K^+-REMOVAL ON THE CIRCULAR MUSCLE OF THE GUINEA-PIG STOMACH.
TOKUNO, H., TAKAI, A. AND TOMITA, T. Dept. Physiol., Nagoya Univ., Nagoya 466

In the circular muscle of the guinea-pig stomach antrum, the slow wave (30-40 mV) appears at a regular rhythm of 4-5/min. The membrane potential was highly K dependent at a range higher than 5.9 mM, the slope being 50-55 mV for a tenfold increase. However, the membrane was hyperpolarized only a few mV by reducing K to 1 mM, and in K-free solution it was slowly depolarized by 5-10 mV. In the absence of K the slow wave became small and its frequency was often decreased. Ouabain also produced similar depolarization, but it did not reduce the frequency. In the presence of ouabain, removal of K repolarized the membrane and reduced the frequency. Readmission of K transiently hyperpolarized and increased the slow wave, and these process was potentiated by increasing Ca concentration. When the external Na was removed, the membrane was depolarized by 10-30 mV. This depolarization was prevented by K removal, and after a prolonged exposure to K-free solution Na removal produced hyperpolarization, the degree of which increased with the time of pretreatment with K-free solution.

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IONIC MECHANISMS OF ELECTRICAL RESPONSES TO NA AND ATP IN THE GUINEA-PIG VAS DEFERENS.
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Noradrenaline(NA) evoked a contraction in the guinea-pig vas deferens consisting of two components. With a single sucrose-gap method, the initial contraction appeared synchronously with membrane depolarization, and the second one with spike discharges when preceding depolarization became large enough to initiate them. Most of these responses to NA was abolished by treatment with a reduction of external Ca ions. The membrane resistance contrary to the widly accepted view was increased by NA with a double sucrose-gap method. Furthermore, depolarization by NA was reduced in its amplitude by artificial polarization to negative site. While, adenosine triphosphate(ATP) evoked a contraction consisting of single component which was produced by spike discharges resulted by a rapid membrane depolarization. This depolarization was observed even in a Ca-free solution. ATP reduced membrane resistance, and the reversal potential of ATP was around 30mV more positive site than the resting potential. It is suggested that NA initially evokes Ca entry from external solution which may reduce K permeability responsible for membrane depolarization. While, ATP mainly induces the increase in Na permeability which is also resulting in depolarization.

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THE MODE OF ATP-INDUCED VASOCONSTRICTION IN THE CANINE INTERNAL CAROTID ARTERY.
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The mode of ATP-induced vasoconstriction in the isolated canine internal carotid artery was studied from the physiological and pharmacological points of view. Adenosine triphosphate (ATP) caused a dose-dependent vasoconstriction. The concentration required to elicit the threshold response was 5×10^{-6} M, and the maximal effect was seen with 2×10^{-4}M ATP. It was about 62% of the maximal constriction caused by 5×10^{-6} M of 5-hydroxytryptamine. Penoxybenzamine (5×10^{-7} M), propranolol (10^{-7}M), atropine (10^{-7} M), cyproheptadine (5×10^{-7} M), and tetrodotoxin (5×10^{-7} M) did not significantly influence the median effective doses (ED_{50}) of ATP and the maximal responses to 2×10^{-4}M ATP. Verapamil (10^{-6}, 10^{-5} M) suppressed the contractile response of the arterial preparation to ATP. ATP produced no vasoconstriction 60 minutes after replacing a physiological saline in the organ bath with a calcium-free solution containing 1mM EGTA. Addition of 2×10^{-4} M ATP to the preparation in a high-potassium solution caused no further vasoconstriction. These results suggest that ATP causes dose-dependent vasoconstrictions in the canine carotid artery due to its direct action on the smooth muscle cells and that an increase of potential-dependent Ca^{2+} influx into the effector cells may play a major role in the ATP-induced vasoconstriction.

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INTRACELLULAR Ca KINETICS BASED ON 'TRUE' INTRACELLULAR Ca TRANSIENTS RECONSTRUCTED FROM MEASURED Ca TRANSIENTS WITH AEQUORIN. KONISHI, M., KURIHARA, S. AND SAKAI, T. Dept. of Physiol., Jikei Univ. Sch. of Med. Tokyo 105

Single muscle fiber of R. temporaria or rat ventricular preparation was injected with aequorin and intracellular Ca transients and tension were simultaneously recorded. We reconstructed 'true' intracellular Ca transients from the measured light signals and calculated the kinetics of Ca bound to intracellular Ca binding proteins. Time to peak 'true' Ca transients in both skeletal and cardiac cells was approximately 8msec shorter than that of aequorin light signals. In rat cardiac preparation, 0.1mM caffeine potentiated the peak of 'true' Ca transient, but suppressed peak tension. These effects of caffeine were well explained by the decrease of the association rate constant of troponin.

These results show that 'true' intracellular Ca transients in both skeletal and cardiac muscle cells have considerably faster time course than aequorin light signals. Compared with the results of methallochromic indicators, our 'true' Ca transient is 65-70% shorter in its time to peak and 2.5 times higher in its peak amplitude. These discrepancies may be due to the difference in experimental condition, such as sarcomere length, and intracellular concentration gradient of aequorin and Ca.

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EFFECTS OF Mg^{2+} AND MgATP ON THE GLYCERINATED CAT PAPILLARY MUSCLE ACTIVATED WITH Ca^{2+} (OR Ba^{2+}). SATO, C., SAEKI, Y. AND YANAGISAWA, K. Dept. of Physiol., Tsurumi Univ. Sch. Dent. Med., Yokohama

At a muscle length Lo(just taut), Ca^{2+}(or Ba^{2+})-activated isometric tension and the transient tension responses to step length stretches (less than 1% of Lo within 2 msec) in glycerinated cat right ventricular papillary muscles (1.5 - 2.5 mm long, 95 - 170 μm in diameter) were measured in solutions containing 50μM, 1mM and 10mM-MgATP and 0.3mM, 3mM and 10mM-Mg^{2+} but at constant ionic strength(0.2M), pH(6.8) and temperature(20°C). Increasing the Mg^{2+} shifted the pCa-tension relation in the direction of increasing Ca^{2+} required for activation, independently of MgATP. The maximum tension was inversely related to the MgATP but increased with increasing Mg^{2+}. These features were also observed in the Ba^{2+}-activated muscles. The transient tension responses were characterized by at least three distinct phases; an immediate tension rise coincident with the stretch(first phase), an exponential tension decrease(second phase) and a delayed tension rise(third phase). The time constant of the second phase(τ) increased with increasing Mg^{2+}. At 50μM MgATP, the value of τ was 47 msec at 0.3mM Mg^{2+} and 56 msec at 10mM Mg^{2+} in the Ca^{2+}-activated muscle, and 44 msec at 0.3mM Mg^{2+} and 56 msec at 10mM Mg^{2+} in the Ba^{2+}-activated muscle. Increasing MgATP to 10mM decreased τ by a factor of about 2.

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ESTIMATION OF INTRACELLULAR CALCIUM TRANSIENTS BY THE CONTRACTION MODEL FOR THE CARDIAC MUSCLE. MASHIMA, H. AND KABASAWA, K. Dept. of Physiol., Sch. of Med., Juntendo Univ., Tokyo 113

A mathematical model for the cardiac muscle contraction was developed. The model consists of three subsystems: the first is the activation of cross-bridges by calcium, the second is the kinetics of the cross-bridge cycle and the third is the dynamics of contraction. Inputs of the model are the rate constants of calcium release and uptake and outputs are the tension or shortening curves of twitch contractions. If the output curve is fit to the experimental twitch curve by adjusting the input parameters, the calcium concentration curve during the twitch can be calculated using these parameters. As the muscle length was increased, the amount of calcium release was increased and the rate of calcium uptake was decreased. As a result, the calcium concentration curves as well as the tension curves were increased both in amplitude and duration. In the isotonic twitches, when the load was decreased, the shortening curve was increased in amplitude and at the same time the calcium concentration curve was prolonged. These results are similar to the calcium transient curves obtained by aequorin injection in the cardiac muscle [Housmans et al., Science 221, 159-161 (1983)].

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INITIATION AND PROPAGATION OF CONTRACTION IN ISOLATED RAT CARDIAC MUSCLE CELLS.
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To examine whether the Ca-induced Ca release mechanism works in intact cardiac cells, we studied propagation of contraction in isolated muscle cells from the ventricle of rat heart by partly sucking them into closely fitting pipette to divide the cells into two halves. When both halves were immersed in the standard solution with 1.8 mM-Ca, contractions initiated at one half rapidly propagated to another. If Ca ions were removed from the exterior of the pipette, contractions initiated at one half inside the pipette did not propagate to another, and vice versa, indicating the inhibition of excitation-contraction coupling in Ca-free medium. On the other hand, local application of caffeine initiated a contraction wave, which propagated over the entire cell even when both halves were immersed in Ca-free medium. The propagation velocity (about 200 μm/sec) was too rapid to be explained by the diffusion of caffeine in the interior of the cell, indicating the involvement of the Ca-induced Ca release mechanism. The extent of contraction at each elementary segment was the same in both electrically and caffeine-induced contractions. These results indicate that the Ca-induced Ca release mechanism may not work in the physiological contraction associated with the action potential.

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EFFECTS OF IONIC STRENGTH ON TENSION DEVELOPMENT IN CHEMICALLY SKINNED FIBERS OF FROG ATRIUM.
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The influence of varying ionic strength on the contraction of chemically skinned frog atrial muscle fibers was studied. The calcium-activated isometric tension falls and the rate of tension development slows as the ionic strength of the bathing solution is elevated. The size of caffeine contracture, however, was larger in the fiber preloaded with calcium at a higher ionic strength. There was a decrease in the maintained tension at a pCa 6 in a high ionic strength solution and an increase at a low ionic strength. Lowering the ionic strength caused a transient tension increase. The response was also observed in a relaxing solution. In the caffeine treated fiber or in the fiber of rigor condition, only the transient responses were blocked. The response was not much influenced by species of salts used to change the ionic strength. When osmolarity of the medium was altered by sucrose, the transient responses disappeared. The results could qualitatively explain the isometric tension change of an intact fiber with the change in tonicity.

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THE PAUSE IN THE SHORTENING OF ISOLATED SINGLE FROG VENTRICULAR CELLS.
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The microscopic image of the single cells (5 - 10 μm in diameter) isolated from bullfrog ventricles was video-taped at 200 frames/sec during shortening in response to electrical stimulation to examine the mode of sarcomere shortening at myofibrillar level. The video-taped record displayed on the TV screen was analyzed by measuring the change of the spacing between natural markers in the cell, which covered about 10 striations. In 13 out of 14 preparations, the shortening curve of the cell segment exhibited a clear pause following the initial shortening over a distance of about 10 nm/half sarcomere. Several preparations showed the second pause following the initial one. The pauses were shorter in cells with large average shortening velocities than those with smaller velocities. Discussion was made on the possible causes of the pause such as (1) friction between the cell and the bottom of the chamber, (2) internal resistance against shortening due to double overlap of the thin filaments, (3) fluctuation of data points, (4) stepwise increase of intracellular free Ca ion concentration and (5) actomyosin (cross-bridge) system.

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DEPRESSIVE EFFECT OF TERBIUM(Tb^{3+}) ON CARDIAC MUSCLE CONTRACTION AND THE INFLUENCE OF CATHECHOLAMINE. TANAKA, S., NISHIYE, H. AND MASHIMA, H. Dept. of Physiol., Sch. of Medicine, Juntendo Univ., Hongo 2-1-1, Bunkyo-ku, Tokyo

Tb^{3+} has the depressive effect on the muscle contractions(Nishiye and Mashima,1983). The twitch tension was depressed depending on the concentration of Tb^{3+}, in the copetitive manner. The duration of action potential was shortened. After withdrawal of Tb^{3+}, the developed tension usually was enhanced to a level larger than that of the control and then returned to the control level. It was noticed that the contraction time was shortened during this enhanced period. The depressive effect of Tb^{3+} was profoundly reduced in the presence of adrenaline and the recovery from depression after withdrawal of Tb^{3+} became more rapid. In the case of much slower recovery after the application of higher Tb concentration, adrenaline promptly quickened the recovery from the depression. The effect of Tb^{3+} on tetanus was not so influenced in the presence of adrenaline. The depressive effect of Tb^{3+} and the influences of adrenaline were similar even in the coexistence of atropine. W-7 and trifluoperadine(TFP) which are calmodulin antagonists, showed slower depressive effect on the contraction. But the degree of depression was not so larger as that by Tb. Under the depressive state by W-7, the depressive effect by Tb became less prominent. And after depression by Tb, W-7 became almost not effective.

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CALCIUM DEPENDENCE OF ELECTRICAL COUPLING IN EARLY EMBRYONIC HEART MONITORED BY MULTIPLE-SITE OPTICAL RECORDING. YADA, T., HIROTA, A., SAKAI, T., KOMURO, H. and KAMINO, K. Dept. of Physiology, Tokyo Medical and Dental University School of Medicine, Bunkyo-ku, Tokyo 113.

The effects of Ca^{2+} on the electrical propagation on early embryonic precontractile chick hearts were optically studied using a voltage-sensitive merocyanine-rhodanine dye. Spontaneous optical signals, corresponding to action potentials, were recorded simultaneously from 25 separate regions of the 8-9 somite embryonic primitive heart using a 25 element square photodiode array. The electrical coupling was assessed from short delay among firing times of the signals obtained in the different regions. The electrical coupling in the heart was suppressed by either the external low Ca^{2+} or the external high Ca^{2+}. Similar effects were produced by a Ca^{2+}-ionophore (A23187). We also have found that the electrical coupling in the primordial fusion line at the midline of the heart was strengthened by elevation of the external Ca^{2+} and weakened by lowering the external Ca^{2+}. Thus, it is concluded that electrical coupling in the embryonic precontractile heart is regulated by the level of the intracellular Ca^{2+}-concentration, and that the electrical coupling in the primordial fusion line probably depends on the external Ca^{2+}.

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OPTICAL DEMONSTRATIONS OF ELECTRICAL ACTIVITY IN EARLY EMBRYONIC CHICK DOUBLE-HEART USING A VOLTAGE-SENSITIVE DYE. KOMURO, H., YADA, T., HIROTA, A., SAKAI, T. and KAMINO, K. Dept. of Physiology, Tokyo Medical and Dental University School of Medicine, Bunkyo-ku, Tokyo 113.

Double-hearted embryos were produced by organ culture of chick embryos which were microsurgically cut through the tissue of the anterior intestinal portal at the 1-6 somite developmental stages. The cultured embryos were removed from an incubator at the 7-16 somite developmental stages, and then spontaneous electrical action potentials and/or contractions were optically recorded simultaneously from both the right and left half-hearts. 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 observed usually 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 incidence of occurrence of the regular activity was significantly higher than that of the irregular rhythm. Furthermore, it was found that 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. We also have measured electrical activity in a quadruplicate-prebeating-heart.

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OPTICAL INDICATIONS OF EXCITATION-CONTRACTION COUPLING IN EARLY EMBRYONIC HEART.
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Excitation-contraction coupling in embryonic initial beating chick heart at 9-10 somite developmental stages was studied by means of optical method together with a voltage-sensitive merocyanine-rhodanine dye. The optical signals were recorded simultaneously from many areas of the heart using a square photodiode matrix array. In the embryonic primitive hearts at time of around initiation of heartbeat, spontaneous optical signals consisting of two components were often detected. The first component is dye-related absorption change due to action potential, and the second component is light-intensity change accompanying contraction coupled with the excitation. Measureing the signals in the various low concentrations of Ca^{2+} in bathing solution, a characteristic curve was obtained between the size of the 1st signal and that of the 2nd signal, corresponding to the excitation-contraction relation curve. Furthermore, it was found that the magnitude of the contraction was correlated with the rate of Ca^{2+}-influx accompanied with action potential. Hence, it is concluded that in the early embryonic initial beating heart, contractile system is directly activated by Ca^{2+}-influx across the sarcolemma accompanying the action potential, and suggested that Na-Ca exchange mechanism perticipates in the relaxation phase of the heartbeat.

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MECHANISMS OF SPONTANEOUS TACHYCARDIA INDUCED BY SINGLE PREMATURE STIMULUS IN THE VENTRICULAR SHEET OF THE DOG.
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Sliced preparations within 1 mm in thickness were obtained from subepicardial muscle of the canine ventricle as a two dimensional model. In these sheet preparations, spontaneous tachycardia was frequently induced by single premature stimulus. From action potentials recorded from more than 40 points in a sheet preparation, the courses of propagation in both the depolarizing wave and the repolarizing wave were mapped. When both the waves spread on the similar radial course, spontaneous tachycardia were never induced. On the other hand, when the courses of spread of both waves were extremely different, tachycardia were easily brought about. Moreover, it was ascertained that 10^{-5} g/ml lidocaine suppressed these tachycardia and that mechanism of such suppression was to make uniform the courses of spread of both the depolarizing and the repolarizing wave. A course of porpagation of premature impulse, block line and the portion of re-entrance were decided by the calculated propagation of premature impulses.

From the results obtained, it was concluded that the spontaneous tachycardia on the two dimensional model of ventricular excitation was induced by the re-entrant mechanism even in the preparation without any obstacle and such re-entrance was due to unhomogeneity of repolarizing process in each cell.

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IONIC CHANNELS AND INTRACELLULAR ATP IN CARDIAC MUSCLE.
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The patch clamp technique was applied to enzymatically isolated single ventricular cells to demonstrate a class of K channels, activated by a decrease in the intracellular ATP level. During exposure to isotonic KCl solution containing 1 mM EGTA, the cell was partially skinned by local application of saponin while the single channel recording was continued from another region of the cell surface (open-cell attached patch). Under these conditions, the activity of the K channel decreased with increasing ATP concentration in the bulk solution. In the whole cell current recordings, an outward current, most probably attributable to this ATP-sensitive K channel, was activated reversively when intact cells was dialysed with an internal solution containing no ATP, while other time dependent currents were depressed.

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ELECTRICAL CHARACTERISTICS OF THE ISOLATED SINGLE PACE-MAKING NODAL CELLS. NAKAYAMA T., KURACHI Y., NOMA A. AND IRISAWA H. National Institute for Physiological Sciences, Okazaki 444

The electrical activities of the single pace-making cells isolated from rabbit S-A and A-V nodes using collagenase were studied. The frequency of the rhythm was 150-260 /min. and was round in shape. Amplitude, duration and max. rate of rise of the depolarization were similar to those obtained from multicellular preparations. The membrane capacitance which was normalized to the apparent size of the cell surface (plane oblate spheroid shape) was 1.30 ± 0.24 $\mu F/cm^2$ (n=18) and the membrane resistance was 14.9 ± 4.0 Kohm.cm^2 (n=12). The inactivation time course of the slow inward current (i_{si}) was fitted with a sum of two exoponential components (6.7 ± 0.6 msec, 46.6 ± 15.3 msec, n=4) and the deactivation of the outward K current (i_K) was also fitted with two components. The amplitude of fully activated i_K crossed over the zero current level at -65~-75 mV and showed an inward-going rectification. The peak amplitude of the i_{si} at 0~10 mV was 18.2 ± 8.4 $\mu A/cm^2$ (n=11) and the amplitude of the fully activated i_K at 0 mV was 4.1 $\mu A/cm^2$ (n=4).

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RELAXATION OF MUSCARINIC ACh-SENSITIVE K^+ CURRENT IN SINGLE NODAL CELL OF RABBIT HEART. KURACHI, Y. AND IRISAWA, H. National Institute for Physiological Sciences, Okazaki, Aichi 444

Characteristics of m-ACh sentitive current were examind in single nodal heart cell. Application of ACh in the bath evoked marked outward current at holding potential of -50 mV. The current showed slight desensitization and reached a steady state within a minute. The ACh-sensitive current showed a clear relaxation and reversed E_K. The relaxation of the current could be explained by differneces in steady state open probability and in the rates of transitions between closed and open states of the channel at differnet potentials. The magnitude of ACh-sensitive current became bigger and the time constant of the relaxation became smaller with higher concentration of ACh (10^{-7}M to 10^{-5}M). These characteristics of ACh-sensitive current were confirmed by averaging the current of ACh-sensitive channel using patch clamp technique. Our results indicate that the relaxation phenomenon of ACh-sensitive current is an inherent nature of a single species of channel modulated by ACh.

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OSCILLATORY POTENTIALS IN OUABAIN-TREATED SINO-ATRIAL NODE CELLS. MIYAMAE, S., GOTO, K., SUDO, S. AND NAKANO, S. Dept. Kanazawa Med. Univ., Uchinada, Ishikawa 920-02

When isolated rabbit sino-atrial node cells were perfused in the presence of 10^{-5}M ouabain which is an inhibitor of electrogenic sodium pump activity, electrical activities ceased rapidly. Further removal of extracellular Ca produced an increasing oscillatory activity within 154 ± 64 sec and transiently led to a small hyperpolarization before the generation of the oscillations. The oscillations increased from spontaneous voltage fluctuations of less than 0.5 mV to a peak amplitude of 43 ± 6.4 mV. Resting potential before the generation of the oscillations was -33 ± 8.2 mV. Replacement of NaCl by Na-methane sulfonate did not affect the generation of the oscillations.

The oscillations developed in a 162.1 mM Na solution containing 2.5 mM Ca after 5 min exposure to a solution containing 25.2 mM Na but did not develop in a low Na solution containing 2.5 mM Ca or free Ca, while 4 mM Mn or 10^{-5}M-acetylcholine suppressed the oscillations. The present results suggest that an increase in I_{si} and a decrease in G_k, induced by a reduction in increased intracellular Ca, must be considered in the genesis of the oscillation. On the other hand, the small hyperpolarization seems to arise from an increase in K-efflux.

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AN ANALYSIS OF THE EFFECTS OF ETHANOL ON THE BULLFROG MYOCARDIUM.
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The effects of ethanol (0.1-10.0%) on the bullfrog atrial muscle were studied, using double sucrose-gap techniques. Ethanol inhibited the contractile tension and the action potential (AP), dose-dependently. The amplitude and duration of AP and the plateau height were all depressed with a slight depolarization of the membrane. The percent grade of inhibition, however, appeared stronger in an order of tension > plateau height > AP-duration > AP-amplitude. Under the voltage clamped conditions, all the membrane currents diminished in the presence of ethanol but differed in grade, depending on the current components. The inhibitions were stronger in an order of $I_{si} > I_{Naf} > I_x > I_{k_1}$. Thus, it was concluded that ethanol, affecting first the I_{si} and then the I_{Naf}, produces a depression of the mechanical and pacemaker activity, an increase of the A-V delay, and a decrease in conduction velocity.

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CHARACTERISTICS OF ACONITINE-INDUCED DELAYED AFTERDEPOLARIZATION IN THE FROG ATRIUM AND GUINEA-PIG PAPILLARY MUSCLE.
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Aconitine is known to cause repetitive activity based on the early and delayed afterdepolarization (DAD). The action of aconitine has been suggested to increase the Na^+-permeability. In order to examine whether aconitine-induced DAD is related with the intracellular Na^+ load, effects of aconitine on the electrical activity of frog atrium and guinea-pig papillary muscle in Ca^{2+}-free solution were studied. In the Ca^{2+}-free solution, aconitine induced DAD and triggered activity (TA) in frog atrium. These activities were elicited in the presence of verapamil, Co^{2+} or Mn^{2+}. The application of $LaCl_3$ inhibited the generation of DAD. Low external Na^+ (50%), high K^+ (6 mM) or the application of TTX suppressed the amplitude of DAD. DAD was observed at the voltage range between -40 and -80 mV, with its peak at about -65 mV. Under the influence of aconitine, depolarizing clamp pulses were followed by a transient inward current (TI) in guinea-pig papillary muscle exposed to the Ca^{2+}-free condition. The TI amplitude showed sigmoid dependence on the duration of the preceding depolarizing pulse. Stronger depolarizations increased the TI amplitude. These results suggest that aconitine induces DAD and TI through an intracellular Na load.

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INHIBITION OF OUABAIN-INDUCED INCREASE IN Na CONTENT OF CULTURED MYOCARDIAL CELLS BY QUINIDINE AND PROCAINAMIDE.
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Addition of ouabain caused gradual increase of both the Na content of cultured myocardial cells and the rate of Ca^{++} uptake by the cells. Ouabain-induced irregular beating of the cells appeared to develop when the Na content and the rate of Ca^{++} uptake exceeded about 1.5 and 2.0 times, respectively, the normal levels. Quinidine and procainamide prevented ouabain-induced increases of the Na content and the rate of Ca^{++} uptake as well as ouabain-induced toxicity. The problem of how quinidine and procainamide counteract the effects of ouabain was then studied. Quinidine and procain amide did not affect the Na^+-Ca^{++} exchange activity, Na^+-pumping out activity or ouabain-binding activity of myocardial cells, but inhibited passive Na^+ influx, which is achieved by a simple diffusion system. From these observations, it is suggested that inhibition by quinidine or procainamide of passive Na^+ influx indirectly prevents ouabain-induced increase in the intracellular Na content of myocardial cells and that this presumably explains at least in part the inhibitory effect of quinidine and procain amide on ouabain-induced irregular beating. (K. Goshima and S. Wakabayashi: 1983, J. Pharmacol. Exp. Ther. 224(1), 239 - 246)

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Na^+-DEPENDENCE OF THE TRANSIENT INWARD CURRENT IN VENTRICULAR MUSCLES.
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The transient inward current (TI) is suggested to be caused by the process of Ca^{2+}-release from the stores under the Ca^{2+}-overload and it is carried mainly by Na^+ ions. So, the effects of changes in $[Na^+]_o$ on the delayed afterdepolarization (DAD) and TI were examined with guinea-pig ventricular muscle fibers. The amplitudes of the DAD and TI first decreased followed by its restoration when $[Na^+]_o$ was increased from 150 mM to 200 mM. On the reduction of $[Na^+]_o$ by a step of 25 or 50 mM, the DAD and TI increased their amplitudes transiently followed by a later decrease. The coupling interval of the DAD and the peak timing of the TI became delayed at the lower $[Na^+]_o$. The TI transiently suppressed upon the return of $[Na^+]_o$ from 50 mM to 150 mM followed by the recovery. The voltage dependence of the TI activation also shifted to the negative direction at low $[Na^+]_o$. The reversal potential of the TI was +6 mV at 150 mM-$[Na^+]_o$ and it decreased with a slope about 52 mV with a decade reduction of $[Na^+]_o$. These results suggest that the TI is mainly carried by Na^+ ions, and its amplitude and the time course are governed by the operation of the Na^+-Ca^{2+} exchange mechanism.

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INWARD RECTIFIER SINGLE K^+ CHANNEL CURRENT AND ITS KINETICS IN THE GUINEA PIG MYOCARDIAL CELL.
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I recorded the single K^+ channel current from the guinea pig ventricular cell in Tyrode solution, using the micropippete filled with 140 mM KCl and 10 mM HEPES. It had a constant slope conductance of 50.7 pS between RP(resting potential)-100 mV and RP+70 mV. Its open probability was augmented with depolarization, while no detectable signal was recorded at the potentials E_K(equilibrium potential of K^+).

A voltage jump did not alter the open and closed state of the channel, so that its state before the jump could be known from whether it was open or close after the jump. When the membrane potential was jumped from the potential E_K to RP, the signal showed most channels open at the previous potential before the jump. Furthermore, the open time histogram made from the first open signals immediately after the jump from the potentials RP+120 mV to RP showed the open to close transition was not the first order reaction different from that in the steady state. These results suggest that the gate of the inward rectifier K^+ channel, that makes it open or close at the potentials E_K, was almost open at the potentials E_K and that there should be another rectifying gate whose open-close transition is carried out around E_K.

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EFFECT OF LYSOPHOSPHATIDYLCHOLINE ON INWARD RECTIFIER K CHANNEL IN GUINEA PIG VENTRICULAR CELLS.
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We studied the effect of lysophosphatidylcholine (LPC), a potent arrhythmogenic metabolite of phospholipid, on the electrical properties of enzymatically isolated guinea pig ventricular cells. LPC (10 - 20 μM) added to the bulk solution decreased the resting membrane potentials and induced spontaneous repetitive action potentials in these cells. To elucidate the mechanism of the depolarization, the effect of LPC on the single channel current of the inward rectifier K channel was examined using patch clamp technique. LPC (100 μM) contained in the pipette with 150 mM K decreased the magnitude of the single channel conductance measured in cell attached mode from 45 ± 7 pS (mean ±SD, n=6) to 14 ± 7 pS (n=9), while no consistent changes in the probability of the channel to open were found. The mean current values obtained by averaging the single channel current in the range of membrane potentials (-80 to 0 mV from resting potential) were significantly lower in the presence of LPC. As the inward rectifier K channel is supposed to play an important role in maintenance of resting potentials near K equilibrium potential, the LPC-induced reduction of single channel conductance may have a pivotal role in alleged, LPC-related depolarization in multicellular ventricular tissue.

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EFFECTS OF Ba ON K-RELATED CURRENTS IN THE BULLFROG ATRIAL MUSCLE.
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The effects of Ba ions on K-related currents in the frog atrtial muscle were studied using voltage clamp with double sucrose-gap techniques. Ba, in doses over 0.01 mM, inhibited the background current (I_{k1}), and 1.0 mM Ba abolished the anomalous rectification of the membrane. Thus, when the K-depletion for hyperpolarization was diminished, a funny inward current (I_f) was evident in the atrial muscle. This current increased with increasing $[K]_o$, and in 5 mM $[K]_o$, a threshold was seen at -80 mV and a saturation occurred at over -140 mV. The delayed outward current (I_x) for depolarizations was also depressed by Ba. Low concentrations of Ba (~0.1 mM) inhibited the slow component of the current (I_{xa}) without affecting the next rapid component (I_{xs}). The remaining I_{xs} showed a reversal potential of -85 mV, thereby suggesting that this current is largely K one. In the presence of higher concentrations of Ba, a depression of I_{xs} occurred in a voltage- and time-dependent manner, and an unblocking-like phenomenon for larger and longer depolarizations became apparent.

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THE EFFECTS OF Ba^{2+} ON THE K^+ CURRENTS OF MAMMALIAN VENTRICULAR MUSCLE
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The effects of extracellular application of Ba^{2+} on the K^+ currents of guinea-pig ventricular muscle fibers were studied using the single sucrose-gap voltage clamp technic. During the depolarizing pulses beyond -40 mV, the time course of current changes showed that Ba^{2+} decreased both time-dependent and time-independent current fraction, suggesting the suppression of both I_{k1} and I_k at these voltage levels. However in the presence of 20 mM Cs^+ + Ba^{2+}, an increase in time-dependent fraction was observed, despite a supression of I_k by Cs^+ alone. On clamping back to -40 mV, Ba^{2+} increased the total amplitude of tail currents with their time course having double exponentials, which indicated the existence of another time-dependent fraction besides the deactivation of I_k. On hyperpolarization below -80 mV, time-dependent decrease in inward current was observed, which was never seen before the Ba^{2+} application. These observations seemed to be explicable if I_{k1} has a time-dependent component due to the time- and voltage-dependent blocking action of Ba^{2+}

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RECONSTRUCTION OF CA CHANNEL TAIL CURRENTS FROM UNITARY CURRENTS IN CARDIAC MYOCYTES.
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Utilizing patch pipettes with large tips containing 50mM Ba, noise-like currents produced by the openings of numerous Ca channels were obtained from the isolated guinea pig ventricular myocytes. In order to facilitate to analyze the kinetic nature of the Ca channel currents we utilized BAY K 8644(1μM) which prolonges the duration of unitary Ba currents by about ten times. On repolarizations to the resting potential from the step depolarizations, inward current pulses with large unitary amplitude of 2.7pA and various durations were elicited frequently. The open time histogram of the unitary currents could be fitted with a double exponential curve having time constants of 1.8msec and 10.4msec. The ratio of the integrals of the fast and slow components of openings was 12.1. Both time constants were independent of the duration and the membrane potential of the preceding depolarizations but were affected by repolarized levels. They were almost doubled at the resting potential plus 20mV. The tail current reconstructed from the integral of the double exponential open time histogram and the amplitude of the unitary current approximately coincided with that obtained by averaging the original Ca channel currents.

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NON-RANDOM OPENINGS OF SINGLE CALCIUM CHANNALS AND EFFECT OF ADRENALINE IN CARDIAC MUSCLE. OCHI, R., HINO, N., OKUYAMA, H. AND NIIMI, Y*. Dept. of Physiol. and Dept. of Anesthesiol.*, Sch. of Med., Juntendo Univ., Tokyo 113

Single Ca channel currents were recorded from the isolated guinea pig ventricular cells by the cell-attached patch clamp method with the pipettes containing 50mM Ba. When constant depolarizing pulses were applied repetitively, a single Ca channel opened in bursts only in some pulses. The ratio of the number of successes to that of failures was 0.1 to 0.3 in normal Tyrode. Adrenaline (2μM) applied to the bath increased the ratio to increase the open probability markedly with little affecting the rate constants in the reaction scheme of C-C-O model of Ca channel gating. The failures or successes to induce openings occurred often successively. This was assumed to be produced by another gate which open and close with time intervals of the order of seconds. The duration of its open state was evaluated by counting the number of successes between failures. The open state duration histograms could be approximated by single exponentials having time constants of 1.4sec at the pulse frequency of 1Hz, 1.2sec at 2Hz in normal Tyrode and 3.7sec at 2Hz in the presence of adrenaline. This time- and metabolism- dependent slow gating process should affect profoundly the amplitude of the total Ca currents in cardiac muscles.

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ION SELECTIVITY OF CALCIUM CHANNELS IN INTERNALLY DIALYSED VENTRICULAR CELLS FROM GUINEA PIG. MATSUDA, H., NOMA, A. AND IRISAWA, H. Natl. Inst. Physiol. Sci., Okazaki, 444

The ion selectivity of the Ca channels in single ventricular cells of guinea pig was studied using a 'giga-ohm seal' patch electrode for voltage clamp and internal dialysis. A time- and voltage-dependent outward current attributed to Cs was observed at potentials above +30 mV in Na-, K-free external solution with Cs-rich internal solution. Cd not only suppressed the inward Ca current but also caused some reduction in outward current. Thus the Cd-sensitive peak current reversed at around +75 mV. However, the outward Cs current was not inactivated by a conditioning prepulse to +15 mV, suggesting that it flows mainly not through Ca channels but through some voltage-dependent leakage conductance. The Ca channel current was not significantly changed when external Na was replaced with Tris in the presence of 1.8 mM Ca, while a slow inward Na current was developed in a Ca-free solution containing 0.1 mM EGTA. These findings indicate that Na do not contribute significantly to Ca channel current under physiological conditions, but flow through the Ca channel at a very low concentration of external Ca.

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A NEW MEMBRANE CURRENT IN GUINEA-PIG PURKINJE FIBERS. MITSUIYE, T., EHARA, T. AND GOTO, M. Dept. of Physiol., Faculty of Medicine, Kyushu University.

The membrane currents in the Guinea-pig Purkinje strands (100~120/150 μm) were studied using the two-microelectrode voltage clamp method. In normal Tyrode solution, spontaneous pacemaker activity was not observed, but train stimulation elicited an oscillatory afterpotential and triggered activity (TA). The plateau currents consisted of I_{Ca}, I_x and I_{to}, though I_{to} was rudimental. The currents associated with repolarization were analysed in the presence of Cd (0.2 mM). The I_x was activated at a voltage above -30 mV and was reversed between -85 mV and -95 mV, thus assuring a rather small K accumulation. When the voltage clamp was released from Vh = -35 mV, the repolarization was greatly retarded, suggesting the presence of a prolonged inward current. Similarly, when the membrane was held at -25 mV, the hyperpolarizing pulse elicited an inward jump of the semi-steady state current which slowly decayed (λ= 3~5 sec). The decay was also observed in the presence of 0.2 mM Ba. The slowly decaying inward current increased with hyperpolarization and became saturated below -65 mV. We conclude that the inward current is not due to the slow inactivation of dynamic currents (I_{Na}, I_{Ca}) nor to induction and removal of the inward rectifying potassium current (I_{k_1}). In this specimen, TA appeared to be induced by this inward current.

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ELECTROGENIC Na-Ca EXCHANGE AND RELATED MEMBRANE CURRENT IN THE BULLFROG ATRIUM. GOTO, M., UMENO, T. AND IKEDA, K. Dept. of Physiol., Faculty of Medicine, Kyushu University.

The membrane current related to the Na-Ca exchange diffusion was studied on the atrial muscle under voltage clamp. Na-deficient Tris Ringer (10~30 mM Na) produced an outward current and a contracture with a decrease in membrane conductance, and resulted in a less-steep voltage-current relationship either in the presence or absence of $[Ca]_o$. Hence, $[Ca]_o$-induced increase of K-permeability (Meech effect) or $[Ca]_o$ activated $I_{Na\ K}$ could be excluded as a cause of the I-V changes. When depolarizing pulses were applied in the presence of TTX, Co and Ba, and the effects of Na-deficiency on the outward current were examined, Na-sensitive current ($I_{Na(X)}$) was identified. It was diclosed that 1) $I_{Na(X)}$ is inward at the resting potential, 2) during depolarization, the current gradually changes from the inward to outward direction, 3) after repolarization, the reverse change occurs. Thus, the $I_{Na(X)}$, as an important component of the membrane current, modifies the time-course of the action potential and hence the contractile tension.

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THE EFFECTS OF CALCIUM-FREE EGTA SOLUTION ON THE ACTION POTENTIAL AND MEMBRANE CURRENTS IN ISOLATED GUINEA-PIG VENTRICULAR MYOCYTES. IMOTO, Y., EHARA, T. AND GOTO, M. Dept. of Physiol., Faculty of Medicine, Kyushu University, Fukuoka 812, Japan

The effects of Ca-removal on the electrical activity of enzymatically dispersed guinea-pig ventricular cells were studied, using a suction pipette method. In nominally Ca-free solution, the resting potential slightly decreased and the action potential (AP) was depressed. When the cells were exposed to Ca-free, EGTA-containing solution, the initial changes were similar to the above, but AP prolonged later, and a long-lasting (2-10s) AP developed in a few min. Under voltage clamp, the EGTA solution produced a biphasic change in the slow inward current (i_s), i.e. I_s initially showed a marked decrease and then recovered to about 40% of control. The recovered I_s exhibited a much slower inactivation compared with the control I_s, had a strong dependence on $[Na]_o$, and was abolished by verapamil. Its reversal potential was ~20 mV. When $[Na]_o$ was 0 (no I_s), a slowly decaying outward current was observed at voltages above 0 mV, and this current was also inhibited by verapamil, suggesting a possible outflow of K ions through Ca-channel under this condition. These results indicate that reduction of $[Ca]_o$ to nanomolar range induces an I_s carried mainly by Na whose inactivation is very slow. This, together with a small decrease in the background outward currents which was also observed in EGTA solution, may explain the long-lasting AP in this solution.

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EFFECTS OF INTRACELLULARY APPLIED CALCIUM-ANTAGONISTS ON THE SLOW INWARD CURRENT IN SINGLE VENTRICULAR CELLS OF THE GUINEA-PIG. IIJIMA, T., YANAGISAWA, T. AND TAIRA, N. Dept. of Pharmacol., Tohoku Univ. Sch. of Med., Sendai 980

Single cells from the guinea-pig ventricle were prepared by using a collagenase digestion procedure. Membrane potential recording and voltage clamping with a one-mictroelectrode technique were carried out using a patch-clamp microelectrode. After establishing a giga-ohm seal on the cell, the membrane patch was disrupted by suction, making the patch electrode an intracellular electrode. Calcium antagonists, nifedipine, nicardipine, D-600, diltiazem and KB-944, decreased the action potential duration and blocked the slow inward current when applied extracellulary. Decreases in the action potential duration and the slow inward current were also induced by intracellulary applied D-600, diltiazem and KB-944. However, intracellulary applied nifedipine and nicardipine in lower concentration produced increases in the action potential duration and the slow inward current. From these results it appears that nifedipine and nicardipine can increase the slow inward current when applied intracellulary.

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MECHANISM OF HYPERPOLARIZATION PRODUCED BY DIVALENT CATIONS IN GUINEA-PIG VENTRICULAR MUSCLE HIROSE, H. AND HIRAOKA, M. Dept. Cardiovasc. Dis., MRI, Tokyo Med. and Dent. Univ., Bunkyo-ku, Tokyo 113

The present experiments were performed to elucidate the mechanism of the hyperpolarization which follows the increase in divalent cations concentrations in the bathing solutions. Papillary muscles of guinea-pigs were used to record the resting potential and tension simultaneously. The membrane current was measured using the single sucrose-gap voltage clamp technic. A sudden increase in $[Ca^{2+}]_o$ or $[Sr^{2+}]_o$ from low $[Ca^{2+}]_o$ caused a transient increase in the resting tension followed by a monotonic hyperpolarization of the resting potential, whereas an increase in $[Mg^{2+}]_o$ produced only 8mV of hyperpolarization without the tension generation. The steady-state I-V curve induced outward shift negative to -40mV either in high $[Ca^{2+}]_o$ or high $[Sr^{2+}]_o$, whereas it shifted to the negative direction at all voltages in high $[Mg^{2+}]_o$. 0.5mM Ba^{2+} eliminated the outward current and the hyperpolarization in high $[Ca^{2+}]_o$. These results suggest that increased $[Ca^{2+}]_i$ indrces a change in the properties of IK_1.

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ELECTRICAL AND MECHANICAL ACTIVITIES OF THE ISOLATED VENTRICULAR PAPILLARY MUSCLE OF THE DOG DURING INTRACELLULAR ACIDOSIS INDUCED BY REMOVAL OF EXTRACELLULAR AMMONIUM ION. IMANAGA, I., KAMEI, R. AND MIYASE, T. Dept. of Physiol., School of Med., Fukuoka Univ., Fukuoka, 814-01

Removal of NH_4Cl(40mM) after transient application(10min) induced intracellular acidosis in the myocardial cell(pH_i=6.45). When the removal was done with CO_2-HCO_3^- buffered solution, intracellular acidosis was recovered to control value(7.02) in a few minutes. When it was done with Tris or HEPES(10mM) buffered solution, intracellular acidosis was continued for about 30 minutes. During intracellular acidosis, a prolongation of APD, a slight decrease in RP, a reduction of contraction and an abolition of electrical slow response through slow channel were observed. Intracellular acidosis was postponed remarkably by SITS(10^{-4}M) or DIDS(10^{-4}M), metabolic inhibitors(DNP:10^{-6}M,CCmp :10^{-7}M) even if the removal of NH_4Cl was done with CO_2-HCO_3^- buffered solution. During intracellular acidosis, mechanical response to Caffein(3-5mM) was abolished or reduced, but to Strophanthidin(10^{-7}M), not reduced remarkably, and resting tension was increased often after application of these drugs. These results suggest that intracellular acidosis inhibits an entry of Ca^{++} into the myocardial cell which may result in decrease in $[Ca^{++}]_i$, inhibits reuptake of Ca^{++} into SR system and that active Cl^--HCO_3^- exchange plays an important role in acid extrusion.

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ANTAGONISTIC ACTIONS OF L-PROPIONYL CARNITINE TO ELECTROPHYSIOLOGICAL AND MECHANICAL DERANGEMENTS INDUCED BY LONG-CHAIN FATTY ACID DERIVATIVES IN ISOLATED VENTRICULAR MUSCLE. ARITA, M., AOMINE, M., KIYOSUE, T. AND IMANISHI, S. Department of Physiology, Medical College of Oita, Oita 879-56

Long-chain fatty acid derivatives such as lysophosphatidyl choline (LPC) or palmitoyl carnitine (Pal C) are amphiphile and accumulate in ischemic myocardium to impair the membrane function. Propionyl carnitine allegedly protects respiratory function of mitochondria in canine hearts subjected to experimental hyperlipidemia. These findings prompted us to study the effects of l-propionyl carnitine (PC) on the LPC or Pal C-induced changes in action potentials (AP's) and contractions of guinea pig ventricular papillary muscles, which were perfused with low pH (6.9 - 7.0)-Tyrode's solution and stimulated at 1 Hz. LPC and PC (10^{-4} M) shortened the AP duration, decreased the maximum rate of rise ($\dot{V}_{max}$) with slight decrease in resting potential (RP), and increased the developed tension with an increased resting tension within 30 min. All these effects, but for the effect on $\dot{V}_{max}$ and RP, were reversed by the pretreatment with, or the following application of, PC (10^{-3}-10^{-2}M). Morphological changes in mitochondria and myofilaments were also nulified by simultaneous application of PC. Results suggest that PC may have antagonistic action to the functional as well as morphological derangements produced by long-chain fatty acid derivatives, the amphiphilic compounds.

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THE EFFECTS OF CAFFEINE ON THE ELECTRICAL PROPERTIES OF ISOLATED, SINGLE RAT VENTRICULAR CELL. YATANI, A., IMOTO, Y. AND GOTO, M. Dept. of Physiol., Faculty of Medicine, Kyushu University.

The effects of caffeine on ionic currents in isolated, single cells from rat ventricles were compared with the effects of adrenaline. In current-clamp experiments, caffeine (1.0 to 10.0 mM) increased the height of the plateau and the duration of the action potential. Both caffeine and adrenaline increased the amplitude and duration of the slow responses. Voltage-clamp experiments revealed that the repolarization phase of the action potential in isolated cells is determined mainly by the slow inward current (I_s), which has voltage-dependent kinetics similar to the I_s in intact cardiac tissue. No evidence of a time-dependent outward current (I_x), nor a hyperpolarization-induced inward current (I_f) was found, although a time-independent background current (I_{k1}) was observed. Caffeine increased the amplitude of I_s, but did not change the time course, voltage dependency (of steady-state inactivation and activation), or apparent reversal potential. I_{k1} is not affected by the caffeine. The results indicated that the effects of caffeine were nearly identical to those of adrenaline.

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AN ANALYSIS OF THE EFFECTS OF PROSTAGLANDINS ON THE BULLFROG ATRIAL MUSCLES. HACHISUGA, M. AND GOTO, M. Dept. of Physiol., Faculty of Medicine, Kyushu University.

Effects of prostaglandins (PGE_1, PGE_2, $PGF_{2\alpha}$) on the membrane potentials, currents and tensions of the atrial muscle were studied using double-sucrose gap technique. All these PGs(10^{-7}~10^{-5}M) increased the amplitude, duration and plateau level of the action potential without affecting the resting potential, and produced a marked positive inotropic effect. These effects were not abolished by propranolol (10^{-6}M) and the half-maximal activation of tension occurred at 10^{-7}M for PGE_1 and PGE_2, and 3×10^{-6}M for $PGF_{2\alpha}$. Under voltage clamp, PGs increased I_{k1}, I_{si} and I_x. The maximal conductance of I_{si} increased but the activation and inactivation kinetics were unaltered. As for I_x, the activation was rapid while the deactivation was slow with PGs, and a voltage-dependent enhancement of I_x was produced, although the threshold voltage remained unchanged. Post-overdrive (1 Hz, 4 min) hyperpolarization, which reflects an activation of electrogenic Na-K pump, was enhanced by PGs. Two exponential components of the hyperpolarization were both increased without altering the time constants. The K-activated hyperpolarization which was induced when K was reintroduced after K-free condition, also increased with PGs.

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EFFECTS OF HEAD-UP/DOWN TILTING AND WATER IMMERSION ON MUSCLE SYMPATHETIC ACTIVITIES IN MAN.

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The purpose of this study is to clarify how the positional change, by head-up/down tilting, and water immersion influence the anti-gravitational compensatory function of sympathetic vasomotor activities in man. The subjects were 22 adult males, ranging in age from 20 to 60. A tungsten microelectrode, with the tip diameter of approximately 1 μm and impedance of approximately 10 MΩ, was inserted into muscle nerve fascicles of the tibial or the median nerve. Pulse synchronous burst discharges were recorded and identified as postganglionic muscle sympathetic (vasomotor) activities. Changes of sympathetic burst rate (number of sympathetic bursts per minute) and burst incidence (number of sympathetic bursts per 100 heart beats) before and during the head-up/down tilting and water immersion procedure were observed. During head-up tilting up to 60°, burst rate/incidence increased in accordance with tilting gradient, while it decreased during head-down tilting down to -15°. Significant correlations were observed between the sine of tilting gradient (-15° to 60°) and burst rate/incidence. However over the tilting degree of 60° and under that of -15°, burst rate/incidence became settled and unchanged with tilting of further degrees. In the course of water immersion, burst rate/incidence was significantly reduced with the increase of immersion level up to the neck of standing subjects. The increase of the muscle sympathetic activity during head-up tilting might be induced by body fluid shift to the lower body due to gravity. Meanwhile, its decrease during head-down tilting and water immersion would depend on the body fluid shift towards the head.

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EFFECTS OF HYPOXIA AND HYPERCAPNEA ON SYMPATHETIC NERVE ACTIVITIES. KUJIME, K., MATSUKAWA, K. AND NINOMIYA, I. Dept. of Cardiac Physiol., National Cardiovascular Center Research Institute, Fujishirodai, Suita, Osaka, 565

To investigate the effects of arterial gas tension on the sympathetic nervous system, rabbits were anesthetized with pentobarbital. Arterial pressure, heart rate, renal (RNA), cardiac (CdSNA) and cervical sympathetic nerve activities (CvSNA) were recorded simultaneously. PaO2, PaCO2 and pH was measured directly and continuously, and changed by artificial respiration with mixed gas of N2, O2 and CO2. Both hypoxia and hypercapnea caused bradycardia and hypertension. When PaO2 decreased from 110 to 20 mmHg, RNA and CvSNA were increased by 147% and 27%, respectively, in contrast, CdSNA was decreased by 64%. When PaCO2 increased from 20 to 90 mmHg, RNA and CvSNA were increased by 100% and 32%, respectively, but CdSNA was decreased by 64%. After denervation of bilateral carotid sinus and vagoaortic nerves, hypoxia and hypercapnea produced bradycardia but no hypertension. Hypercapnea caused small changes in three nerve activities with similar pattern as in the control. Hypoxia increased CvSNA and RNA slightly but did not change CdSNA.

It is concluded that changes in PaO2 and PaCO2 caused a specific response in each RNA, CdSNA and CvSNA reflexly through chemoreceptor and also directly through the central nervous system.

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EFFECTS OF HYPERCAPNEA ON RENAL SYMPATHETIC NERVE ACTIVITY. SHIRAHATA,M. NISHINO,T.*, HONDA, Y.** Dept. Anesth., National Chiba Hospital, Dept. Anesth., Chiba Univ.*, Dept. Physiol., Chiba Univ.**, Chiba, Chiba 260

We investigate the responses of renal nerve activity (RNA) to hypercapnea and the contribution of peripheral chemoreceptors to that response. Twenty-one cats weighing 2.2-4.1 kg were used. They were anesthetized with α-chlorarose and artificially ventilated. With increasing P_{ETCO2}, RNA significantly increased without significant change in mean arterial pressure (MAP) at 5 min and 10 min after P_{ETCO2} reached to steady state. After the denervation of carotid sinus nerves, RNA increased at 5 min after P_{ETCO2} reached to steady state, but at 10 min RNA did not show significant change. Change in MAP was not significant. In the aortic nerves denervated cats, RMA and MAP did not change signifi cantly. In the cats whose carotid and aortic nerves were cut, the changes in RNA and MAP were not statistically significant. These results indicate that hypercapnea increases RNA, but this increase is not mainly caused by baroreflex. Peripheral chemoreceptors play an important role in increasing RNA during hypercapnea and the contribution of aortic chemoreceptors may be important than carotid chemoreceptors.

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SYNAPTIC RESPONSES OF THE PTERYGOPALATINE GANGLION CELLS OF THE RABBIT TO THE STIMULATION OF THE POSTGANGLIONIC NERVES IN VITRO. HOSHINO,T., YOSHIZAKI,K., KOYANO,H. Dept. of Physiol. Akita Univ. Sch. of Med., Akita 010.

Extracellular recording was carried out on the post nasal nerves. The stimulation of each of three post nasal nerves evoked the activity of other post nasal nerves. This functional connection was recorded among all of the post nasal nerves. In about 10 % of neurones studied intracellularly, the fast e.p.s.p.s were recorded in response to stimulation of the post nasal nerve as well as the Vidian nerve (preganglionic nerve), which were inhibited by d-TC (10^{-5}M). The afferent impulses were recorded from the post nasal nerve by the application of a cooling physiological solution to the nasal membrane. The fast e.p.s.p.s were also recorded from some pterygopalatine ganglion cells in response to the cooling stimulation of the nasal membrane. From the results above mentioned, it was confirmed that the pterygopalatine ganglion cells and the afferent thermoreceptive fibers constitute the peripheral reflex (ganglionic reflex) arc.

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FUNCTIONAL INNERVATION OF THE AUTONOMIC FIBERS IN THE INFERIOR ALVEOLAR NERVE TO THE SALIVARY GLAND MINOR. MORITA,M., SHIOZAKI,K. AND NISHIMURA,K. Dept. Physiol., Tokyo Dental College, Chiba 270

There are sympathetic and parasympathetic fibers in the inferior alveolar nerve of rabbits. And a part of the mental nerve reached the salivary gland minor (lip gland) by macroscopic observation.

Functional relation between the salivary gland and autonomic fibers in the inferior alveolar nerve were investigated in anesthetized rabbits. Responses of the salivary gland, which were caused by the electrical stimulations of the cervical sympathetic trunk or the inferior alveolar nerve, were recorded using stain-less needle electrode (tip: 5μ, extracellular recording). Frequency dependant responses were observed by the stimulation of each. Optimal frequencies were different, 10-20Hz at the cervical sympathetic trunk stimulation and 50-70Hz at the inferior alveolar nerve stimulation in sympathectomized rabbits. The latter response was abolished by the administration of atropin. The salivary gland minor is one of the effector organs of autonomic fibers in the inferior alveolar nerve.

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STUDY ON TIME-COURSE OF SECRETORY POTENTIALS AND SEVERAL IONS IN HUMAN SALIVA. INOMATA, K., TAMAGAWA, K., TAKAKUWA, M., NAKAMURA, H°. AND KURAHASHI, M°. Dept. of Physiol. and Oral Physiol°, Higashi-Nippon-Gakuen Univ., Sch. of Dent., Ishikari-Tobetsu, Hokkaido, 061-02

When tongue is stimulated with 3 % tartaric acid, salivation increases and time-course of secretory potentials are clarified. In this study, individual time-course of secretory potentials sometimes differed from each other. Sometimes the time-course of secretory potentials in one subject dose not show the same patterns in spite of similar salivation volumes(2.5-3.0 ml). The deflection in secretory potentials recorded on the suction capsule is about 10 mV, while the deflection recorded on the mucous membrane at about 10 mm distant from papilla parotidea and on the skin over parotid gland are -0.3 mV. The differece in secretory potentials due to the different positon of recording electrodes is too large. Time-course of secretory potentials showed the different patterns due to the different psychological conditions of subject (hunger, irritation etc .). We consider time-course of secretory potentials is related not to individual ion charge but to total ions charges in saliva.

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REFLEX RESPONSES OF SINGLE SUPERIOR SALIVATORY NEURONS TO STIMULATION OF THE GLOSSOPHARYNGEAL AND VAGUS NERVE IN THE CAT. ISHIZUKA, K., MURAKAMI, T., YOSHIHARA, M., UCHIYAMA, M. AND NAKAYAMA, K. Dept. of Oral Physiol., Nippon Dental Univ., Niigata, Niigata

The reflex responses of 39 single superior salivatory neurons to stimulation of the glossopharyngeal(GL), vagus(VA), chorda tympani(CT), lingual(LN) and inferior alveolar(IA) nerve were investigated in the urethan-chrolarose anesthetized cat. Twenty seven neurons(69%) polysynaptically responded with spike potentials to any of these nerve stimulation (R neuron), while remaining 12 neurons(31%) did not respond to stimulation of any of them (NR neuron). Eighty nine percent of the R neurons responded to stimualtion of LN or IA; 60% of them responded to stimulation of GL; 55% of them responded to stimulation of VA; 26% of them responded to stimulation of CT.Sixty nine percent of the R neurons received the inputs from both of GL or VA and LN or IA. Forty nine percent of R neurons received the inputs from GL,VA and LN or IA. It is concluded that many of single salivatory neurons receive the excitatory inputs from GL or VA and also receive the inputs from LN, IA or CT.

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Effect of glucose and 2DG on the efferent discharge rate of the autonomic nerves innervating visceral organs in the rat. Niijima, A., Fukuda, A. and Yamaguchi, K., Dept. of Physiol., Sch. of Med. Niigata Univ., Niigata, 951)

Efferent discharges were recorded from axons dissected from the nerve bundles of the gastric (VGE), celiac (VCE) and hepatic (VHE) branch of the vagus nerve, and also those of pancreatic (SPE) and hepatic (SHE) branch of the splanchnic nerve. Simultaneous recordings were made in VGE-VCE, VCE-SPE and VHE-SHE. An increase in discharge rate in VCE and VHE, and a decrease in VGE SPE and SHE was observed following i.v. injections of glucose in the normal and decerebrated rats.

An i.v. administration of 2DG caused an opposite response in these nerves; i.e. an increase in VGE, SPE, SHE and a decrease in VCE and VHE in normal and decerebrated rats.

The results suggest multiplicity and hierarchic control mechanism of the autonomic responses in relation to the blood glucose levels.

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ELECTROTONIC ANALYSIS OF GUINEA-PIG ENTERIC NEURONS STAINED WITH LUCIFER YELLOW. KATAYAMA, Y., HIRAI, K., MORITA, K., °MIHARA, S. & °NISHI, S. Dept. Autonom. Physiol., Med. Res. Inst., Tokyo Med. and Dent. Univ., Tokyo 101 and °Dept. Physiol., Kurume Univ. Sch. Med., Kurume 830.

The passive membrane properties of enteric neurons of the guinea-pig were studied *in vitro*. Intracellular recordings from submucous neurons were followed by intracellular injection of Lucifer Yellow CH. The submucous neurons showed a great variety of soma shape and size; long axis: 15∿40 μm and short one: 7∿20 μm. There was marked variation in dendrite number and length. The longest processes sometimes extended for 1 mm. Electrotonic potentials were induced by constant-current pulses within the linear range in the V/I curve. The "peeling" technique was used to determine the electrotonic structure of the submucous neurons by assuming a simple equivalent cable model with a sealed end. The average electrotonic length (L) was 0.7 and the average dendrite-to-soma conductance ratio (ρ) was 3.1. The mean specific membrane resistance (Rm) and capacitance (Cm) were about 3800 $\Omega \cdot cm^2$ and 2.0 $\mu F/cm^2$, respectively. Investigations with myenteric neurons are in progress to compare the electrotonic structure of submucous neurons with that of myenteric neurons.

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ACTION OF THE ENKEPHALINERGIC NEURONS IN THE GASTROINTESTINAL TRACT IN GUINEA PIGS AND RATS. NAKAYAMA, S., YAMASATO, T., MIZUTANI, M. AND ITANO, N. Dept. of Physiol. Okayama Univ. Med. Sch., Okayama 700

Enkephalins have been immunohistochemically demonstrated in the neurons of the gastrointestinal tract in various mammals. Action of enkephlinergic neurons on the gastrointestinal motility was studied in vitro in guinea pigs and in vivo in rats. (1) Spontaneous contractions of isolated guinea-pig ileum were enhanced by naloxone(5×10^{-9} - 10^{-7}g/ml). (2) The ileal contractile response generated by transmural stimulation at 0.1 Hz was increased by naloxone(3×10^{-9} - 5×10^{-8}g/ml). (3) Naloxone(10^{-7}g/ml) produced a considerable blockade of the inhibitory response elicited after transmural stimulation of ileal segment at 10 Hz for 5 min. (4) The contractile response of isolated ileal segment to mesenteric nerve stimualtion was enhanced by naloxone(3×10^{-8}g/ml) and was completely abolished by atropine. (5) The peristaltic reflex activity of isolated ileal segment elicited by distention of its lumen(3 cmH_2O) was enhanced by naloxone(3×10^{-7}g/ml). (6) In vivo experiments of the rat, the contractile response of the stomach to efferent stimulation of the cervical vagus was increased after naloxone(1 - 3 μg/kg·min, i.v.), while the response of the duodenum and jejunum was reduced. The results provide evidence for modulatory role of enkephalins released from the myenteric neurons in the cholinergic control of the gastrointestinal motility.

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EFFECT ON DRUGS ON THE CELL-TO-CELL COMMUNICATION PROPERTIES IN THE CULTURED AUERBACH'S PLEXUS CELLS. MARUYAMA, T. Dept. of Applied Physiology., Tohoku Univ. Sch. of Med., Sendai, 980 Japan.

The cultured Auerbach's plexus cells which were derived from the small intestine were studied in this experiment. The cells proliferated well in the culture system. During the culture, the Auerbach's plexus cells connected with each other, some by their processes and the other by their cell bodies. These cells are coupled by low resistance channels that permit the cell-to-cell passage of the electrical current and florescein dyes. Several antipsychotics, local anesthesia and naphtalenesulfonamide derivates were found to block the electrical communication in the cultured Auerbach's plexus cells, and to inhibit the transfer of florescent dyes for the junctional membrane channels or processes of their cells. Other anesthetic compounds, procaine and pentobarbital, did not block the cell-to-cell communication in the cultured Auerbach's plexus cells.

These results suggest that calmodulin antagonists which antagonize calcium through the interaction with intracellular calcium receptors may control the physiological roles of the cell-to-cell communication in the cultured Auerbach's plexus cells.

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CHEMOSENSITIVE NEURONAL UNITS WITHIN THE NUCLEUS OF THE SOLITARY TRACT WHICH ARE ACTIVATED BY HEPATOPORTAL AFFERENT DISCHARGES. KOBASHI, M. AND ADACHI, A. Dept. of Physiol., Okayama University Dental School, Okayama, Japan.

Neuronal units which are activated by afferent discharges from the hepatoportal region within the nucleus of the solitary tract (NTS) were identified by electrical stimulation delivered to the hepatic branch of the vagus. Antidromic response of vagal motor units within the dorsal motor nucleus of vagus were eliminated by a collision test.

The units which decreased firing rates during portal infusion of isotonic glucose solution were glucose- sensitive so that they decreased the firing rates during a topical application of glucose by means of micro-electro- osmotic techniques.

Activities of the osmo-sensitive units which were inhibited by portal infusion of hypertonic saline were suppressed by the topical application of Na^+. The result indicates that convergence of hepatoportal glucose sensitive afferent to glucose sensitive neuronal units within the NTS and of hepatoportal osmosensitive one to osmosensitive neuronal units within the NTS as well.

Finding of many inhibitory interneurons suggests that there might be unknown integrating action as well as relay of primary afferent signals from the liver in the NTS.

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THE MEMBRANE PROPERTIES AND CHEMOSENSITIVITIES OF RAT DORSAL VAGAL MOTORNEURONS.
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Intracellular recordings were obtained from the rat brainstem slices maintained in vitro. The dorsal motor nucleus of the vagus (DMV) could be easily recognized as a translucent area under a light microscope and most DMV neurons, although not all, were identified by orthodromic (the tractus solitarius) or antidromic stimulation, or by HRP injection after recordings. Mean resting potential, input resistance and time constant were - 57mV, 102MΩ and 13ms. DMV neurons had a long afterhyperpolarization (AHP, up to 500 ms) following action potential elicited by either direct or antidromic stimulation. Ca spikes could be generated by addition of TTX to the bath medium. The long AHP was supposed to be caused by Ca-activated K conductance. Bath- and/or electrophoretically-applied drugs, glutamate, ACh, glucose, vasopressin, oxytocin, noradrenalin (NA), 5-HT and GABA were tested on the DMV neurons. The former four were mainly excitatory, while GABA showed a strong inhibitory effect. Glucose-induced depolarization was accompanied with input resistance increase. NA and 5-HT had weak excitatory effects on only a small number of the neurons. The excitatory actions of the two hormones on the DMV neurons have been shown for the first time in this study and support a peptidergic link between the hypothalamus and the DMV as one of the autonomic circuits.

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INFLUENCE OF INDOMETHASIN ON THE SECRETION OF GASTRIC ACID AND PEPSIN INDUCED BY CHOLINERGIC NERVE STIMULATION IN CONSCIOUS DOGS. FUJII, K. AND MUKAI, M. Dept. of Physiol. and Surg., Hiroshima Univ., Sch. of Med., Minami-ku, Hiroshima 734

Influence of indomethacin on the neural and neuro-humoral secretion of gastric acid and pepsin induced by intravenous injection of insulin (0.5 μ/kg) was studied in conscious dogs. (1) The secretion of gastric acid and pepsin induced by insulin consisted of a neural response which was abolishable by cimetidine (5 mg/kg,i.v.) and neuro-humoral response which was not. (2) The secretion of gastric acid and pepsin induced by tetragastrin (1 μg/kg,s.c.) and histamine (2 mg/kg,i.v.) was abolished by cimetidine. (3) The neural secretion of gastric acid and pepsin induced by insulin was increased significantly ($p < 0.05$) by indomethasin (1-2 mg/kg,s.c. daily for 8 days). However, the neuro-h-moral secretion was unaffected by indomethacin.

These findings suggest that endogenous PGs liberated by stimulation of cholinergic nerves have an inhibitory effect on the mechanism of neural secretion of gastric acid and pepsin.

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CENTRAL ACTIONS OF D-GLUCOSE ANOMERS ON ENHANCED MOTILITY OF RAT STOMACH. SAKAGUCHI, T., *OKUDA, J. AND NIIJIMA, A. Dept. of Physiol., Niigata Univ. Sch. Med., Niigata 951. *Dept. of Clin. Biochem., Facul. of Pharm., Meijo Univ., Nagoya 468.

The injection of α, β, or equilibrated (α: 36%, β: 64%) D-glucose solution into the cranial side of carotid artery decreased gastric pressure caused by insulin in rats with bilateral adrenalectomy. This effect was not reproduced after vagotomy at the cervical level. The effect of β-D-glucose was more rapid and potent than that of α-D-glucose. The injection of isotonic NaCl solution, however, produced no change in pressure. Similar findings were obtained when the two anomers were infused in or near the nucleus of the vagus nerve. These results suggest that β-D-glucose in the blood may play an important role in activating a brain mechanism vagally controlling gastric motility.

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REFLEX RESPONSES IN THE EXTERNAL URETHRAL AND ANAL SPHINCTER FOLLOWING DISTENSION OF THE URINARY BLADDER AND THE RECTUM IN DOGS. YAMANE, M. AND NANBA, Y. Dept. Physiol., Kawasaki Medical School, Kurashiki, Okayama 701-01

Electromyograms of the external urethral and anal sphincter or the outflow in the urethral and anal branches of the pudendal nerve were recorded simultaneously in decerebrated or urethane-chloralose anesthetized dogs. In dogs with the intact pontine micturition and defecation reflex centers, activity of the external urethral sphinater (EUS) was only decreased during spontaneous contraction of the urinary bladder, but that of the external anal sphincter (EAS) was increased in some cases and decreased in other cases. By distension of the rectum, activity of EUS was only increased, however that of EAS was increased or decreased. During spontaneous contraction of the rectum, activity of EUS was increased or decreased, but that of EAS was decreased alone. After acute transection of the spinal cord (Th_{13}), activities of EUS and EAS were increased or decreased by distension of the urinary bladder and rectum. During spontaneous contraction of the rectum, the increase in activity of EUS occurred, but in the activity of EAS either increased or decreased responses appeared. These changes in activities of EUS and EAS were completely abolished by cutting of the bilateral pelvic nerves.

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DEFECATORY POSTURE AND THE STRAINING REFLEX CENTERS OF THE DOG. FUKAI, K. AND FUKUDA, H. Dept. of Physiology, Kawasaki Medical School, Kurashiki 701-01

During defecation, dogs take a defecatory posture and do strainings repeatedly, which accompanied a more flexion of the back and rear legs, and a more lift of the tail. The postural changes can be elicited by distention of the rectum of decerebrate dogs. So we aim in this study to define the brain part which participates these postural changes. By distention of the rectum, activities of the abdominal compressors and closers of the glottis were first increased tonically (tonic straining), then the repeating strainings appeared, with which these activities were transiently increased further. Activities of extensor muscles of the hip joint, flexors of the stifle joint and lifter of the tail increased simultaneously with the tonic and repeating strainings, however extensor of the stifle joint did not. After destruction of the pontine reflex center of the repeating strainings, the transient increases in activities of these leg and tail muscles disappeared. Their tonic increases, however, still remained after the tonic straining was stopped by transection at the lowest medulla oblongata.

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EFFECTS OF STIMULATION AND NEURONAL ACTIVITIES OF THE PONTINE REFLEX CENTER OF THE REPEATING STRAININGS FOR DEFECATION, MICTURITION AND PARTURITION IN THE DOG. FUKUDA, H. AND FUKAI, K. Dept. of Physiology, Kawasaki Medical School, Kurashiki 701-01

Strainings can be repeatedly elicited in decerebrate dogs as in conscious dogs by distention of the rectum, urinary bladder and vagina. Position of the reflex center was defined as Kölliker-Fuse nucleus by experiment using partial cuts of the brain stem. We aim in this study to elucidate the effects of stimulation and neuronal activities of the reflex center. Many neurons in the area responded with the mean latency of 137 msec (n=155) to activation of the pelvic afferent fibers. About 60% of them discharged synchronously with the repeating strainings elicited by distention of these organs and activation of the ipsiralateral pelvic afferent fibers, and about 30% responded to these stimuli with a tonic increase of the discharges. Stimulation (5-10 Hz, 20-200 μA) of a small area in the Kölliker-Fuse nucleus elicited the repeated strainings. These results show that the reflex center of the repeating strainings for defecation, micturition and parturition exists in the Kölliker-Fuse nucleus in the anterolateral ponse.

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THE INHIBITORY EFFECT OF OPIOID PEPTIDES AND MORPHINE APPLIED INTRATHECALLY AND INTRACEREBROVENTRICULARLY ON THE MICTURITION REFLEX IN THE CAT.

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Intrathecal(i.t.) and intracerebroventricular(i.c.v.) administration of enkephalins and enkephalin analogs inhibited reflex contractions of urinary bladder and firing in vesical parasympathetic postganglionic nerves. Leucine- and methione- enkephalin(L-ENK and M-ENK,mean threshold doses:m.t.d. 110μg,i.t. and 17μg,i.c.v.) elicited short-lasting inhibition(3-30min), whereas D-Ala^2-leucine- and D-Ala^2-methionine- enkephalinamide (m.t.d. 23 g i.t.) produced more prominent and prolonged inhibition(1.5-3h). DESLET, a selective δ opiate receptor agonist, blocked bladder activity when injected i.c.v. or i.t.(m.t.d. 0.08μg and 13μg), whereas morphine, which has greater affinity for μ opiate receptors, was effective by i.c.v. and intraveneous injection(m.t.d. 5μg and 300μg/kg),but not by i.t. injection(200-400μg). Thiorphan(50-200μg i.t. i.c.v.) enhanced the responces to L-ENK, whereas naloxone(10-50μg i.t. i.c.v. i.v.),blocked the responces to all opioid substances. In untreated animals thiorphan depressed and naloxone enhanced bladder reflexes. These results suggest that bladder reflexes are controlled by tonic enkephalinergic inhibitory mechanism in both the brain and spinal cord and that different opiate receptors may mediate inhibition.(e.g. δ at spinal cord, μ and δ in the brain)

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LEFT VENTRICULAR SYSTOLIC MECHANICAL MODEL BASED ON THE LARGE DEFORMATION THEORY.
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We proposed a viscoelastic model for the ejecting left ventricle under a constant ejection pressure. The left ventricle was assumed to be a thick spherical shell and its deformation was analysed based on the large deformation theory. The model is consisted of a time-varying elastic element connected to a parallel viscous element. At isovolumic phase, the pressure(P) - volume(V) relation can be written in the form: $P_I(t) = a(t)[\ V - V_0(t)\ ]$, according to our previous study (Abe, 1981). At ejection phase: $P_E = 1/g \cdot [\ dV(t)/dt\ ] + a(t)[\ V(t) - V_0(t)\ ]$, where a is the stiffness depending on the contraction of ventricular muscles, $V_0(t)$ the volume axis intercept of the regression line of the P-V relation, 1/g the viscosity coefficient and V(t) the instantaneous volume. The equation can be transformed to, $[\ \Delta V(t)/dt\ ] + a(t) \cdot g \cdot \Delta V(t) = b \cdot g \cdot [\ P_I(t) - P_E\ ]$, where $\Delta V(t)$ is the instantaneous ejected volume, b constant depending on the ejection pressure. The calculated value of g showed no dependency on preload, afterload and time course of ventricular contraction. Our results indicate that the viscoelastic model could account for the mechanical behavior of the left ventricle.

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THE DEPENDENCY OF THE END-SYSTOLIC PRESSURE-VOLUME RELATIONSHIPS IN THE ISOLATED BLOOD PERFUSED CANINE LEFT VENTRICLES ON THE CORONARY PERFUSION FLOW RATE.
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To elucidate the influence of increasing the coronary blood flow rate on the slope of the regression lines for the end-systolic pressure-volume relationships(S-ESPVR) in the left ventricles, we made the 9 isolated blood-perfused canine left ventricle equipped with the balloon connected to a volume servo pump, with which the volume of the balloon were able to be controlled, therefore left ventricular cavity volume were controlled and we determined S-ESPVR with this system. The coronary blood flow rate were altered by changing the pumping rate of the pump placed in the coronary perfusion circuit. There was a positive correlation between the altering ratio of the coronary blood flow and changing ratio of S-ESPVR($Y=0.51x+0.56, r=0.87$). These results indicate that S-ESPVR depends on the coronary blood flow rate in the range above normal coronary perfusion rate.

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RELATION BETWEEN OUTPUT RESISTANCE AND STROKE VOLUME IN FROG ATRIUM PREPARATION.
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The relation between output resistance and stroke volume was investigated in atrium preparations of the bullfrog. The preparations were electrically driven once every 5 sec. The output resistance was altered from minimum (Rmin, 0.5 $cmH_2O \cdot sec/cm3$) to maximum (Rmax=Ca. 17 times Rmin) by a change in the cross-sectional area of the cannula tip. Venous pressure was changed within the range of 0.5 and 7 cmH_2O. When the output resistance was increased, both the stroke volume and the maximum contraction velocity were monotonously decreased, but the duration of the contraction curve was monotonously increased. The decrease in stroke volume was never accompanied by a change in end-diastolic volume. The stroke volume (percentage of that at Rmin) at the maximal output resistance (Rmax) increased as venous pressure increased, and this relation was never modified by isoproterenol application. The action potential duration didn't seem to be related with this phenomenon. The intersection of two curves of normal and isoproterenol Ringer was observed in stroke volume and venous pressure relation.

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EFFECTS OF THYROXINE ON EVERTED SINUS-ATRIUM PREPARATION. TAMURA,T., HAYASHI,H.,HORIUCHI,E.,and DOHI,Y.* Dept. of Physiol., *2nd Dept. of Internal Medicine, Saitama Medical School,Iruma-gun,Saitama 350-04

Chronic repetitive administration of thyroxine shortened the cardiac interval of an in situ bullfrog. In acute experiment, however, thyroxine(5×10^{-8}M) for 30 minutes prolonged cardiac interval(+20%) on everted sinus-atrium preparation. Thyroxine also prolonged sinus-atrium delay, and shortened the action potential duration of atrial muscle fiber(-10%). Epinephrine(7×10^{-8}M) did not modify this thyroxine-induced prolongation of cardiac interval, but pretreatment of Cd(10 μM) significantly inhibited this prolongation. Thyroxine restored the contractility which was previously weakened by Cd.

It is suggested that acute effects of thyroxine on the cardiac muscle might be explained by increase in intracellular calcium.

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Control of cardiac output by peripheral vascular parameters. (2) Vector presentation of vasoactive substances. SATO, T., TAKEUCHI, A., SHIRATAKA, M., AND IKEDA N. Dept. Med., Physiol., Inform. Sci., School of Med., Kitasato Univ., Sagamihara, Kanagawa 228

Effect of peripheral vascular parameters on cardiac output and blood pressures was studied by computer simulation of the whole circulatory system. A capacitance, unstressed volume, arterial and venous resistances were assumed for the models of the systemic and pulmonary vascular beds. EMAX model (Suga) was used to calculate cardiac output for a given pre and afterload (pressures) and to estimate cardiac work. Zsc, myocardial ischemic index, was calculated based on the cardiac work and the model of autoregulated coronary perfusion. The model could simulate the behavior of hemodynamics in response to changes of the peripheral vascular parameters under the conditions of normal and failing heart.

Right heart bypass experiments and pulmonary vascular perfusion experiments were performed with the dog to study the effect of various vasodilating substances. As the result, unstressed volume and resistances proved to be the main parameters affected. Thus the control of cardiac output was presented on a plane of resistances and unstressed volumes with the constraints of tolerable ranges of pressures and coronary matching. A treatment vector was suggested to maximize cardiac output on this plane.

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THE CHANGE OF THE SEGMENTAL PULSE WAVE VELOCITY IN THE HUMAN AORTA
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In a series of twenty subjects(m:7,f:13),the aortic pressure wave was studied by introducing a micro-tip manometer(Miller 8Fr.pig-tail catheter)from the femoral artery to the root of the aorta. The pressure wave at the root of the aorta was recorded with the carotid pulse wave and ECG,then the catheter was withdrawn 10cm and a new recording made. This procedure was repeated until the tip was at the bifurcation of the iliac artery. The pulse wave velocity was calculated by these recordings,using the carotid pulse wave or the R wave of ECG as a time marker. The mean and SD of the segmental pulse wave velocity in the aorta was as follows,ascending aorta:1032±330 cm/sec,upper descending aorta: 604±145 cm/sec,lower descending aorta:594±164 cm/sec,upper abdominal aorta:633±230 cm/sec, lower abdominal aorta:983±262 cm/sec. The pulse wave velocity of the ascending aorta was faster than the others. The segmental pulse wave velocity decreased in the descending aorta and increased again in the abdominal aorta. These results were not explained by the changes of the blood pressure,the aortic diameter,the aortic wall thickness and the aortic wall elasticity. It was suspected that the segmental pulse wave velocity change in the aorta was mainly influenced by the wave reflection.

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Relationship of arterial flow wave form and vascular parameters. TAKEUCHI, A., SATO, T., SHIRATAKA, M., NARA, Y., AND IKEDA, N. Dept. Med., Physiol., Inform. Sci., School of Med., Kitasato Univ., Sgamihara, Kanagawa, 228

We have previously reported that after vibration of flow wave form seen in the aorta and large arteries of the dog disappeared after injection of vasodilating drugs. (The same meeting, 1983) It was postulated then with a help of a lumped constant model of the arteries that these characteristic changes of the blood flow pattern might have been caused by a change of capacitance of proximal arterial segments rather than that of arteriolar resistances. In the present study, the mechanism of the after vibration was further pursued by experimental as well as theoretical studies. In the experimental study, the blood velocity was measured at various sites of aorta and arteries by injecting different vasoactive substances and physically manipulating the arterial parameters: clamping of aorta and intra-aortic injection of beads of 100 μm diameter. The model used in the theoretical study was modified from Avolio (1980). It is a distributed model composed of 128 arterial segments with different radii. The model was shown to well simulate the wave pattern under the control condition and after the physical manipulation. The computer simulation confirmed the previous conclusion that the change of flow wave may be attributed to the change of Young's moduli of the proximal arteries.

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NONINVASIVE MEASUREMENT OF ARTERIAL PRESSURE-VOLUME RELATIONSHIP IN HUMAN BODY SEGMENTS

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Static and dynamic arterial pressure-volume relationship and viscoelastic properties were noninvasively measured in human forearms, upperarms and fingers. The beat-to-beat arterial pressure was detected by a noninvasive measurement system using the vascular unloading technique in the index finger. While, the arterial volume and its dynamic change were simultaneously detected by electrical admittance plethysmography. The arterial viscoelastic properties in these arteries were determined at various transmural pressure levels from 0 mmHg to around the mean arterial pressure by changing pneumatic cuff pressure on the segments.

The values of arterial volume elastic modulus were different among these three vascular segments; it showed the lowest in the finger. The elasic modulus in the upperarm was lower than that in the forearm.

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HEMODYNAMIC RESPONSES TO OCCLUSION OF LIMB ARTERIES.

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Five mongrel dogs weighing 8 to 14kg were used for the experiment. They were anesthetized with sodium pentbarbital(30mg/kg, iv). A thoracotomy was performed and an electric flow meter was inplanted at the asending Aorta to measure arterial blood pressure. The responses of cardiac output to 1 min occlusion of femoral(left and right) and abdominal arteries(lower and upper) were observed with infusion of noradrenalin(NADR, 5μg/kg), isoproterenol(ISP, 0.5μg/kg) and acetylcholine(Ach, 5μg/kg). The acrdiac outputs were compared during and after occlusion. The results showed that 1) the changes of cardiac output during occlusion are less than those after the release of occlusion; 2) compared to cardiac output before arterial occlusion, the cardiac output changed less than 5% by the occlusion of right femoral artery during infusion of NADR and Ach, 3) the cardiac output changed less than 10% by the occlusion of both femoral artery during infusion of NADR and Ach as well as by the occlusion of right femoral artery during infusion of ISP; 4) the blood pressure of the right brachial artery increased slightly during occlusion of femoral artery, and showed a remarkable increase during abdominal occlusion.

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INFLUENCE OF TRAINING ON LOCAL BLOOD FLOW IN RAT SKELETAL MUSCLE BY USING HYDROGEN GAS CLEARANCE METHOD. MAEDA,J., FUJITA,Y., TAKEMIYA,T. AND FUJITA,T. Div. of Physiol. Inst. of Health & Sports Sci., Univ. of TSUKUBA. Sakura, Niihari, Ibaraki 305

Wistar rats were devided into two groups at 7 weeks of age; exercise groups(Ex) and non-exercise groups(nEx). Ex were grown up in cage with rotating wheel as they were able to run voluntarily for additional 10 weeks. Measurement of muscle blood flow (ml/min/100g) was made in accordance with the hydrogen gas clearance method by Aukland. The resting blood flow in M.Gastrocnemius and M.Soleus of Ex were 16.1±3.76(16) and 13.4±3.26(15), whereas that of nEx were 14.6±1.97(14) and 10.8±1.78(14), respectively. Exercise hyperemia at 1Hz in both muscles of Ex were 20.9±4.03(9) and 15.8±2.96(6) whereas that of nEx were 16.2±2.15(11) and 12.7±2.40(11), respectively. Exercise hyperemia at 5Hz in both muscles of Ex were 34.6±5.50(10) and 26.8±2.74(10), and that of nEx were 26.1±3.63(7) and 26.8±6.86(7), respectively. Under condition of approximately equal blood pressure(mean), our data showed significant increases of resting blood flow in M.Soleus and exercise hyperemia in M.Gastrocnemius of the trained groups.

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STRUCTURE OF EXPERIMENTAL PULMONARY EDEMA MODEL. Ueda,G., Sakai,A., Takeoka,M., Koshihara,Y., Kobayashi,T.*, Kubo,K.*, Fukushima,M.*, Yoshimura,K.* and Shibamoto,T.*. Dept. of Adapt. Physiol. and Internal Med.*, Shinshu Univ. School of Med., Matsumoto

Sheep were exposed to a simulated altitude of 6,600 m. The hemodynamic parameters were measured by Staub's technique and the data were analyzed by Kedem-Katschalskys' equations. This was regarded as a high-altitude pulmonary edema model. The lymph flow was related thereby to a hydraulic filtration and an osmotic reabsorption. Among them, the first factor contributed greatly and the second one was in general small. The ratio was 18 to 24 for the control and 8 to 9 for the test period. For the protein clearance, the convection term was greater, compared with the diffusion term. In cases of the atrial pressure elevation and endotoxin injection, the diffusion term in the second equation increased relatively compared with the 6,600 m effect. From these findings, the components which contribute mainly to the lymph flow was recognized quantitatively, though the practical structure is expressed in equal weight for four terms in the phenomenological equations.

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FUNCTIONAL AND MORPHOLOGICAL ASPECTS OF THE VENOUS SYSTEM. OHHASHI, T., MURASE, K. AND *AZUMA, T. Dept. of Physiol., Shinshu Univ., Sch. of Med., Matsumoto 390, and *Juntendo Univ., Tokyo 113

In order to evaluate the function of the venous system with regard to its integrated control in managing the capacity of the circulation, we have attempted to investigate regional differences in the venous distensibilities, the structure of the veins at different sites, the pharmacology of the venous smooth muscles, and their innervation. Non-uniform distensibility characteristics in the venous system were considered on the basis of the local differences in architecture of the muscular and fibrous components in the walls. The venous valves with two cusps, which prevent backflow or collapse due to the effects of gravity or other sources of pressure, were proximally observed at the positions of the venous angle and inguinal ligament in the human, monkey, dog and rabbit. The valves were located distal to the junction of tributaries with the main vein in the extremity deep and superficial veins. The pharmacological, histochemical and electorn microscopic studies suggested that noradrenergic fibers were densely innervated in the trunkal venous walls, such as the abdominal inferior caval and common iliac vein. Thus, we discussed structure-function relationships.

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SPONTANEOUS CONTRACTIONS OF THE ISOLATED RAT ARTERIES.
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Spontaneous contractions and membrane potentials of vascular smooth muscles were recorded in the isolated large arteries(ringed preparation) of the rat.Spontaneous synchronized contractions(SC) and fine irregular contractions(FIC) were observed in the thoracic and proximal abdominal aorta.The distal abdominal aorta and some aortic branches showed FIC only.All SC were accompanied by changes in membrane potential.In some cases,FIC were also accompanied by changes in membrane potential. No action potential(spike) was observed.The frequency of SC and vascular tension decreased by low temperature or Ca^{2+}free sulution.The effect of low temperature or Ca^{2+}free solution was smaller in FIC than in SC. SC may be initiated by electrical and mechanical synchronization of many smooth muscle cells.The result suggests that both SC and FIC may play a role in the regulation of vascular tension.

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ROLE OF ENDOTHELIAL CELLS IN THE VASOCONSTRICTIVE AND VASODILATORY RESPONSE TO VASOACTIVE SUBSTANCES IN THE ISOLATED CANINE RENAL ARTERIES.
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The present study was designed to investigate wheter or not endothelial cells are involved in the vasoconstrictive and vasodilatory response in the isolated intact and everted cylindrical segments of the canine renal artery. The segments were cannulated with needles, stretched to their in-situ length and fixed within a specimen chamber. The vessel segment was perfused intraluminally at a constant pressure (80mmHg) with a normal Krebs solution. The normal solution in the organ bath was circulated at a rate of 3.2 ml/min. The same concentration of the vasoactive substances was administerd to both intra- and extraluminal perfusates. The change of the external vessel diameter was recorded continuously by means of a photoelectric device. Intimal side infusion of vasoconstrictive agents (norepinephrine, epinephrine, 5-hydroxytryptamine and prostaglandin $F_{2\alpha}$) caused a more marked decrease of the external diameter of both intact and everted segments than adventitial side infusion. The endothelial cells also played an important role in the dilatory response of both arterial segments to acetylcholine and papaverine. These results suggest that the endothelium can mediate the ACh-induced vasodilation and in part the vasoconstrictive agents-induced vasoconstriction.

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A MATHEMATICAL EXPRESSION FOR DISTENSIBILITY CHARACTERISTICS OF CONTRACTED OR RELAXED ARTERIES.
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There have been no mathematical expressions that describe a possible effect of smooth muscle cells(SMCs) on arterial distensibilty. The present study was carried out to obtain a constitutive equation of arterial wall with or without activated SMCs. Pressure(P)-diameter(D) relationships of dog renal arteries were investigated in in vitro under cyclic loading and unloading in the pressure range of 5-180 mmHg. SMCs were activated by norepinephline of 10^{-5}M. In the case of highly activated conditions, the vessel diameter change showed that the diameter has a minimum value of Dmin at a lower pressure and a maximum value of Dmax at a higher pressure. Based on the experimental results, a constitutive equation was derived as $D=Dmin+K/(1-Mexp(-P/E))$, where $K=Dmax-Dmin$, $M=(Dmax-D(0))/(D(0)-Dmin)$, and D(0) is the diameter at P=0. The constant, E, has the same dimension as the modulus of elasticity. Four unknown parameters, Dmax, Dmin, M, and E, were determined by a nonlinear regression analysis from the P-D relationship ships obtained experimentally. The calculated P-D relationships were coincided well the experimental data and the correlation coefficients were the value of above 0.99. Most significant change by the activation of SMCs occured in the magnitude of M's value which gives a good index of the degree of activation.

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A NEWLY DEVELOPED SYSTEM FOR SIMULTANEOUS MEASUREMENT OF INTERNAL DIAMETER AT DIFFERENT SITES IN SMALL ARTERIES. SADA, K., SHIRAI, M. AND NINOMIYA, I. Department of Cardiac Physiology, National Cardiovascular Center Research Institute, 5-125 Fujishiro-dai, Suita, Osaka 565, Japan

A new digital subtraction angiographic system, which consisted of (1) X-ray apparatus and X-ray sensitive 1 inch vidicon camera having very high power resolution; (2) on line image processing apparatus coupled with microcomputer; (3) monitor TV and video recording device, was developed to measure the internal diameters(ID) of small arteries at the speed of 30 frames/sec. In anesthetized cats, small pulmonaly arteries(about 500-100 μm in ID) were clearly detected by digital subtraction mode of this system. In a given arterial site, ID and peak density(PD) of contrast medium were analyzed. PD could be expressed as a function of ID,i.e., $\mathrm{Log(PD)} = a + b \cdot \mathrm{Log\ (ID)}$ with high positive cross correlation coefficient. This result indicates that ID of small pulmonary artery can be estimated by PD of the contrast medium.

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EFFECTS OF BARORECEPTOR REFLEX ON MICROCIRCULATORY BLOOD FLOW IN THE RABBIT MESENTERY. MINAMIYAMA, M. AND HANAI, S. Dept. of Vascular Physiol. National Cardiovascular Center Rseach Institute, Suita, Osaka 565

Direct studies of microcirculatory vessels and blood flow provide a detailed interpretation of circulatory control mechanism. We studied the changes of blood flow in arterioles, true capillaries and venules under the effects of baroreceptor reflex in the rabbit mesentery.

We measured aortic blood pressure, inner diameter of the vessels and center-line red blood cell (RBC) velocity in the vessels by a newly developed off-line system.

The inner diameter of arterioles and venules in which we measured RBC velocity did not change during the electrical stimulation of the depressor nerve. When the aortic blood pressure fell rapidly by the depressor nerve stimulation, the RBC velocity in microcirculatory vessels decreased lineally with aortic blood pressure. But, when the RBC velocity returned to the control level after the stimulation, the aortic blood pressure stayed at 90% of the control value. We obtained the time delay by making a comparison between the recovery of the aortic blood pressure and the recovery of the RBC velocity in mesenteric arterioles. This time delay was about 8 sec under the effective baroreceptor reflex.

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EFFECTS OF HISTAMINE ON INTERNAL DIAMETER OF SMALL PULMONARY ARTERIES IN THE CAT. SHIRAI, M., SADA, K. AND NINOMIYA, I. Department of Cardiac Physiology, National Cardiovascular Center Research Institute, 5-125 Fujishiro-dai, Suita, Osaka 565, Japan

Using soft X-ray vidicon camera coupled with image processing systm,effects of histamine on internal diameter (ID) of small pulmonary arteries (500-100 μm) were studied in 12 anesthetized cats. Arteriograms were taken before and after intravenous administration of histamine (10-15 μg/kg). The control ID's measured at 448 sites were classified into four different groups according to their sizes,i.e., A)500-400 μm,B)400-300 μm,C)300-200 μm,D)200-100 μm. The relative ID's with histamine were calculated at the same sites in the four groups. The mean relative ID in A) was not changed,but those in B) and C) decreased significantly to 91% and 97% of the control values,respectively. The magnitude of the constriction in B) was statistically larger than that in C). On the other hand,the ID in D) dilated significantly to 108% of the control value. These results indicate the effects of histamine on ID of small pulmonary arteries are varied depending on the control ID size.

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NONLINEAR ASPECT OF THE DUAL REGULATION BY LOCAL AND CENTRAL CONTROL MECHANISMS ON THE MICROCIRCULATION IN THE RABBIT SKELETAL MUSCLE. SHIBATA, M.*, YOSHIDA, M.*, KAMIYA, A.* AND FUKUOKA, M.** *Res. Inst. of Appl. Electr., Hokkaido Univ., Sapporo 060, **Dept. of Physiol., Kyorin Univ., Mitaka, Tokyo 181

Effects of the local and cetral control mechanisms by the tissue PO_2 and the autonomic nervous system on the microcirculation were investigated in the rabbit tenuissimus muscle under vital microscopic observation. After the preparation, the muscle was placed on a pair of prisms so as to locate the main supplying artery and vain at the center in the space between them. The microcirculation was observed a TV monitor through a microscopic-TV camera. Microcirculatory responses to the electric stimulation of the carotid sinus nerve were measured at the state of different PO_2 in Tyrode solution suffusing the muscle. When PO_2 in Tyrode solution was elevated, capillary red cell velocity and the perfused capillary density were decreased both at control and during stimulation but different extents. Regression lines between these changing rates and PO_2 levels revealed that the reflex effect on velocity and density at 20mmHg was some 1.3 and 4.8 times enhanced at 80mmHg respectively. These results suggested that resistance change due to the arteriolar smooth muscle contraction elicited by the sympathetic nerve discharged was significantly influenced by the ambient PO_2 level.

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ORIGIN OF THE HYPOTENSIVE RESPONSE CAUSED BY RAPID BLOOD TRANSFUSION INTO AORTA OR BRAIN ARTERY. HATAKEYAMA, I., KATAKURA, T., SAWAKI, Y. AND NISHIDA, S. Dept. of Physiol., Kitasato Univ. Sch. of Med., Sagamihara, Kanagawa 228

Previously we reported that elevation of local arterial blood pressure of rabbit by rapid blood transfusion (RBT) into aorta was opposed by transitory but considerable hypotensive response, while RBT into cerebral artery via internal carotid artery produced marked drop of the systemic arterial pressure. In the present paper, some of the studies on the origin of this hypotensive effect will be shown. These effects were suppressed or disappeared under lower level of blood pressure as a result of hemorrhage. Propranolol did not show any significant influence on the both effects, while phentolamine inhibited the hypotensive effect by RBT into cerebral artery but the hypotensive effect by RBT into aorta was kept. This fact suggests that the origins of both hypotensive effects are different. Internal carotid artery of rabbit usually has several small branches which distribute facial region and ear. It was shown that RBT into the branch which distributes to ear also produces remarkable drop of arterial pressure with transitory rise of brain arterial pressure. It is suggested that the extracranial branch of internal artery has a collateral which connects with intracranial brain artery where a blood pressure receptor exists.

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EFFECT OF AGING ON RESPONSIVENESS OF THE RAPIDLY ACTING ARTERIAL PRESSURE CONTROL SYSTEM IN CONSCIOUS RABBITS. HOSOMI, H., KATSUDA, S., TATEISHI, J., AND HAYASHIDA, Y. Dept. of Physiol., Kagawa Med. Sch., Kagawa 761-07 and Dept. of Physiol., Kobe Univ. Sch. of Med., Kobe 650.

Changes in responsiveness of the rapidly acting arterial pressure control (AP) system with aging were investigated. Two catheters, one for pressure measurement and the other for inducing hemorrhage from the aorta were chronically implanted in 25 rabbits from the same colony (aged 6 to 30 months). A few days after the operations, each animal was quickly bled (2 ml/kg body weight) while it was conscious. The hemorrhage experiment was repeated 16 times and the mean arterial pressure responses were sampled with an A/D-converter and stored in a digital computer, the 16 strings of data being pooled for each animal. The overall open-loop gain (G) of the AP-system was estimated from the individually pooled responses. In the present study, aging exerted no significant effect on the value of G (7.1) as evaluated by Kruskal-Wallis' non-parametric one criterion variance analysis ($p<0.05$). The reflex sensitivity of the AP-system over the pressure ranges used in this experiment thus appears to be unaffected by aging over the range from 6 to 30 months.

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HEART RATE AND ARTERIAL BLOOD PRESSURE AT THE BEGINNING OF EATING IN AWAKE CAT. MATSUKAWA, K. and NINOMIYA, I. Dept. of Cardiac Physiol., Nat. Cardiovascular Center Res. Inst., Suita, Osaka 565

Changes in heart rate (HR) and arterial pressure (AP) coupled with eating were studied in 12 awake cats. We determined the time relation that exists between the onset of eating and changes in HR and AP in animals which were trained to eat in an experimental condition. In each cat, the food was presented automatically for a period of 11 seconds at intervals of 100 seconds and 10 - 60 feeding trials were performed. HR, AP and head movements were recorded simultaneously. Feeding response occurred immediately by 1.5 sec after the presentation of the food. HR and mean AP increased significantly during presentation and ingestion of the food. A new finding is that the onset of HR increase preceded by 0.2 sec, while the onset of AP change followed by 1.0 sec, the beginning of eating. When ganglionic blocker (C6) or β - blocker (Propanol) were given intravenously, HR response to eating decreased. This suggests that HR change at the beginning of eating was induced directly from higher nervous center through the sympathetic nervous system.

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CONTROL OF RENAL VASCULAR RESISTANCE BY THE BAROREFLEX SYSTEM. TATEISHI, J., HAYASHIDA, Y. AND HOSOMI, H. Dept. of Physiol., Kagawa Med. Sch., Kagawa 761-07

The purpose of this study was to investigate effects of renal blood flow (RBF) on control of renal vascular resistance (RVR) by the baroreflex system. The left kidney of 22 dogs anesthetized by Nembutal was perfused by blood from the left femoral artery with a pump, while the carotid sinus and vagus nerves and the left renal nerves were intact. Left RBF and renal perfusion pressure (RPP) were monitored. Systemic arterial pressure was measured at the abdominal aorta. Blood (2 ml/kg body weight) was quickly withdrawn from the aortic arch. RVR was calculated by deviding RPP by RBF. A reflexogenic maximum increase in RVR (ΔRVR) in response to the hemorrhage was measured at different levels of RBF. ΔRVR was about 8.9 mmHg·min·g/ml at RBF of 0.5 ml/min·g and decreased with increasing RBF. The reflexogenic increase in RVR was not observed after sectioning the carotid sinus and vagus nerves or the renal nerves. Thus, the reflexogenic increase in RVR in response to the hemorrhage was attributed to neural control via the baroreflex system and decreased with increasing RBF.

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DISCHARGE PATTERN OF CARDIAC SYMPATHETIC NERVE ACTIVITY DURING ACUTE CORONARY ARTERIAL OCCLUSION IN AWAKE CATS. NINOMIYA, I., KUJIME, K., MATSUKAWA, K, TOMISAKA, J* and NISHIURA,N. Dept. of Cardiac Physiol., National Cardiovascular Ctr.Research Institute, Suita, Osaka, 565

We attempted to determine the relative role of sympathetic (CSNA) and vagal (VNA) nerve activities on heart rate (HR) at the early stage of myocardial ischemia in awake cats. CSNA was measured simultaneously with ECG, HR and art. pressure (AP) before and during acute left ant. descend. coronary arterial (LAD) occlusion in both anesthetized and awake conditions. Time course and magnitude of AP, HR and CSNA in response to LAD occlusion varied significantly among the different cats in a given condition and also between two conditions in an animal. Mean AP decreased 20 and 37 mm Hg at the time of 54 and 37 secs after occlusion in anesthetized and awake conditions, respectively. HR decreased 7 and 46 bpm at the time of 83 and 37 secs in two conditions, respectivley. In many awake cases CSNA increased initially and then decreased with LAD occlusion. Change in CSNA showed no parallel relation to HR or AP at the early stage of occlusion. This suggests that the bradycardia at the early stage of occlusion was induced initially by an increase in VNA.

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INFLUENCE OF SLEEP-INDUCED APNEA ON ARRHYTHMIAS IN RATS.

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The purpose of this study was to evaluate whether the occurrence of bradyarrhythmias observed in rats was related to apneic episodes. Five male Wistar rats, 7 to 9 weeks of age, were used for this study. Respiration was monitored by the recording of chest movements using impedance transducer. A silicone tube containing saturated $CuSO_4$ solution, bipolarly plugged with copper electrodes, was implanted beneath the skin around the chest. The light was kept on from 5:00am to 7:00pm. Food and water were available ad libitum. Ambulatory ECG, EEG and respiration monitoring was continued for 6-10 days. Sleep states were classified into alertness, slow wave sleep and paradoxical sleep (PS). Respiratory abnormalities were observed most frequently during the PS in all rats. These sleep-induced apneas were classified into three grade: Grade 1, an interruption of the ventilation record shorter than 2.5 sec; Grade 2, a hypopneic episode longer than 2.5 sec; Grade 3, an apneic episode longer than 2.5 sec. Influences of the apneic episodes on bradyarrhythmias were investigated. As a result, 88.1% of arrhythmia episodes were related to apneic episodes. In accordance with the grading, the incidence of arrhythmias increased: Grade 1, 13.8%; Grade 2, 25.6%; Grade 3, 48.8%. These results suggest that the sleep-induced apnea is one of the mechanisms of bradyarrhythmias during sleep.

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EXPERIMENTAL STUDY ABOUT THE RELATIONSHIP BETWEEN SLEEP STATES AND ARRHYTHMIAS.

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The purpose of this study was to evaluate the role of the vagus nerves on the relationship between sleep states and the arrhythmias, or on the circadian rhythm in bradyarrhythmias. Male Wistar rats, 11-33 weeks of age, were used for this experiment. Animals were divided into 3 groups: control (n=3), transection of left vagus nerve (n=5), and right vagus nerve (n=3). Vagus nerves were transected at the cervical level. Ambulatory ECG-EEG monitoring was started on the 14th day after the transection and continued for 6-10 days. Transection of the left or right vagus nerve resulted in a decrease in the bradyarrhythmia incidence to 55.7% and 27.1%, respectively. Arrhythmogenic properties of the paradoxical sleep were kept in each rat of the transected groups. Circadian rhythm in bradyarrhythmias was affected by the transection: phase shifts of the acrophases, examined by Halberg's technique of cosine fitting by the least-squares method, were observed in both transected groups. These results suggested that the vagus nerves are related closely not only to the occurrence of bradyarrhythmias, but also the expression of circadian rhythm in bradyarrhythmia occurrence.

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THE ROLE OF THE CENTRAL NERVOUS SYSTEM IN THE OCCURRENCE OF CARDIAC BRADYARRHYTHMIAS.

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The purpose of this study was to evaluate : 1. whether bradyarrhythmia episodes observed in rats show a circadian rhythmicity. 2. whether or not, the sleep state is related to the occurrence of arrhythmias. The ambulatory ECG-EEG monitoring was continued for 6-10 days. Sleep states were classified into alertness, slow wave sleep (SWS) and paradoxical sleep (PS). Under the 14/10 light-dark illumination schedule, circadian periodicity was demonstrated in bradyarrhythmia episodes as well as SWS, PS and heart beats. This diurnal variation shows two major peaks. The highest peak coincides with the time zone immediately after the start of illumination, and the second peak coincides with the acrophase of the diurnal variation in PS. Such circadian rhythm was disclosed even under constant illumination. It was enhanced in rats that NGF was administered for 13 weeks since a newborn, and abolished after lesions of bilateral suprachiasmatic nuclei (SCN). These results suggest that the autonomic nervous system as well as the SCN are involved in the generation of the circadian periodicity. In addition, definite increase in the incidence of bradyarrhythmias during the PS was observed in every rat, which suggests the arrhythmogenic property of PS in rats.

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ENDOCRINOLOGICAL CONTROL OF CIRCULATION. IRIUCHIJIMA, J., TERANISHI, Y., AND SAKATA, S. Dept. of Physiol., School of Med., Univ. of Hiroshima, Hiroshima 734

In the rat in the conscious state, tonic secretion of adrenaline from the adrenal medulla, among other effects, keeps muscle blood vessels in a dilated state through its beta-adrenergic effect. Pentobarbital anesthesia inhibits the tonic secretion and diminishes muscle blood flow. When the sympathoadrenal system fails, pressor amounts of vasopressin are secreted in compensation to maintain arterial pressure. The failure of the sympathoadrenal system seems to be signaled as extreme diminishment of both baro- and volume receptor impulses. When the vasopressin secreting system also fails, secretion of renin is facilitated as the third mechanism of maintenance of arterial pressure, through disinhibition from the inhibitory effect of vasopressin on renin secretion.

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STIGMATIZED VASCULAR AREA IN HYPERTENSION. TERANISHI, Y. AND IRIUCHIJIMA, J. Dept. of Physiol., School of Med., Univ. of Hiroshima, Hiroshima 734

In spontaneously hypertensive rats (SHR), measurements of peripheral blood flow at various arteries with chronically implanted electromagnetic flow probes revealed that elevation of vascular resistance was not uniform over the whole body but exceptionlly marked in the hindquarter. Compared with normal control rats, the decrease in conductance in the hindquarter area in SHR contributed about 40% of the total decrease in conductance. Such an area may be referred to as a stigmatized vascular area in hypertension. Stigmatized area appears to be different depending on the kinds of hypertension. In one-clip, two-kidney renovascular hypertensive rats, the stigmatized area was not the hindquarter but the superior mesentric area. It seems that vascular resistance is markedly elevated in skeletal muscle in SHR but in splanchnic viscera in one-clip, two-kidney renovascular hypertensive rats.

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DECREASE OF VENOUS DISTENSIBILITY IN SPONTANEOUSLY HYPERTENSIVE RAT. MURASE, K., FUKUSHIMA, S., OHHASHI, T. AND AZUMA, T.* Dept. of Physiol., Shinshu Univ. Sch. of Med., Matsumoto 390 and *Juntendo Univ., Tokyo 113

The mechanical characteristics of venous walls in spontaneously hypertensive rats (SHR) were investigated from a rheological and morphological point of view. Pressure-radius relationships in the isolated abdominal inferior caval vein and portal vein of SHR and Wistar-Kyoto rats were examined. Optical microscopic examinations of the venous preparations were also carried out. The distensibilities of both venous walls of SHR decreased significantly with a development of hypertension. Morphological observations revealed that increases in amount of smooth muscle cells, collagen and elastic fibers in the venous walls of SHR. These results suggest that the decrease of distensibility in the isolated venous preparations from SHR may be related to the morphological changes, the increase of thickness of venous walls with an increase of systemic arterial pressure. On the other hand, chronic (35 weeks from 2 weeks old) intramuscular-injection of elastase, which is released from an exocrine gland in pancreas and has an enzymatic action on elastic fibers in arterial and venous walls, tended to inhibit the above-mentioned mechanical and morphological changes in the venous walls of SHR. Further investigations will be needed to elucidate the mode of action of elastase in the metabolism of the venous walls.

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HYPERACTIVE CENTRAL NEURAL INTEGRATION TO RELEASE VASOPRESSIN IN SHR. YAMANE, Y., NAKAI, M. AND OGINO, K. 2nd Dept. Intern. Med., Kansai Med. Univ., Moriguchi, Osaka 570; Natl. Cardiovasc. Ctr. Res. Inst. Suita, Osaka 565

Experiments were conducted on rats under N_2O anesthesia and artificial ventilation. The cervical spinal cord was cut and the vago-sympathetic trunks and aortic nerves blocked by cutting and ligation. The animals were maintained at their normal arterial blood pressure by infusing phenylephrine throughout the entire course of each experiment. Monopolar electrical stimulation was delivered over 4 min to the intermediate portion of the solitary nucleus (SOL). The stimulus current was set at 50 uA. The stimulation elicited significant increase ($P < 0.05$) in concentration of plasma vasopressin (pVP; measured by radioimmunoassay) by 644 ± 99 pg/ml in 13 SHRs, and by 416 ± 47 pg/ml in 16 normotensive Wistar rats. We conclude that neurosecretory function of the central neural integration, which emanates from the intermediate portion of the SOL, is about 1.5 times as powerful in releasing vasopressin in SHR as in normotensive rats.

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CHEMO- AND BARORECEPTOR AREA IN CHELONIA(GEOCLEMYS REEVESII). ISHII, K., ISHII, K.[1] AND KUSAKABE, T.[2] Dept. of Physiol., Fukushima Med. Coll., Fukushima 960. [1]Watari Nakae-cho 9-2, Fukushima 960. [2]Dept. of Anat., Sch. of Med., Yokohama City Univ., Yokohama 220.

In the chelonia superior and inferior truncal nerves contain baro- and chemosensory fibers. The former terminates in aortic arch and the latter in truncal region, giving off fine branches to aortic and pulmonary arches. In anesthetized turtle 2 types of activity were recorded on the peripheral cut end of these nerves. The one was concomitant with the blood pressure fluctuation. The other, non-rhythmic lower potential discharge, increased with the intravenous administration of NaCN(20ug) and decreased with O_2-blowing into the lung. Cutting their peripheral branches one by one and inducing electrical activity on the nerve in connection with a small piece of aortic arch, baro- and chemoreceptor areas were confirmed as follows.

In aortic arch there are baro- and chemoreceptors. Their impulses are transmitted via superior truncal nerve and a fine branch of inferior truncal nerves which arises from the ganglion trunci of vagus. In pulmonary arch baroreceptors are mainly located with a few chemoreceptors. Moreover, baro- and chemoreceptive areas are located in the truncal region.

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POSTNATAL DEVELOPMENT OF THE AORTIC NERVE IN RABBITS. SHIMIZU, T., ISOMURA, G.[1] AND MIYAKAWA, K.[2] Dept. Physiol., Fukushima Med. Coll., Fukushima 960, [1]Dept. Anat., Fujita-Gakuen Univ. Sch. Hyg., Toyoake 470-11, [2]Dept. Physiol., Shinshu Univ. Sch. Med., Matsumoto 390

The composition of aortic nerves which are the main afferents in the relfex pathway of the brain ischemic bradycardia was studied in newborn rabbits. The cross section of the nerve at the level immediately caudad to the point of bifurcation from the superior laryngeal nerve was examined by an electronmicroscope. The average numbers of myelinated and non-myelinated fibers of 5 rabbits aged 4 - 6 days were 83 ± 26 and 902 ± 476 in the right side and 136 ± 41, 773 ± 198 in the left respectively. The diameter of axon, the thickness of myelin of the myelinated fiber and the diameter of the nerve trunk were 1.51 ± 0.17(major axis) X 0.68 ± 0.06μm(minor axis), 0.31 ± 0.06 μm and 39.5 ± 6.4 X 25.0 ± 4.9μm in the right and 1.79 ± 0.43 X 0.67 ± 0.13μm, 0.38 ± 0.09μm and 61.6 ± 11.6 X 26.4 ± 5.2μm in the left respectively. These figures showed 25.9 - 42.8% of the corresponding mean values obtained in 6 mature rabbits aged 22 weeks with the exception of numbers of non-myelinated fibers which were 75.3 - 93.2%. However more rapid development of the myelination was observed in 7 days animals, which will promote elicitation of the ischemic bradycardia.

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EFFECTS OF Ca^{++}-BLOCKERS ON THE VAGOSYMPATHETIC NERVE-HEART PREPARATION OF FROGS. WAKITA, Y., OCHI, R.*, IKENAGA, T.*, YONEMURA, K. AND TANAKA, I. Dept. of Physiol., Kumamoto Univ. Med. Sch., Kumamoto 860

In the isolated frog heart with the cardiac (vagosympathetic) nerves, tetanic stimulation of the nerves for 10 sec produced a biphasic effect, an early inhibition followed by a late augmentation of heart activities. Three blockers such as diltiazem (D), verapamil (V) and nifedipine (N) all depressed contractile forces and all lowered heart rates of spontaneous activities in a dose-related manner (10^{-8} to 5×10^{-5} M), although N showed a relatively small effect on heart rates. The early inhibitory effect of the nerves was enhanced by D and V at lower doses but antagonized by D and V at higher doses (after addition of 2 mM $CaCl_2$ to the perfusate). As opposed to this, N antagonized the inhibitory effect of the nerves at lower doses but enhanced the inhibitory effect on contractile forces and antagonized the effect on heart rates at higher doses. The late augmenting effect of the cardiac nerves was not antagonized but rather enhanced by these Ca^{++}-blockers. Thus, the present study indicates that these Ca^{++}-blockers influence more greatly on the vagus nerve actions than on the sympathetic nerve actions in the frog heart.

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RENAL BLOOD FLOW REGULATION DURING RENAL NERVE STIMULATION. HAYASHIDA,Y., MITANI,Y., TATEISHI,J. & HOSOMI,H. Dept. of Physiol. Kagawa Med. Sch. Kita-gun, Miki, Kagawa 761-07

The effects of renal nerve stimulation on renal blood flow (RBF) was studied on mongrel dogs, which were anesthetized with sodium pentobarbital (35 mg/Kg body weight, i.v.). The left kidney was exposed with a retroperitoneal approach. RBF, blood pressure and vascular resistance were monitored. Square pulse stimulus was delivered to the renal nerve at various intensities and frequencies. A 23-gauge hooked needle was inserted into the left renal artery for the intrarenal administration of drugs.

Electrical stimulation of the renal nerve (10 - 20 Hz in frequency and 10 V in intensity) reduced RBF by almost 100 % of the control RBF. During the renal nerve stimulation for 2 min, RBF recovered gradually to the control level at the mean rate of 0.45 $ml/min^2 \cdot g$ kidney (autoregulatory escape). The recovery of the RBF in spite of continued stimulation was not due to adaptation of the contractile elements of blood vessel nor to a decrease in the amount of any transmitter released by repeated stimulation. Calcium chloride which was administered into the renal artery accelerated the rate of recovery of the RBF and papaverine hydrochloride abolished the recovery of the RBF during the renal nerve stimulation, suggested that the autoregulatory escape has related with the genuine autoregulation during the changes in perfusion pressure of the kidney.

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THE ROLE OF ADRENOCEPTORS IN REGULATION OF THE HEPATIC CIRCULATION IN ANESTHETIZED DOGS. TERADA,N., TAKEUCHI,T and KOYAMA,S. Department of Physiology, Yamanashi Medical College, Tamaho, Yamanashi 409-38

The present experiment was designed to study possible participation of adrenoceptors in hemodynamic changes in the hepatic bed caused by occlusions of the hepatic artery or portal vein in anesthetized dogs. Occlusion of the hepatic artery for one minute produced not appreciable changes in central venous, hepatic venous and portal venous pressures, and portal venous flow(PF), but caused a slight increase in abdominal aortic pressure. The intraportal administration of propranolol(0.1mg/kg) or yohimbine(0.1mg/kg) resulted in not significant changes in these responses. The prazosin(0.2mg/kg,IP) administration, however, significantly increased PF and decreased the portal venous resistance. Namely, the administration of alpher-1-blocker caused a significant increase in PF during the hepatic arterial occlusion. Occlusion of the portal vein significantly decreased hepatic arterial resistance, which showed a not significant change after the administration of adrenoceptor antagonists. It is concluded that reflex venoconstriction may be produced in the portal vein during the portal hypotension.

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TARGET BLOOD VESSELS OF SYMPATHETIC VASOCONSTRICTOR TONES. SAKATA, S. AND IRIUCHIJIMA, J. Dept. of Physiol., School of Med., Univ. of Hiroshima, Hiroshima 734

Spinal transection at C8 performed in the rat under ether anesthesia induced a marked lowering of arterial pressure, which was gradually recovered after withdrawal of ether to reach a new plateau level in about one hour. The new level of arterial pressure, which is considered to be maintained by the automaticity of spinal sympathetic cells, was only slightly lower than the level before spinal transection which is considered under the control of the so-called medullary cardiovascular center. However, cardiac output was markedly lower after transection than before. Right atrial pressure was also decreased considerably after spinal transection, which was absent in splanchnicectomized rats. One implication of the above findings would be that target blood vessels of the vasoconstrictor tone from the medullary center are both capacitance and resistance vessels, while those of the spinal tone are mainly resistance vessels.

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EFFECTS OF REPETITIVE ELECTRICAL STIMULATION OF PERIPHERAL AFFERENT NERVE ON THE SOMATOSYMPATHETIC REFLEXES IN ANESTHETIZED CATS. SATO, A., SATO, Y., SUZUKI, A. AND SWENSON, R.S. Dept. of Physiol., Tokyo Metro. Inst. Gerontol., Itabshiku, Tokyo 173

Effects of repetitive electrical stimulation of various hindlimb afferent nerves on sympathetic reflexes in response to a superficial peroneal (SP) nerve stimulation were tested in anesthetized cats. As control reflex responses, sympathetic A and C reflexes of about 60 and 400 msec latencies, respectively, were recorded from the inferior cardiac sympathetic nerves in response to each myelinated A and unmyelinated C afferent fiber stimulation of SP nerve. These A and C reflexes evoked by SP stimulation were not influenced by repetitive electrical stimulation of sural and tibial afferent nerve for 30 min duration at any stimulus intensity. However, the C-reflex was specifically depressed, in about one third of all tested cases, for about 20 min after end of repetitive stimulation of 30 min duration, only when the same SP afferent nerve was stimulated at 2-10 Hz with sufficient stimulus intensity to stimulate C afferent fibers. Although a part of the depression mentioned above was due to a depression of afferent volley of the C fibers itself, the other part of the depression was considered to be due to a long lasting effect of endogenous opioid release from reflex pathways between the SP C afferents and the cardiac sympathetic neurones because the depression was reversed by naloxone (morphine antagonist).

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ROLE OF CARDIAC SYMPATHETIC NERVE AND BUFFER NERVES IN THE PRODUCTION OF HYPERTENSION BY COMPLETE INTERRUPTION OF BLOOD SUPPLY TO THE BRAIN IN RABBITS. HAYASHI, M. AND MIYAKAWA, K. Dept. Med., Shinshu Univ., Matsumoto, Nagano 390

The blood supply to the brain was completely interrupted for 25 minutes in anesthetized rabbits. By the interruption systemic arterial pressure (SAP) rose rapidly drawing a logistic curve, followed by a plateau-like elevation which was maintained throughout the course of it. By resumption of blood supply to the brain SAP gradually fell, depicting an exponential curve, and returned to its preinterruptory value. Under the condition of intact and no cardiac sympathetic innervation, the times for half-increase of SAP after the interruption were 9.3 $\pm$ 0.5 (Mean $\pm$ SE, n=11) sec. and 10.4 $\pm$ 1.6 sec., respectively. The time for half-decrease of SAP after the resumption was 14.1 $\pm$ 1.6 sec. and 13.6 $\pm$ 1.8 sec., respectively. On the other hand, under normal condition and that with the depressor nerves cut, the times for half-increase of SAP were 10.0 $\pm$ 0.5 (n=4) sec. and 10.5 $\pm$ 1.0 sec., respectively. The times for half-decrease of SAP under the same conditions were 21.5 $\pm$ 1.5 sec. and 27.0 $\pm$ 1.5 sec., respectively. Thus, the section of cardiac sympathetic nerves mainly prolonged time of the increase, while the section of buffer nerves mainly prolonged the time of the decrease. These results suggest that cardiac sympathetic nerves contribute to the SAP elevation by the interruption and that buffer nerves contribute to the SAP fall by the resumption.

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VAGUS CARDIOINHIBITORY PREGANGLIONIC NEURONS IN RABBITS: CELL SOMATA DISTRIBUTION AND AXONAL PROPERTIES. NOSAKA, S. AND MURATA, K.※ DEPT. PHYSIOL., MIE UNIV. SCH. OF MED., TSU, MIE 514

It has been reported that the majority of the vagus cardioinhibitory preganglionic neurons (VCIN) in rabbits are located in the dorsal motor nucleus (DMN), while a minority, in the nucleus ambiguus (NA). We have reexamined this subject with HRP and functional studies. HRP was applied on the central cut end of a functionally identified cardiac branch of the vagus nerve in urethane-anesthetized rabbits. More than 90 % of HRP labelled cells were localized in NA region, while the remaining ones, in DMN. Electrolytic destruction of NA region abolished bradycardia elicited by DMN stimulation in C_1-transected hemivagotomized rabbits, whereas lesioning of DMN region did not affect bradycardia produced by NA stimulation. Reflex bradycardia elicited by methoxamine injection or the aortic nerve stimulation was abolished by destruction of NA region. These findings indicate that the VCIN reside mostly in NA region in rabbits.

In another series of experiments, properties of axons of the VCIN were studied electrophysiologically. It was confirmed that vagal bradycardia is mediated by axons of B-fiber group. However, de Neef et al's observation that vagal C fibers exert a prejunctional inhibition on B-fiber mediated bradycardia was not ascertained: It was attributed to anodal block of B-fiber conduction due to improper experimental setup.

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VASODILATATION OF THE BRAIN ELICITED BY ELECTRICAL STIMULATION OF THE INTERMEDIATE PORTION OF THE SOLITARY NUCLEUS IN RATS. NAKAI, M. AND OGINO, K. Natl. Cardiovasc. Ctr. Res. Inst., Suita, Osaka 565

An effect of monopolar electrical stimulation of the intermediate portion of the solitry nucleus (SOL) on the brain circulation was studied in 48 anesthetized (N_2O), artificially ventilated Wistar rats with cordotomy (C_2) and vago-sympathetic trunks cut. A sufficient dose of a vasopressin-analog given in each rat prevented vasopressin-induced hypertension in stimulating the medulla. Stimulation at a current intensity of 60 $\pm$ 4 (SE) uA (n=9), which was equivalent to 2.5 times the threshold for vasopressin-induced pressor response, elicited an increase ($P < 0.05$) in regional blood flow (iodoantipyrine technique) by 82 %, and 43 % on average in the frontal and occipital cortices, respectively, and 25 % in the caudate-putamen. It was found that to the result observed, there were litte detectable contributions of current spreading of stimulus towards adjacent structures surrounding the SOL (the dorsal medullary reticular formation and cuneate fascicles) and retrograde transmission towards the cerebellar fastigial nucleus. Overall, we conclude that a central neural integration emanating from the intermediate portion of the SOL regulates the blood vessels of the brain as a vasodilator.

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CHANGES OF NORADRENERGIC NEURONS IN THE NUCLEUS TRACTUS SOLITARIUS IN SPONTANEOUSLY HYPERTENSIVE RAT. OHTSUJI, M. AND NOMURA, M. Dept. of Physiol., School of Med., Fujita-Gakuen Univ., Toyoake, Aichi 470-11

It is suggested that in spontaneously hypertensive rats a functional disorder of noradrenergic(NA) neurons contributes to an increase in peripheral vascular resistance which causes an increase in arterial blood pressure. We examined histochemical and biochemical changes in the brainstem, including the nucleus tractus solitarius(NTS) in SHR as compared with those of control Wistar Kyoto rats(WKY). After brainstem tissue sections of SHR and WKY were stained with FAGLU-PA-GAS method, the distribution of fluorescent NA neurons was examined. In pre-hypertensive SHR aged 4 weeks, an evident reduction of intensity of fluorescence was observed in cell bodies and axons of NTS, as well as A1 and A2 regions in medulla oblongata. A receptor binding experiment was performed using [^{3}H]-WB4101 and [^{3}H]-Yohimbine as ligands for the alpha-1 and alpha-2 receptor, respectively. The alpha-1 ligand binding to homogenates showed no difference. But the alpha-2 ligand binding K_D and Bmax were lower in SHR than in WKY even the age of 4 weeks. These results suggest that some disorder in synaptic transmission in NA neurons of the brainstem, including the NTS may be involved in the development of hypertension and disorder in the adrenergic control on the peripheral vessels.

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EFFECT OF SINO-AORTIC DENERVATION ON THE LONG-TERM CONTROL OF ARTERIAL PRESSURE.
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In twelve conscious rabbits, arterial pressure was continuously recorded up to 10 weeks by an indwelling catheter in the thoracic aorta. The mean value and standard deviation (SD) of mean arterial pressure (MAP) were calculated for each day and compared before and after bilateral sino-aortic (SA) denervation. Subsequent to SA denervation, the mean value of MAP was initially elevated but invariably returned to the pre-denervation level in 5 to 30 (average 14) days. Subsequent cervical vagotomy also failed to produce a sustained hypertension. The SA denervation, however, brought about certain lasting hemodynamic changes. First, the patteran of the MAP histogram was markedly altered. Before denervation the histogram was bimodal, the two peaks corresponding to the resting and moving states. After denervation, the two peaks merged, as a result of an increased SD of MAP during the rest, and a diminished mean value of MAP during movement. Second, SA denervation modified the circadian rhythm of MAP. Before denervation rabbit's MAP does not typically show a distinct circadian rhythm. After denervation hourly means of MAP were higher than the daily average in the early morning hours but were lower in the daytime.

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NUCLEUS TRACTUS SOLITARII OF THE CAT: THE PROJECTIONS FROM THE CENTRAL STRUCTURES ONTO THREE SUBNUCLEI IN RELATION TO THE EFFECTS OF NALOXONE ON THE CAROTID SINUS NERVE REFLEX, AND THE EFFECTS OF MICROSTIMULATION ON CIRCULATION AND RESPIRATION
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By the application of the specific opiate antagonist naloxone (250 mM, 500 nA, 1.5 s) to small loci in the nucleus tractus solitarii (NTS), the field potentials evoked by electrical stimulation of A-fibers of the carotid sinus nerve were inhibited in the rostral (NTSR) and caudal (NTSC) subnuclei of the NTS, but not in the intermediate subnucleus (NTSI). Following microstimulation (5 μA, 1 ms, 20 Hz) of small loci where the evoked field potentials were recorded, characteristic patterns of cardiovascular and respiratory responses were observed in each subnucleus. The NTSR was characterized by the pressor and apnea responses, and the NTSI and NTSC by a mixture of depressor and pressor responses. By injecting small amount of HRP into each subnucleus, we sought HRP labelings of neurons from the medulla oblongata to the hypothalamus. We found no conspicuous difference in sites of labeling in the central structures, and these sites of labeling were similar to the sites of origin which innervate the intermediolateral nucleus of the upper thoracic cord (JANS, 7, 1983). In addition, it should be noted that there was no labeled cells in the cerebellar fastigial nucleus in which Ross et al. (Brain Res., 223, 1981) found many labeled cells in the similar experiment.

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EFFECTS OF FOCAL STIMULATION OF BRAINSTEM ON INTRACRANIAL PRESSURE AND ARTERIAL PRESSURE.
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Focal electric stimulation was stereotaxically applied to the brainstem of anesthetized cats (sodium pentobarbitone) to investigate the effects on intracranial pressure (ICP) by the activation of different neural tissues. The effects were analysed in relation to the actions on arterial blood pressure (BP) and the stimulated sites were mapped on the schematic drawing of the brainstem. There were sites which produced an increase in ICP associated with an increase or a decrease in BP, sites which produced a decrease in ICP and a decrease or an increase in BP, and sites which produced a change only in BP or ICP. The other stimulated sites showed no responses. However, when changes in BP were produced by a rapid infusion of saline solution into artery or bleeding, the associated changes in ICP always occured in the same direction as the increase or decrease in BP. The observations show that the actions on ICP by the activations of brainstem neural tissues are not always secondary effects to the changes in BP, but include primary actions on ICP.

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VESTIBULO-SYMPATHETIC RESPONSES INDUCED BY ANTIDROMIC STIMULATION OF SPINAL CORD.
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The interval between the lumbar nerve conditioning stimulus and vestibular nerve testing stimulus was changed and recovery curve of renal nerve potential was drawn. We reported the recovery curves for the test response from the somatic nerve and the sympathetic nerve were different. After 3rd thorathic spinal lateral column transection, the sympathetic responses were still present, but the somatic response had disappeared. Spikes of single neuron were extracellulary recorded in the medulla oblongata in chloralose anesthetized Mioblock immobilized cats and observed by stimulations of ventral part of 3rd lumbar segment and ipsilateral veastibular nerve. Vestibular nerve stimulation strength was used 2 xT threshold of nystagmus. Stimulus strength of spinal cord was used less than 50 uA. Post-ganglionic renal nerve potential and blood pressure, heart rate were also recorded. Spinal-induced antidromic spikes in reticular formation were identified by following high frequency stimuli and collision test. The unit response by spinal stimulation cosisted of antidromic early response and multiple late responses. However, on the renal nerve potential, the test response appeared after 250 msec of conditioning stimulus. It is assumed that a part of inhibitory region relating with vestibulo-sympathetic response is located in medulla oblongata.

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A DIRECT PROJECTION FROM THE MOTOR CORTEX TO THE HYPOTHALAMIC VASODILATOR AREA.
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The functional fiber connection between the motor cortex and hypothalamus was studied concurrently with the retrograde horseradish peroxidase (HRP) method and electrophysiological techniques in the cat. By stimulation of the motor cortical hindlimb area, field potential of a short latency (3-5 ms) which followed to high-frequency stimulation was recorded in the ipsilateral anterior hypothalamus. Simultaneously, atropine-sensitive vasodilation was elicited in the femoral artery after vagotomy. The vasodilation, which was considered to be driven through the sympathetic vasodilator system, was also seen following stimulation of this hypothalamic region. Conversely, antidromic spikes were activated at short latencies (2-6 ms) in the motor cortex following stimulation of the anterior hypothalamus, and some of these units successfully collided with the spontaneous discharges descending from the motor cortex. HRP was applied iontophoretically to the hypothalamic site when either the orthodromic or antidromic response was obtained with the same electrode. A small number of HRP-positive pyramidal neurons were found in laminae 3 and 5 of the motor cortex. It is suggested that the anterior hypothalamus receives a direct projection from the motor cortex, serving for active blood supply to the skeletal muscle.

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PHYSICAL ANALYSIS OF BLOOD SMEARING AND ITS APPLICATION. NAKAGAMI, Y., SASAKI, S. AND SAKAKI, T* Dept. of Physiol., Fujita-Gakuen Univ., Sch. of Med., Toyoake, Aichi 470-11, and Dept. of Chem.,College of Gen. Educ., Nagoya Univ., Nagoya.

When a blood sample is smeared with conventional wedge method, a relation, $h=C\cdot u^{n}\cdot r_{e}$, exists between h(thickness of the film), u(speed of smearing), and r_e(radius of a circle inscribed to the glasses). C and n are parameters decided by the condition of smearing.

A programm of smearing speed enabling to produce a smear with uniform thickness from end to end was derived from the above equation. Computer-assisted smearing device was constructed so that the smearing is made according to the programmed speed. With this device, smears having uniform thickness were obtained, and the distribution and differential count of leukocytes were compared to those obtained with conventional two other methods, wedge method and centrifugal method. The new method gave most satisfactory results.

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MOBILIZATION OF LYMPHOCYTES WITH MACROMOLECULAR SULFATES. KARASAWA, K. AND SASAKI, S. Dept. Physiol., Fujita-Gakuen Univ. School of Med., Toyoake, Aichi 470-11.

It was reported that macromolecular sulfates caused lymphocytosis in experimental animals. This work was performed to clarify the mechanism of the lymphocytosis and to detect the organs which contribute to the lymphocytosis.

An apparatus was designed and used to estimate the lymphocyte outputs from spleen and from thoracic duct of rat separately and continuously. After administration of dextran sulfate, the lymphocyte outputs from spleen and from the duct were both increased, in which peaks of the outputs were observed much earlier than the peak of the lymphocyte count in blood. Lymphocyte output from spleen contributed approximately 70 % of the increment of lymphocytes in blood, and output from thoracic duct about 35 %. The results indicate that the lymphocytosis is caused by mobilization of lymphocytes from spleen and lymphnodes, and not by the interruption of the return of lymphocytes from blood to their storage.

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SEXUAL DIFFERENCE OF CHANGES IN THE ADHESIVENESS OF LEUKOCYTES DURING DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS IN THE RABBIT. OHKUBO, C. AND ASANO, M. Dept. of Physiol. Hygiene, the Inst. of Public Health, Tokyo 108

Rabbits of both sexes whose ear lobes had a transparent round chamber for vital-microscopic observation were fed a regular diet(control group) or that supplemented with 1.5% cholesterol(CH goup) for 12 weeks. Changes in the adhesiveness of leukocytes were vital-microscopically investigated and concurrently measured by a newly developed in vitro assay method. In the ear chamber of CH groups, intravascular leukocytes became more adhesive, quite remarkably in males and less so in females, not only to post-capillary vessel walls but also to arterioles, although no changes were observed in control groups. Concerning macrocirculatory changes in CH groups, anemia and hyperlipidemia were also progressively developed during observation period, especially in males. In vitro leukocyte adhesiveness measured with a hematocrit glass capillary was investigated every 3 weeks. The specimens of CH groups showed a significant increase in the adhesiveness($p<0.005$) within 3 weeks of cholesterol feeding, although no effect was observed on the adhesiveness in those of control groups throughout the experiment. The changes in the adhesiveness of leukocytes were also much more pronounced in males. The results showed that the in vitro assay well corresponed with vital-microscopic observations on the leukocytes.

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A LIBERATION OF Na^+ AND HCO_3^- IONS FROM HUMAN RED BLOOD CELLS INTO PLASMA DURING A PASSAGE OF A CO_2-RICH GAS THROUGH HUMAN WHOLE BLOOD.
ASANO, K., SHIMABUKURO, S., TANASE, K., OGAWA, A., OHIRA, T., MINAGAWA, T. AND HIRAKAWA, S. 2ND. DEPT. INTERN. MED. GIFU UNIV. SCH. MED. GIFU 500

We have previously reported that there occurred a liberation of Na^+ and HCO_3^- ions from dog's red blood cells(RBC) into plasma in one-to-one relations as well as a chloride shift in a reversible fashion, when a CO_2 -rich gas was passed through dog's whole blood. In the present study, we passed a CO_2-rich gas mixture of 15% CO_2, 25% O_2 and 60% N_2 through human whole blood in order to see whether or not a liberation of Na^+ and HCO_3^- ions would occur from human RBC into plasma as was the case with dog's whole blood. We found that there occurred a liberation of Na^+ and HCO_3^- from human RBC into plasma in one-to-one relations together with a chloride shift, when human whole blood was exposed to a CO_2 -rich gas mixture, and this was almost reversible in nature. Roughly, the approximate net "efflux" of Na^+ was 0.34 pmol/cm^2/sec, pCO_2 65.3 mmHg and pH 7.25, 5 minutes after starting a CO_2-rich gas passage.

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IN VITRO ERYTHROPOIETIN (Epo) ASSAY BASED ON ERYTHROID COLONY FORMATION IN FETAL MOUSE LIVER CELL CULTURE.
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Critical studies were made on erythroid colony formation from cultured fetal mouse liver cells in an attempt to develop a simple and sensitive Epo assay procedure. The maximum colony formation was observed 24 h after plating of the cells where a dose-response relation was found for Epo added. The colony forming ability decreased steadily as the gestational age of the fetus advanced and was lost during postnatal 10 days. In view of morphological and histochemical criteria almost all the colonies were concluded to be erythroid. ^{59}Fe-label experiment revealed a good correlation between the colony numbers and ^{59}Fe incorporation into the cells and also into the heme fraction. Based on these findings we developed a procedure which could measure as least as 0.001 U of Epo without ^{59}Fe and, therefore, was 1000 times more sensitive than the standard starved rat procedure. Using this method plasma Epo titers were determined in 39 normal and 76 anemic subjects.

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DETECTIONS OF I AND i ANTIGEN POSITIVE RED BLOOD CELLS IN ADULT PERIPHERAL AND UMBILICAL CORD BLOOD BY IMMUNOFLUORESENT STAING METHOD.
MATSUDA,M., TERASAWA, T., UGIIE, K. ITO, T AND KASAI, S., Dept. of Physiol., Tohoku Dental Univ., Koriyamashi, Fukushima.

In order to investgate the changes of I and i expressions in the human erythrocytes during the course of human development, we examined the frequency distributions of I and i positive red blood cells in adult peripheral and umbilical cord blood by immunofluoresent staining method. Fresh venous blood samples were drawn from adult healthy volunteers and umbilical cord (38-42-wks gestation). The anti-I serum was obtained from a patient with chronic cold aggultinin disease. It is a monoclonal IgM antibody with an aggultination titer of 1/80000 against adult erythrocytes and 1/128 against cord erythrocytes at 4 C. The anti-i serum (monoclonal IgM antibody) with aggultination titer 1/16800 against cord erythrocyte and 1/128 against adult erythrocytes was obtained from a patient with Waldenstrom macroglobilinemia. RBC were washed in PBS and were subsequently incubated with anti-I or anti-i serum at 4 C for 3 hrs. They were rinsed in cold PBS, and FITC anti-IgM was then applied for 2 hrs. Frequency distributions of I and i-cells in adult blood were 56 ± 8% (n=19) and 1.5 ± 1.6% (n=12), respectively and in cord blood were 1.6 ± 1.2% (n=12) and 63 ± 14% (n=10), respectively.

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SHAPE CHANGE AND MECHANICAL FRAGILITY OF ERYTHROCYTES MEMBRANE. UYESAKA, N., KAWASAKI, K.*, SHIO, H.** and SHINAGAWA, Y. Dept. of Physiol., Nippon Med. Sch., Sci. and Engin. Res. Inst., Doshisha Univ.*, Dept. of neurol., Fac. of Med., Kyoto Univ.**

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 such as isotonic medium, and normal hematocrit(Hct) value. Red cell schape change induced by impact-force was studied by scanning electron microscope, and there were several recognizable stages of echinocyte-spherocyte transformation. Most characteristic shape change induced by impact-force was fragmentation of erythrocytes. Effect of Hct value upon mechanical fragility of erythrocytes was investigated, and the decrease in mechanical fragility and almost linear relationship between Hct value and mechanical fragility was observed in the range of physiological Hct value, namely from about 25% to 50%. Comparing vitamin E deficient rat with normal rat, little difference between osmotic fragility and membrane fluidity studied by spin labeling was observed, however, an increase in mechanical fragility of erythrocytes of Vitamin E deficient rat was detected. These results indicate clearly that mechanical fragility of erythrocytes gives us unique information concerning molecular constituents of membrane, which is not detected by other methods such as osmotic test and spin labeling.

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EFFECTS OF FLUX ON MEMBRANE POTENTIAL OF NEWT ERYTHROCYTES. KAWANO, K., FUKUSHIMA, M., IBUKI, C., SHINAGAWA, Y. Dept. of Physiology, Nippon Med. School, Sendagi, Tokyo 113

Membrane potentials of newt erythrocytes were measured by means of microelectrode method. The observed potentials were -16.2±0.4 mV and -23.0 ± 1.0 mV in Ringer solution at 18°C and 0°C, respectively. The Nernst potential of chloride ions cannot be applied according to two reasons: (1) The membrane potential was almost linear function of the external Cl concentration, (2) as the temperature of the external solution was reduced from 18 °C to 0 °C, the membrane potential hyperpolarized almost linearly. To explain these observations, the following equation was proposed,

$$E = \frac{RT}{F} \ln \frac{P_{Cl}[Cl]_i + \phi_i \exp(\Psi_i F/RT)}{P_{Cl}[Cl]_o + \phi_o \exp(\Psi_o F/RT)}$$

where ψ_i and ψ_o represent the inner and the outer surface potential of the membrane, respectively, ϕ_i and ϕ_o, flux at inner and outer surface of the membrane. Using the values, $\phi_i/P_{Cl}=1.45\times10^{-9}$, $\phi_o/P_{Cl} = 8.09\times10^{-11}$, ψ_i =0.606 and ψ_o =0.699, the theoretical values calculated from the equation shows well fitness to the observed ones.

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OSMOTIC RESISTANCE OF RABBIT RETICULOCYTES
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Dept. of Physiol., Tokyo Med. Coll., Shinjuku-ku, Tokyo 160

Reticulocytosis due to hemolysis was induced by 4 days subcutaneous injection of Phenylhydrazine hydrochloride (PH 7.0, 0.6 mg/kg) in to a rabbit. Blood sample was spun up 2500 g for 15 min, and a final concentration was 95%. Reticulocyte membrane resistance was determined by C.P.C. system, H.S.P.: 87-102 mOsm, Peak: 79-86 mOsm, H.E.P.: 58-65 mOsm. These values were lower than mature erythrocyte, but membrane osmotic resistance was increased.

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CHANGES IN OSMOLARITY AND FLOW PROPERTIES OF SALMON BLOOD DURING TRANSFER FROM SEAWATER TO FRESHWATER. KIKUCHI, Y., KAKIUCHI, Y. AND KOYAMA, T. Div. Physiol., Res. Inst. Appl. Electr., Hokkaido Univ., Sapporo, Hokkaido 060

Changes in blood osmolarity and blood flow properties in salmon following transfer from seawater to freshwater were studied by using chronically cannulated fishes. Blood samples taken via the cannula in the dorsal aorta were used for measurements of plasma osmolarity, plasma total protein, hematocrit, mean corpuscular volume, and an index of red cell deformability (mean passage time of red cells through 8 μm pores). Plasma osmolarity decreased from 400 mOsmol to 300 mOsmol within 1 hour after the transfer and showed no fluctuations thereafter. This reduction in plasma osmolarity appeared to be only partly due to the inflow of water from the outer water to blood but mainly caused by the osmoregulation which was probably switched from seawater to freshwater conditions immediately. A marked improvement in red cell deformability was observed in parallel with the decrease in plasma osmolarity. An inflow of water to the red cells occurred following this change in their deformability. Measurements of red cell deformability at various temperatures showed that salmon red cells were still highly deformable even at 5°C. These observations suggested that the microcirculation in osmoregulatory organs of salmon was supported by deformability properties of the red cells.

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DETERMINATION OF THE OXYGEN PERMEATION CONSTANT OF AN ERYTHROCYTE CELL MEMBRANE HASHIMOTO, M.*, ISOMOTO, A.*, TYUMA, I.*, AND UOZUMI, M.**. *Dept. of Physicochemical Physiol., Med. Sch., Osaka Univ., Osaka 530, and **Division of Environmental Health Research, Osaka Pref. Inst. of Public Health, Osaka 537.

A convenient method was developed to determine the overall oxygen permeability constant of an erythrocyte cell membrane.

The constant was estimated by comparing the deoxygenation rate of the hemoglobin in a hemolysate with that in an erythrocyte suspension. The deoxygenation was carried out by flashing a pure nitrogen gas on the sample film (18～54μ thick) on a slide glass, and the concentration of oxyhemoglobin in the sample was continuously monitored by a dual beam spectrophotometer.

The results said that the oxygen permeability of the cell membrane was one thirtieth fold the oxygen diffusibility from liquid to gas phase.

This method made the determinatin simple and easy by eliminating the need for measuring the free oxygen concentration in the outside of the erythrocytes.

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THEORETICAL STUDY ON CELL MEMBRANE OSCILLATION AND ITS DETECTION BY LASER LIGHT SCATTERING. Y.SHINAGAWA, YASUKO SHINAGAWA*, and I. KUBO** Dept. of Physiology,Nippon Medical School, *Dept. of Physiology, Fac. of Med., Kyoto Univ. **Dept. of Mathematics, Hiroshima Univ. General Sciences

Human red blood cell shows remarkable increase of cell volume in the isotonic glucose-electrolyte solution, as was reported previously by us. The increase of volume shows an overshoot phenomenon around equilibrium point. Computer analysis of the equations on membrane permeability of non-electrolyte revealed that (i) the overshoot phenomena is the part of membarane oscillation and (ii) the equations have solution of the limit cycle oscillation.

Mathematical provement of the existence of the limit cycle was perviously reported by us[1], by means of the computer aided rigorous solution of the differential equations.

Theoretical basis of the detection of membrane oscillation in human erythrocytes by means of the laser light scattering was discussed in this report.

1) Y. Shinagawa (1982) Membrane, 8, 204

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D-GLUCOSE TRANSPORT ACROSS ERYTHROCYTE MEMBRANE STUDIED BY OSMOTIC FRAGILITY AND INVESTIGATION OF IT'S INHIBITOR. FUKUSHIMA, M., KIKUCHI, H., HIROSE, T., SHINAGAWA, Y.*, UYESAKA, N. AND SHINAGAWA, Y. Dept. of Physiol., Nippon Medical School, Bunkyo-ku, Tokyo and Dept. of Physiol., Fac. of Med., Kyoto Univ., Sakyo-ku, Kyoto*

Osmotic fragility of human erythrocytes was tested by a coil planet centrifuge method and usual method. The mixture of NaCl and D-glucose (1:1) solution was produced by iso-osmotic replacement. Degree of hemolysis tested by the mixture solution is more larger than that of done by electrolyte solution. It has been acquired that changes in osmotic fragility induced by the mixture depends mainly upon sugar transport across erythrocyte membrane. Addition of blood plasma (0.3 - 6%, final concentration) inhibits an increase in osmotic fragility induced by the mixture solution. Blood plasma incubated at 56°C for 30 min represents more effective inhibition than native plasma, on the other hand incubated blood plasma (91°C, 30 min) does smaller one than native plasma. These results suggest that there are possible tow kinds of substances in plasma, one inhibits hemolysis induced by the mixture and another accerelates it, and possible role of prostagrandins and amines as accerelator of this phenomenon is discussed. Effects of Ca^{++}, albumin and α-globulin on this phenomenon result in inhibition of it, however, the manner of inhibition is different each other. The mechanism of inhibition and accerelation of this phenomenon will be reported shortly.

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EFFECT OF LECTINS ON WATER CONTENT OF HUMAN RED CELLS.
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Changes in water content of red cells exposed to lectins were investigated. Red cells were added to phosphate buffer saline containing 5 % plasma, 4 μg/ml sucrose and a lectin and centrifuged. Water and sucrose contents of packed red cells were measured by gas-liquid chromatography. Intracellular water content was obtained by correcting trapped water calculated from the sucrose content. Erythroagglutinin (EA), phytohemagglutinin (PHA) and concanavalin A (Con.A) did not markedly affect to water content of red cells at a range of 10 μg/ml to 200 μg/ml. Only wheat germ agglutinin increased it from the control value of 70.96 % to 72.02 % at 50 μg/ml. Disparity of the effects of EA and WGA having strong agglutinating and hemolytic properties suggested that the change in water content of red cells did not directly relate to agglutination and hemolysis of red cells. According to elevated concentration of the lectins, EA increased trapped water, contrarily, PHA and Con.A decreased it and WGA did not almost change it. As a cause to these phenomena, change in deformability of red cells may be speculated.

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GLOBIN-CHAINS BIOSYNTHESIS BY FETAL LIVER ERYTHROPOIETIC BURSTS IN VITRO. TERASAWA, T., UGIIE, K., MATSUDA, M., ITO, T AND KASAI, S., Dept. of Physiol., Tohoku Dental Univ., Koriyamashi, Fukushimaken.

We examined globin-chains biosynthesis by fetal liver erythropoietic bursts (12-wks gestation) in methylcellulose clonal cell culture. Globin-chians were labeled with ^{14}C-amino acid. Alpha, beta, and Gγ and Aγ globin chain biosynthesis by individual erythropoietic bursts were carried out by isoelectric focusing in polyacrylamide slab gels containing 8M-urea and 3% Nonidet P-40 (a nonionic detergent) and fluorography. The relative proportions of alpha, beta, Gγ and Aγ globin chains in fetal liver bursts were 51.2%, 2.4%, 33.2% and 13.2%, respectively. Gamma chains comprised 95.1% of total non-α-chains and the Gγ : Aγ ratio was 0.72. The relative proportions Gγ and total γ chain biosynthesis in 34 separate erythropoietic bursts were 0.70 ± 0.04 and 0.97 ± 0.02.

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DEFORMABILITY OF ERYTHROCYTES IN IRON DEFICIENT RATS MEASURED BY LASER DIFFRACTOMETRY. NAGASAWA, T., KINOSHITA, Y. Dept. Physiol. Osaka City Univ. Med. Sch. Osaka 545, Japan

Red cell deformability was measured by rheocytometry which is a laser diffractometric method using a flat glass cell (0.21x7x30 mm^3) and helium-neon laser. For the measurement 50 ul of blood were mixed with 10 ml of 15% dextran T40 dissolved in phosphate-buffered saline (290-295 mosmol, pH 7.4) and red cells were subjected to shear stresses by causing the red cell suspension to flow through the flat cell and diffraction pattern was obtained on the screen. The deformability index (DI) is calculated using equation; DI= (L-W)/(L+W) where L is the measured vertical dimension of the diffraction pattern and W is the holizontal dimension.

The deformability curve for iron-deficient red cells was below that of normal cells and the deformability of iron-deficient cells was recovered to normal level by providing control diet, correlating with the hematologic parameters (RBC, Ht,Hb, serum iron). The osmotic fragility and MCHC of iron-deficient red cells were decreased and spectrin extractability in 1 mM EDTA solution was reduced, suggesting that reduced deformability of iron-deficient red cells is mainly contributed to increased membrane stiffness.

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ROLE OF PLASMA PROTEINS AND RED CELL SURFACE CHARGE ON THE RED CELL AGGREGATION. MAEDA, N., IMAIZUMI, K.* AND SHIGA, T. Dept. Physiol., Sch. Med., Ehime Univ., Shigenobu, Onsen-gun, Ehime 791-02 (* Dept. Health Sci., Joetsu Univ. of Education, Joetsu, Niigata 943)

The effects of various plasma proteins and the negative charge of red cell surface on the human red cell aggregation was studied in a low shear rate (7.5 sec^{-1}) by the TV-image analyzer, kinetically. (1) The red cell aggregation was remarkably augmented by increasing fibrinogen concentration in the presence of albumin. (2) The effect of fibrinogen was further accelerated by reducing the negative charge of red cell surface (which was mainly dependent upon the sialic acid content and was removed by treating with neuraminidase). However, the desialylated red cells never aggregated in the fibrinogen-depleted medium. (3) Immunoglobulins were also effective in the order of IgM > IgA $\geq$ IgG. Fragment, $F(ab')_2$ was more effective than IgG.

In conclusion, the attractive force of macromolecules bridging among red cells was dependent upon the molecular weight, the stereospecificity (molecular shape, flexibility, ..) and the net charge, and was essential and more effective to red cell aggregation than the contribution of the electrostatic repulsive force due to the surface negative charge of red cells.

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KINETICS OF HEMOGLOBIN INJURY IN NITRIC OXIDE EXPOSED RAT BLOOD. SHIGA, T., MAEDA, N. KON, K. AND IMAIZUMI, K.* Dept. of Physiol., Sch. of Med., Ehime Univ., Ehime 791-02 and *Dept. of Health Sci., Joetsu Univ.Educ., Joetsu, Niigata 943.

Nitric oxide (NO) passing through alveoli binds with hemoglobin in circulating blood, then nitrosylhemoglobin (NO-Hb) is formed. The reaction between NO-Hb and dissolved oxygen yields methemoglobin (MetHb), which can be reduced to deoxyhemoglobin by MetHb-reductase in red cells. The time course of NO-Hb and MetHb production, in NO-exposed (100 and 200 ppm) rat blood were measured with electron spin resonance. The amount of NO-Hb reached to steady level within 1 hr, while that of MetHb did not saturate up to 3 hr at 200 ppm NO. The amounts of MetHb were 7-15 times higher than those of NO-Hb (25-300 ppm NO, for 1 hr). In order to estimate the kinetic parameters, the computer simulation was performed.

The rate limiting step was MetHb reduction (first order rate constant was ca. $2x10^{-2}$ min^{-1}, at 100 ppm NO exposure), which became slower as increasing the NO pressure. The rate constant of MetHb formation was constant (ca. $2.5x10^{-1}$ min^{-1}).

Though NO is toxic due to NO-Hb production, the inevitable MetHb formation acts as a detoxication process because of the reduction to Hb at lower levels of NO exposure. The overproduction of MetHb is main injury at higher levels of NO exposure.

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NANOSECOND FLUORESCENCE STUDIES ON THE MICROVISCOSITY OF ERYTHROCYTE MEMBRANES —EFFECT OF TEMPERATURE AND MEMBRANE PROTEINS—. ARAISO, T., KOYAMA, T., ARAI, T., AND SHINDO, T. Div. of Physiol., Res. Inst. of Appl. Electr., Hokkaido Univ., Sapporo, 060

Fluorescent probe DPH (1,6-diphenyl-1,3,5-hexatriene) in lipid bilayers makes wobbling motion with characteristic wobbling diffusion rate which can be detected by means of nanosecond fluorescence depolarization technique. The wobbling diffusion rate (D) relates to microviscosity (η) as: $D=kT/6\eta Vef$, where Ve represents an effective volume of DPH and f, shape factor. For normal erythrocyte membranes the microviscosity has been determined to be 0.4 poise at 37°C. At low temperature, increase of the microviscosity was measured (0.9 poise, at 20°C), while high temperature measurement did not show its decrease (0.4 poise, at 50°C). Glutaraldehyde treatment for fixation of membrane proteins gave very low values (<0.1 poise, at 37°C). On the other hand spectrin free membrane showed rather high viscosity (0.6 poise, at 37°C). Those results indicate that the membrane proteins control the microviscosity of membranes by regulation of free thermal motion of phopholipid molecules. When the structure of the network of the membrane proteins is rigid, the microviscosity was low. Thus, it would appear that the role of the protein network is not only to keep the shape of erythrocytes but also to decrease the microviscosity of lipid bilayers.

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GAMMA CHAIN COMPOSITION OF FETAL HEMOGLOBIN IN HUMAN ADULT AND NEWBORN BLOOD. ENOKI, Y., NAGANO, R* AND OHGA, Y. Second Dept. Physiol., Nara Med. Univ., Kashihara, Nara 634

This study was intended to clarify the molecular mechanisms of the postnatal synthesis of three types of the γ chains, Gγ(136 Gly, 75 Ile), AγI(136 Ala, 75 Ile) and AγT(136 Ala, 75 Thr) under a marked de-expression of the respective genes. Fetal hemoglobins(Hb F) were isolated by alkali denaturation-salting out technique from 121 samples of normal adult blood. Their γ chain compositions were determined by a reversed phase high performance liquid chromatography to compare with those of cord blood from 70 newborns. In the newborns with the average Hb F of 80 %, Gγ and Aγ levels were controlled in a very narrow range as shown by the average Gγ value of 66.5±5.7 %. Fifteen cases of heterozygosity for AγT gene were found and the gene frequency was estimated as 0.107. In the adults with the average Hb F of 0.5 %, the γ chains were present in highly variable levels, which suggested that the postnatal de-expression of the genes might proceed in a very variable and non-coordinated fashion. Thirty-one cases heterozygous and 3 cases homozygous for AγT gene were detected and the gene frequency was 0.153.

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QUANTIFICATION OF PLATLET AGGREGATION BY ELECTRIC PARTICLE COUNTING METHOD WATANABE, M., HIGASHI, T., ISOMOTO, A., AND TYUMA, I. Dept. Physicochem. Physiol., Med. Sch., Osaka Univ., Osaka 530.

ADP induced human platelet aggregation was quantitatively estimated using electric particle size analyzer. 50 µl of platelet rich plasma was diluted in 40 ml of buffer solution and stirred at 330 rpm. 0.8 ml of 5.0×10^{-3}M ADP was added to induce platelet aggregation. Distribution of particle size was analyzed at 10 minutes interval during aggregation of platelet occured. From the rate of decrease of particle counts which size was thought to represent a single platelet, the kinetics of platelet aggregation was estimated. Also with a least-square fitting method, the observed distribution curves of log-scaled platelet cell volume was analysed as the superpositions of the six Gaussian functions, each representing the single and aggregated (2 to 5 and above 6) cells. Of the 18 parameters, 12 were fixed at the theoretical values. And kinetics was analysed from the reduction of the single cell counts.

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ENHANCING EFFECT OF COLLAGEN ON PLATELET AGGREGATION IN WHOLE BLOOD. FUNAHARA, Y., DHARMA*, R., KUROKAWA, W., UCHIYAMA, T., OGAWA, K., MIKI, M., HIRATA, M. AND KITAGUCHI, H. Dept. of Physiol., Kobe Univ. School of Med., Kobe, Hyogo 650 and *Dept. of Clin. Pathol., Indonesia Univ., Jakarta, Indonesia

In order to know whether or not red cell or/and white cell influence on collagen, epinephrine or ADP-induced platelet aggregation, the following experiments were done by using human citrated blood and Coulter Counter®. The aggregation reaction was performed in an aggregometer at 37°C, and the reaction was monitored by checking residual free-platelet number in whole blood or platelet rich plasma (PRP) of which platelets were fixed by 2% formaldehyde-Isoton II® before platelet number determination. Concentration of agents in whole blood was adjusted by heamatocrit value of 45%. Obtained results are the followings: 1. Collagen induced aggregation-reaction in whole blood was more distinct than that in PRP. 2. The enhancement was not observed when ADP or epinephrine was used as an inducer of the aggregation-reaction. 3. At very low concentration (9-18 μM), Dilazep®, red cell membrane stabilizer, inhibited the reaction more effectively than that of Aspirin (ASA). 4. Combination use of the drugs was shown to be very effective method for suppressing the collagen induced aggregation reaction in whole blood.

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CHANGES IN COAGULABILITY OF BLOOD RELATED TO AGE IN FISCHER RATS. MATSUDA,T., OGAWARA,M., MIURA,R., AND SEKI,T. Division of Clinical Physiology(II), Tokyo Metropolitan Institute of Gerontology, Tokyo 173.

Levels of factors XIII, XII, XI, X, IX, VIII, V, II and I (fibrinogen), antithrombin III and α_2-antiplasmin in plasma were determined in 92 Fischer rats, 6, 12, 24 and 30 months of age. Factor XIII activities were assayed using factor XIII deficient fibrinogen. Plasma fibrinogen content was measured using thrombin time method. Levels of antithrombin III and α_2-antiplasmin were determined using chromogenic substrate. Determinations of levels of the other clotting factors were carried out using one-stage method. In the Fischer rats, activities of factors XII, V and XIII were higher than human, whereas concentrations of factors XI, X, IX and VIII were lower. Levels of antithrombin III and α_2-antiplasmin were also higher in the rats than in human. In the Fischer rats, levels of factor VIII and of fibrinogen increased and concentrations of factors IX and XIII decreased with ageing. Activities of factors X and II, levels of antithrombin III increased with age in the male rats. There were significant correlations between levels of factors VIII and V, factors XII and X, factors X and II, as well as factor II and antithrombin III. As a whole, cogulability of blood in Fischer rats was lower than in human and was not enhanced with ageing unlike in human.

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ENZYMATIC PROPERTIES OF THROMBIN-LIKE ENZYME FROM VENOM OF TRIMERESURUS FLAVOVIRIDIS. ARIGA, Y., KOSUGI, T. AND KINJO, K. Department of Physiology, School of Medicine, University of Ryukyus, Uehara 207, Nishihara-cho, Okinawa

It has been reported that thrombin-like enzymes was purified from venom of Trimeresurus flavoviridis and some physicochemical properties were previously investigated. In this paper, it was studied in detail that the differences on the enzymatic properties between this enzyme and bovine thrombin. On behaviour of this enzyme to fibrinogen originated from various mammalians, it was clarified that the only fibrinogen from rabbit could be converted into fibrin and during the process of the fibrinogen-fibrin conversion this enzyme released a peptide identifying as the fibrinopeptide A by means of determining a mobility on paper chromatography. The peptidase activity of this enzyme was significantly smaller than it of bovine thrombin on the measurement using S-2238 (H-D-Phe-Pip-Arg-pNA) and specificity to chromogenic substrates was different from bovine thrombin. Futhermore, this enzyme was inhibited by DFP and Leupeptin, however, was not by TLCK, hirudin and AT III-heparin.

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INCREASE OF FIBRINOLYTIC ACTIVITY IN THE LUNG AND SPLEEN OF STREPTOZOTOCIN-INDUCED DIABETIC RATS.

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Diabetes in man is associated with a high incidence of thromboembolic disorders. Many investigators reported increased activities of coagulation and platelet aggregation, but there is no such agreement about fibrinolysis in diabetic patients. This report deals with the behavior of the activities of plasminogen-activator and non-plasmin fibrinolytic protease (Okamoto,U. et al. Thromb.Haemostas.,1979,42,726-733) in streptozotocin(STZ)-induced diabetic rats.

In the diabetic rats, although activated partial thromboplastin time was elongated, dilute blood clotlysis time was shortened in most cases, indicating the activation of thrombolysis. As to the tissue fibrinolysis, non-plasmin fibrinolytic protease activity in the lung and spleen increased significantly ($P<0.001$) and tissue plasminogen-activator activity was also increased in the lung. Studies on the variation of plasminogen-activators of the lung indicated that either soluble activator or insoluble activator was nearly simultaneously increased, which suggests the possible release of plasminogen-activator from the tissue to the blood. It is likely that plasminogen-activator of usual type and non-plasmin protease as well are also involved in diabetic thromboembolic disorders.

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NMR BEHAVIOR OF WATER IN COAGULATION AND FIBRINOLYSIS.

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Fibrinogen in solution was made into fibrin gel by coagulation and the gel was lysed to sol by fibrinolysis. In each stage, the hydration was examined by using the relaxation time (T_1 and T_2) of water protons (D_2O in 10 % for NMR field lock) resonant at 80 MHz on Bruker's NMR-spectrometer. The T_1 and T_2 at 24 $\pm$ 0.5 °C were measured to within 3 % deviations from the mean by adjusting the 180° pulse width between 95.4 and 109.0 microsec for each sample. As the solution sample, 5 ml borate-saline buffer solutions of 1 to 50 mg/ml fibrinogen (Miles-Sankyo's, 90 % clottable) were taken into the 20 mm tubes. To some of them were added 0.3 ml solution of thrombin (Mochida's) to make the gel within a few minutes. To others were added a few drops of human serum (our clinic's) diluted with an SK solution (Lederle's Varidase) and also 0.3 ml of thrombin solution to make the sol in from 0.3 to 30 hours. With an increase of fibrinogen in each stage, both T_1 and T_2 decreased exponentially and reached a plateau. The T_1 changed from 3.4 to 2.2 sec, though there was little difference among those of three stages. However, the T_2 changed from 2.2 to 0.2 sec for the gel, to 0.4 for the solution and 0.9 for the sol, suggesting the extent of hydration in g/g to be 75, 50 and 25, respectively.

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FIBRINOLYTIC ENZYMES EXTRACTED FROM THE EARTHWORM.

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Our recent studies revealed a very strong and novel fibrinolytic enzyme extracted from the earthworm, Lumbricus rubellus. Purification of the enzyme was performed. Three crude active fractions were obtained and designated F-I, F-II and F-III. These three fractions were further purified respectively. F-I was divided into three fractions, and F-III into two fractions. Each of the total six fractions revealed a single band on SDS-PAGE. The molecular weights ranged between 21,500 and 34,200, and the isoelectric points were between pH 4.12 and pH 3.52. LBTI and DFP completely inhibited all enzymes. SBTI completely inhibited the II, and III fractions, but showed little effect on the I fractions. From these results, the enzymes were considered to be serine proteases. The amino acid compositions of each enzyme indicated very abundant asparagine or aspartic acid, which may be related to the fact that the enzymes were acidic and stable to heat and pH. However, there was very little proline and lysine. The earthworm enzymes are clearly distinct from other well-known fibrinolytic enzymes. The III fractions readily split arginine and lysine bonds, while the I fractions split tyrosine bonds. Based on these findings, the fraction III enzymes are thought to be trypsin-type enzymes, and the fraction I enzymes to be chymotrypsin-type enzymes.

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FIBRINOGEN-FIBRIN CONVERSION MEASURED BY USING OSCILLATING-TUBE DENSITOMETER —— A NEW METHOD FOR STUDYING THE MECHANISM OF FIBRIN-POLYMER FORMATION. HIGASHI, T., TAKASHIMA, N*, SAGAWA, S*, WATANABE, M., NAKAMURA, Y*, ISOMOTO, A. AND TYUMA, I. Dept. Physicochem. Physiol., Med. Sch., Osaka Univ., Osaka 530

The change of the solution density in the thrombin-catalyzed fibrinogen-fibrin conversion is continuously measured with the oscillating-tube densitometer (Anton Paar DMA60, Measurement accuracy: $1 \times 10^{-6} g/cm^3$). The reaction curve is sigmoid. This fact may suggest the rate determining step of the fibrin polymerization is co-operative. Chemical kinetics study of the reaction rate at the point of inflexion shows that the reaction rate is directly proportional to the concentration of thrombin, and the K_m for fibrin-polymer formation in Tris·Cl buffer (pH 8.5, 0.05M, ionic strength 0.45) is about 2×10^{-5}M. The gel-filtration by high performance liquid chromatography (Toyo Soda G4000SW & G6000PW) shows that only a 5% change of the maximum density change is observed when 40% fibrinogen or fibrin monomer has already converted to the small linear oligomers. Kinetics of ligation of fibrin polymers is studied by using cross-linking technique by thrombin-factor XIII and polyacrylamide gel electrophoresis with disulfide reduction to separate the α-,β-,γ-chains. The density change seems to agree with the α-polymer formation rather than the γ-dimer formation. We can briefly follow the entire course of the fibrin-polymer formation by this method.

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STUDIES ON THE FIBRINOLYTIC ENZYME SYSTEM IN HEPATOBILIARY SYSTEM(XII)— TISSUE LOCALIZATION OF BILOKINASE(BK)— OSHIBA, S., IMAI, H., YOSHIDA, M, SAWAI, H., ENDOH, E., OGASAWARA, Y. AND *ARIGA, T. Dept. of Physiol., Nihon Univ. Schol. of Med., Tokyo, *Dept. of Nut. and Physiol., Nihon Univ. Colle. of Agricul. and Veteri., Tokyo

The purpose of present investigation is to elucidated the immunological properties of BK by comparing them with those of UK, and to detect the tissue localization of BK by utilizing an immunofluorolecent technique. Duble immunodiffusion patterns a clear single precepitin line between bovine BK and its anti-serum. There was no cross-reaction between bovine BK and bovine UK. The anti-bovine BK serum showed strong quenching against bovine BK in fibrin clot lysis, but did react against bovine UK. The identical relationship was seen, to a lesser extent, between anti-bovine UK serum and bovine UK. The immunofluorescence of bovine BK was observed to localize on hepatic cells, and gallbladder mucosa. It was also detectable in blood vessel walls. While the localization of bovine UK was limited to urinary tubles. It is of interest to observed that cultured hepatic cells secrete plasminogen activator into these medium.

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THE USE OF A PLASTIC MICROTITER PLATE FOR THE COLORIMETRIC DETERMINATION OF PAH AND OTHER SUBSTANCES IN SMALL SCALE. YOSHIDA, T., Dept.Physiol., Mie Univ. School of Med., Tsu, Mie 514.

A plastic microtiter plate was used for the colorimetric determination of PAH,inulin and creatinin in small scale. For example, PAH was determined by successively adding 30 μl of 2 N HCl, 10 μl of 1 mg/ml sodium nitrite, 10 μl of 0.25 g/ml urea and 10 μl of 0.2 % 1-(diethylaminoethyl)-naphtylamine oxalate(Tsuda reagent) into 120 μl of sample solution previously put in the well of microtiter plate(MP) and, after mixing for 10 min., by reading A_{570} with a MP reader. The anthrone method for the determination of inulin was also performed on a MP, by mixing 20 μl of sample solution and 150 μl of 0.08 % anthrone in 80 % H_2SO_4 in the well of MP while keeping the MP cold, then incubating it at 38 ℃ for 1 hr and by reading A_{630}. With these methods, the detection limit of both PAH and inulin was about 100 ng and the amounts of sample and the reagents required were 1/20 to 1/50 of those in the ordinary methods. A similar modification of the colorimetric method for the determination of creatinin using picrate was also carried out with MP. These methods were applied for monitoring the renal clearances of these substances in rabbits in the investigation of the effect of a physiologically active peptide, bradykinin, on the renal function of rabbit.

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INFLUENCES OF PROSTAGLANDINS ON THE VASOPRESSIN-INDUCED OSMOTIC WATER FLOW OF THE TOAD BLADDER MARUMO,F., SHIMADA,H., SAKAMOTO,H. AND NARA, Y., Department of Medicine, Kitasato Univ. Sch. Med., Sagamihara, Kanagawa 228

Influences of prostaglandins on the vasopressin(Vp)-induced osmotic water flow across the bladder of toad, Bufo bufo japonicus, were examined. After double pithing of the toads, the bladders were excised and halved, and each hemibladder was mounted in a glass chamber. To measure the osmotic water flow volumetrically, Ringer's solution diluted to 1/5 of normal was applied to the mucosal chamber. All drugs were applied to the serosal medium.

PGH_2 inhibited the Vp-induced water flow at 10^{-7}M. PGE_2 inhibited the water flow at 10^{-8}M, while PGI_2, PGD_2 and $PGF_{2\alpha}$ did not at 10^{-6}M. Indomethacin enhanced both Vp- and cyclic AMP-induced water flow, and the maximum activation doses were approximately 5×10^{-9}M and 5×10^{-7}M, respectively. PGE_2 inhibited the Vp-induced water flow with dose-dependent manner, while did the cyclic AMP-induced flow without dose dependency. These results indicate that PGE_2 physiologically affect the Vp-induced water flow and inhibit it by suppressing cyclic AMP production. PGE_2 also inhibits the Vp effect at the point of after cyclic AMP production, however, this effect may be non-physiological.

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H-NMR RELAXATION TIMES T_1 AND T_2 OF WATER SIGNALS IN SOLUTIONS OF URINE COMPONENTS. NAKAYA,S., TANAKA,Y., HONJO,R., YOSHIOKA,Y. and YAGI,S. Dept. of Physiol., Sch. of Med., Iwate Med. Univ., Morioka 020

Using a Bruker's 80 MHz H-NMR-spectrometer, the hydration of different human urine components was examined from the NMR relaxation time at room temperature (24 ± 0.5 °C) by the inversion recovery T_1 and the CPMG T_2 methods.

Each sample was prepared in the 20 mm NMR-tube as a 5 ml aliquot of differing concentration (0.1 to 5 M) by dissolving pure reagents (Wako's for NaCl & KCl, Nakarai's for urea) in water and adding 0.5 ml heavy water for the internal lock of NMR. The FT-spectrum of each sample, taken by checking the 180° pulse width in microseconds, showed a single peak of water at 4.78 ppm, from which T_1 and T_2 were obtained. T_1 values among the different components were not significantly different from those of pure water (3.34 ± 0.24 sec). But, with an increase of the concentration, T_2 values showed a tendency to decrease from 3.81 ± 0.84 sec exponentially to 2.47 ± 0.07 sec for NaCl and 0.33 ± 0.08 sec for urea. However, they increased with an increase of KCl above 0.4 M. At heavier concentrations of the KCl solution, elongations in T_2 corresponded to reductions of the increasing rate in the specific gravity, suggesting that the T_2 increase depends upon an increase of the free water volume.

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CONTINUOUS MONITORING OF REDISTRIBUTION OF BODY FLUIDS IN DOGS USING ^{51}Cr-EDTA. ITOH,T., MIKI,K.* AND MORIMOTO,T. Dept. of Physiol., Kyoto Pref. Univ. Med., Kyoto, *Dept. of Physiol., Univ. of Occupational and Environmental Health, Kitakyushu

In order to analyse the reaction of intracellular fluid (ICF) to fluid modifications, plasma volume (PV) and extracellular fluid (ECF) volume were continuously determined in dogs. After the equilibrium of the duistribution of ^{51}Cr-EDTA, ECF volume tracer, was attained, two kinds of fluid modifications were carried out for 10 min at a rate of 1 ml/min-kg: (1)isotonic dialyzation (2)infusion of hypertonic mannitol solution. In the former, PV decreased by 4 ml/kg and ECF by 5 ml/kg despite the water loss of 10 ml/kg, and continuous the profile of ECF showed a significant mobilization from ICF. An osmotic force mediated by hyperglycemia was considered as the mechanism of this shift of water. The results of the experiment (2) were served to assess the filtration coefficients in capillary bed and in cellular membrane. A mathematical model was postulated using Kedem-Katchalsky equation, and non-linear minimization was performed to obtain the best-fit of the model to the transient data. The values of Lp were 3.2×10^{-11}ml/sec-dyne in capillary and 0.05×10^{-11}in cellular membrane, respectively. Using these parameters, dynamic changes of body fluids after body fluid modifications were satisfactorily simulated.

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ON THE PHASE ASSIGNMENT IN PULMONARY VENTILATION CYCLE IN BULLFROGS·

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In the bullfrog pulmonary ventilation cycle(PVC) currently accepted, similar pattern in the sternohyoid nerve(SH) activity appears before and after expiration-inspiration(E-I) phases. Whether or not both activities should be incorporated in the PVC was investigated. The respiratory electrical activities were simultaneously taken from the SH and trigeminal(V) or hypoglossal nerve(XII), two or three hours after urethane injection (1.0 - 1.5 g/Kg) and surgical operation. The peak integrated SH activity in the first phase was significantly higher(1.2 times) than that in the last phase of PVC, which was the same magnitude as that during buccal ventilation cycle(BVC). When ventilation was stimulated by N_2 inhalation, SH activity in the first phase, not in the last phase, proportionally increased with the inspiratory XII activity. When the moderate pinch stimulus(0.2 - 2 sec duration) was applied to the finger of the fore leg during apnea which was drived by urethane anaesthesia and artificial hyperventilation with air, tonic and long lasting SH activity preceded the E-I nerve activities, followed by slight SH activity. When the same stimulus was given at various points during time course of BVC, which appeared under light anaesthesia, highly augmented SH activity was obtained only before E-I activities. All of these results led us to conclude that only the first SH activity should be incorporated in a PVC.

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MODIFICATION OF PHYSIOLOGICAL FUNCTION IN CATS UNDER HYPERBARIC ENVIRONMENT AT $PICO_2$=0.16 bar (1.02 & 11ATA, $He-O_2$, N_2-O_2)

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We dicussed about the effects of high $PICO_2$ (0.16 bar) on heart frequency(fH), respiratory frequency(fR), brain temperature(TB) and sleep of cats in the different ambient pressure (1.02 ATA, 11 ATA) and inert gases ($He-O_2$, N_2-O_2). Results shows that the difference in ambient pressure and breathing gases did not produce change in fH which tend to decrease as the exposure time elapsed. As for fR, it remarkably increased when the cats breathed either $He-O_2$ or N_2-O_2 at 1.02 ATA, or $He-O_2$ at 11 ATA. However, no noticeable increase was observed in N_2-O_2 at 11 ATA. In regard to the sleep stage, there appeared paradoxical sleep(PS) in two cats out of four in N_2-O_2 at 1.02 ATA and in one cat out of four in $He-O_2$ at 11 ATA. In many cases cats died in a state of and coma with brain temperature of 32-33°C.
These results suggest the correlation of inert gas (N_2, He) and CO_2 in hyperbaric environment.

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AN ASSESSMENT OF OVERALL 'GAIN' OF CO_2-VENTILATION FEEDBACK CONTROL SYSTEM IN HYPOXIA.

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Overall gain of the CO_2-feedback control system in hypoxia (Gco_2H) was determined on 8 male and one female healthy subjects. The external dead spaces used were plastic tubings of 250, 500 and 750 ml in capacity. The subjects breathed in the closed circuit, and were subjected to the progressive hypoxia test. Gco_2H was calculated from the ratio of the slope of the CO_2 response curve (S) to that of metabolic hyperbola (S_L). Gco_2H was fairly larger than the overall gain of the O_2-feedback control system (Go_2) previously obtained. This was explained by both higher S and smaller S_L in the present experiment than in the previous one obtained Go_2.
With addition of 250, 500, and 750 ml of external dead spaces, the end-tidal Pco_2 increment (ΔP_{ETCO_2}·actual) was experimentally observed. The ΔP_{ETCO_2}·actual thus obtained was found to be in good agreement with the end-tidal Pco_2 increment deduced from Gco_2H (ΔP_{ETCO_2}·expected). This result was similar to that previously seen in normoxia experiment.

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EFFECTS OF SITS ON THE PH AND ELECTROLYTES CHANGES IN DOG BLOOD PRODUCED BY CO_2 TITRATION. HATA, N., AKIYAMA, S. AND HONDA, Y. Dept. of Physiol., Sch. of Med., Chiba Univ. Chiba 280.

The acid-base and electrolytes changes in SITS (10^{-3}M) treated and untreated dog blood were studied by titration with low ($5\%CO_2 + 18\%O_2$) and high CO_2 gas ($12\%CO_2 + 18\% O_2$). After equilibration to low or high gas for 60 min, plasma (pHp) and cell pH (pHc), plasma $[Cl^-]p$ and cell chloride concentration $[Cl^-]c$, blood and plasma total CO_2 contents, water contents and Ht were determined in the blood with and without SITS. Plasma bicarbonate increments due to CO_2 titration were inhibited by SITS, while cell bicarbonate increments were not. $[Cl^-]c$ in the control blood was increased by CO_2, but $[Cl^-]c$ in the SITS blood was not. pHp–pHc relationship of the control blood was pHc=7.215+ 0.956(pHp-7.40) (r=0.993, n=22), and of the SITS blood was pHc=7.217+0.908(pHp-7.40) (r=0.977, n=22), both being highly significant. Their correlations were not statistically differed each other. Regression lines of electrolyte distribution ratio (r_{H^+}, r_{Cl^-}, $r_{HCO_3^-}$) against pHp were slightly higher in the SITS than in the control blood. Their distances were also no statistically significant.

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A STUDY ON THE OXYGEN PASSAGE THROUGH ERYTHROCYTE MEMBRANES. KOYAMA, T. AND ARAISO, T. Division of Physiology, Res. Inst. Appl. Electr., Hokkaido Univ. 060 Sapporo

The effects of lipid bilayers of erythrocyte membranes on the passage of oxygen into erythrocytes was investigated by means of a nanosecond fluorometer and a fluorescence probe, diphenylhexatriene (DPH). Thermal motion of DPH molecules entrapped in erythrocyte membrane ghosts showed a microviscosity of 0.4 poise (a viscosity caused by a two dimensional collision in a microstructure) and wobbling angle of 40 degree at 37°C. This wobbling angle indicates that the space of the wobbling motion has a diameter of 13 A. The bulk viscosity (due to a three dimensional collision) of dipalmitoylphosphatidylcholine which is the main component of the lipid bilayers of erythrocyte membrane was estimated to be greater than 4 poise. An extraporation from these results suggests that the practical membrane microviscosity (one dimensional collision) against the passage of oxygen molecules is smaller than 0.4 poise by a factor of 1/10. Thus, the lipid bilayers of erythrocyte membranes form no barriers for oxygen diffusion.

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DEVELOPMENT OF MICROFLUOROMETER FOR pH MEASUREMENT. NIIZEKI, K. AND MOCHIZUKI, M. Dept. of physiology, Yamagata University, School of Medicine, 990-23 Yamagata.

A new device for measuring pH in a microphotometric region was developed by using pH-sensitive fluorescence of 4-methylumbelliferone. An UV excitation spot of ca. 50 μm diameter was injected in a thin layer of hemoglobin (Hb) solution and RBC suspension with highly concentrated carbonic anhydrase (C.A.). The pH change following a sudden change in P_{CO_2} in the gas phase was recorded through a photomultiplier, R-268. The S/N ratio was significantly increased by a use of a long-pass-filter. The measured half-time was linearly related to Hb concentration or the hematocrit. In the observed layer, HCO_3^- ions are hydrated from CO_2 molecules catalyzed by C.A., and diffuse together with molecular CO_2. Thus, we solved the simultaneous diffusion equations for CO_2 and HCO_3^- by using a modified Henderson-Hasselbalch equation, and attempted to estimate the diffusion coefficient of HCO_3^-. The coefficient was reduced as the Hb concentration was increased, as seen in our previous observation. In RBC suspension the extracellular pH change was slower than that of Hb solution in a hematocrit range of 30 to 50 %, whereas it was faster in SITS pretreated RBC suspension. That is, the extracellular pH change seemed to be limited dominantly by the HCO_3^- shift across the RBC membrane despite presence of C.A..

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CHANGE IN Pco_2 IN RED CELL SUSPENSION FOLLOWING HCO_3^- SHIFT AND CO_2 DIFFUSION

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In order to clarify the CO_2 diffusion process into and out of the red blood cell (RBC), Pco_2 changes following the HCO_3^- shift (primary HCO_3^- shift) and the CO_2 diffusion (primary CO_2 diffusion) were examined. In the primary HCO_3^- shift, RBC suspension was mixed with saline solution containing different HCO_3^- concentrations at the same Pco_2, and equilibrated in a closed chamber at 37°C. The Pco_2 change depended on both the initial intracellular HCO_3^- concentration and the hematocrit as well as the amount of HCO_3^- shift. In the primary CO_2 diffusion, RBC suspension was mixed with the extracellular fluid separated preliminary from the same RBC suspension, after an equilibration at different Pco_2. The above Pco_2 changes were compared with the theoretical ones calculated from intracellular Henderson-Hasselbalch equation and a relation between both changes in buffer base and intracellular pH. The calculated Pco_2 changes in each equation coincided well with the measured one : The differences between them were 0.4 ± 3.4 and 5.8 ± 8.3 Torr (mean ± SD), respectively, suggesting the validity of the theoretical equations. These equations would be important conjugate ones to describe the mechanism of the coupled HCO_3^- shift and CO_2 diffusion through the RBC *in vivo*.

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EFFECT OF HCO_3^- SHIFT ON THE CO_2 DIFFUSION IN THE RED BLOOD CELLS.

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A computer program for a numerical solution of the CO_2 diffusion in the red blood cell including the HCO_3^- shift was developed. Comparing the calculated extracellular Pco_2 curve with the experimental data on the primary and secondary CO_2 diffusions, the permeabilities of CO_2 and HCO_3^- across the red cell membrane, $\eta(CO_2)$ and $\eta(HCO_3^-)$, were determined. The dependencies of the CO_2 diffusion rate on the hematocrit and initial in- and extracellular HCO_3^- concentrations were examined by using the above permeabilities. The half-time of the extracellular Pco_2 change in a closed RBC suspension was obviously reduced as the hematocrit and initial extracellular HCO_3^- increased, while the effect of the initial intracellular HCO_3^- was not significant. The CO_2 diffusion *in vivo* was simulated by using pulmonary and tissue capillary models. The hlaf-time of the extracellular HCO_3^- change was about 0.15 sec, while that of the intracellular HCO_3^- change ranged from 40 to 60 msec.

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MORPHOLOGY OF PHRENIC MOTONEURONS IN THE CAT.

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Physiologically identified single phrenic motoneurons(PMs) were intracellularly stained with horseradish peroxidase and reconstructed three-dimensionally. Intracellular recordings were obtained from 29 PMs and measured each axonal conduction velocity. Thirteen of these cells were successfully stained. Each cell soma was located in the ventral horn which corresponded to the nucleus latero-medialis(LM) of Rexed' lamina IX. The dendrites were distributed in the three separated area. The main bundle of dendrites extended rostro-caudally within the phrenic motor nucleus, and its length was over 1 mm from cell soma. The dorso-laterally directed dendrites ran through lamina VII and entered into the lateral funiculus, while those directed dorso-medially reached the anterior funiculus and some dendrites crossed the midline, then entered into the contralateral gray matter. Each of 13 stem axons had no axon collaterals. The axonal conduction velocities ranged from 35m/sec to 75m/sec(n=29). The size of PMs was defined as the mean of major and minor diameters in the horizontal plane and ranged from 44μm to 69μm(n=13). The axonal conduction velocities was plotted against the size of cell soma and there was the positive correlation between them.

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PROJECTIONS OF CERVICAL AND MEDULLARY RESPIRATORY NEURONS TO THE PHRENIC NUCLEUS IN THE CAT.

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We have recently demonstrated that respiratory neuron activities could be recorded within the upper cervical (C1-C2) cord in lightly anesthetized cats. In the present study, we examined descending axonal projections of the cervical and medullary respiratory neurons to the phrenic motor nucleus by electrophysiological analysis and the HRP retrograde tracing method. In the electrophysiological analysis, most of the cervical units(92%) were antidromically activated by the stimuli delivered to the ipsilateral C5-C6 ventral funiculus close to the phrenic nucleus, while 65% of the respiratory neurons of N.Retroambigualis were activated by the contralateral stimuli. In the HRP study, retrograde transport of HRP injected into the phrenic nucleus(1.0 μg) in C5-C6 segments was utilized. HRP-labeled neurons were localized in the lateral margin of the intermediate zone of the gray matter of C1-C2 segments ipsilateral to the injection site and mostly in the contralateral N.Retroambigualis and N.Tractus Solitarius.

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SLOW DEPOLARIZING POTENTIALS PRECEDING RESPIRATORY SPIKES INTRACELLULARY RECORDED BEHIND THE TRIGEMINAL EMINENCE IN THE BRAIN-STEM OF LAMPREYS.

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In vertebrates, the precise mechanism of rhythmic respiration is not fully understood. In an effort to find the central neurons essential for breathing, medullary respiratory activities were investigated throughout the entire body of the adult lamprey. Brain sectioning transversally immediately behind the trigeminal eminence (TE, a ventricular prominence of the motor nucleus of V) revealed that TE is not indispensable for rhythmic respiration. Partial slitting of the midline of the brain indicated that bilateral commissures do not localize within the medulla but diffusely distributing throughout the brainstem. After immobilization (i.v., d-tubocurarine) the viscero-motor zone in the medulla was explored by a glass microelectrode; efforts were concentrated on the area of the VIIth nucleus. Slow and smoothly increasing depolarization preceding respiratory spike potentials strongly support the hypothesis that respiratory burst generators in the lamprey are essentially similar to heart pacemakers; prepotentials (spontaneously or endogenously occurring membrane depolarization) may be directly and primarily responsible for generation of respiratory excitation.

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PERIODIC APNEUSIS INDUCED BY STRYCHNINE.

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Change of peripheral inspiratory and expiratory activities caused by strychnine was investigated in anaesthetized, vagotomized and paralyzed cats being maintained by artificial ventilation. Reciprocity between phrenic and expiratory(pharyngeal constrictor nerve) activities decreased by i.v. administration of strychnine. Tonic phrenic and expiratory activities appeared with increase of disinhibitory effect of strychnine. The tonic phrenic activity was interrupted by periodic pausing phase. Discharge pattern of interrupted tonic phrenic activity was similar to so-called apneusis. Duration of strychnine apneusis changed gradually with disinhibitory effect of strychnine. Range of change was from 5 min to several sec. Normal respiratory rhythmicity superimposed small inspiratory fluctuation upon the tonic component of the apneusis. Duration of the pausing phase elongated slightly with the passage of time, but the range of change was very small as compared with duration of the apneusis. Periodic interrupted pattern did not appeared in expiratory activity. These results lead to the conclusion that inspiratory activity keeps a rhythmicity as periodic apneusis in a considerably disinhibitory state.

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THE EFFECT OF INTRACEREBRAL INJECTION OF TRH ON RESPIRATION. OOUCHI, M., HOMMA, I. AND ICHIKAWA, S. Department of Physiology, Showa University, School of Medicine, Shinagawa-ku Tokyo, 142

Thyrotropine releasing hormone (TRH) has been shown to be distributed in the brain-stem and to affect autonomic functions. We examined TRH effects on phrenic nerve activity in vagotomized rabbits. Experiments were performed on 8 rabbits anesthetized with Urethan-Chloralose (initial dose; 450mg(Ure) + 45mg(Chl)/kg I.P.). Injection of saline solution into the fourth cerebroventricle produced no apparent effect, but injection of 1.0μg of TRH solution (30 μl saline solution) increased respiratory rate and facilitated phrenic nerve activity. The peak of the integrated activity either increased slightly or did not change. A marked effect of TRH was the rise of tonic phrenic nerve activity between bursts. This activity was eliminated by the inspiration of CO_2. CO_2 decreased the respiratory rate that was increased by TRH. Results suggest that CO_2 facilitates central respiratory activity to increase tidal volume, while TRH facilitates it to induce tonic activity and increase respiratory rate.

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THE CHEMORECEPTOR RESPONSE OF THE RAT RESPIRATORY CENTER IN VITRO. HARADA, Y., WANG, Y.Z.* AND KUNO, M. Dept. Physiol., Kyoto Univ., Fac. Med., Sakyo-ku, Kyoto 606

The primary stimulus for the central chemoreceptor has been assumed to be H^+, rather than CO_2. In order to test this assumption, the excised respiratory center preparation was devised, using newborn rats. Under ether anesthesia, the brain was transected immediately above the pons. The brain stem and cervical cord were excised together with attached phrenic nerve on the right side. The cervical cord was saggitally hemisected, and the left half of the cord was removed. All the cranial nerves and dorsal roots were cut to abolish the sensory input. The excised preparation was perfused with physiological saline equilibrated with CO_2 (0-7%) in O_2. The pH of the solution was maintained at desired levels (7.0-7.6) by adjusting the $NaHCO_3$ concentration. The respiratory activity was recorded from the phrenic nerve with a suction electrode. Phrenic activity was half-wave rectified and integrated for each 0.1 sec period. Respiratory minute activity was calculated as the product of the peak integrated value and respiratory frequency. Respiratory activity was found to be increased by an increase in P_{CO_2} at a constant level of pH as well as by a decrease in pH at a constant level of P_{CO_2}. It is concluded that both H^+ and CO_2 act as a stimulus for the central chemoreceptor.

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FACILITATORY EFFECTS OF THE DIENCEPHALON ON RESPIRATION IN RABBITS. SAWAKI, Y., TAKIZAWA, N., NISHIDA, S. AND HATAKEYAMA, I., Dept. of Physiol., Sch. of Med., Kitasato Univ., Sagamihara, Kanagawa 228

Effects of electrical stimulation of the diencephalon on respiratory rate were studied in anesthetized rabbits. Repetitive stimulation (2 ms, 2 or 4 V; 20 Hz; 10 s) induced a marked decrease in inspiratory and expiratory duration when the stimulating points were localized in ventromedial, dorsomedial and posterior nuclei of the hypothalamus; mammillary and supramammillary nuclei; lateral hypothalamic area; and Forel's fields H1 and H2, and zona incerta. Stimulation with brief train pulses (2 ms, 4 or 8 V; 100 Hz; 10 pulses) induced switching to the inspiratory phase when the pulses were applied during the expiratory phase in 25 out of 53 points tested. Diencephalic area which induced switching to the inspiratory phase after brief pulse stimulation and that which produced a marked increase in respiratory rate after repetitive stimulation overlapped each other. These results suggest that this area of the diencephalon controls respiration by affecting facilitatory effects on the medullary respiratory center. Computer simulation on model system of the respiratory center pointed out the same results.

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EFFECTS OF VIBRATION ON RESPIRATION RELATED NEURAL ACTIVITY. HOMMA, I., ONIMARU, H. AND OOUCHI, M. Department of Physiology, Showa University, School of Medicine, Shinagawa-ku, Tokyo 142

Respiration is affected by activation of mechanoreceptors located in various parts of the body. Most respond well to mechanical vibration. It is interesting and important to examine how vibratory stimulation affects pulmonary mechanoreceptors. Air oscillations produced by a diaphragm attached to a moving coil were applied in the trachea, and mass phrenic efferent and afferent discharges from the vagus nerve of rabbit were recorded. Airway vibration at 100 Hz did not alter the phrenic bursts, but the silent periods, which correspond to expiration, were markedly prolonged. Vagus afferent activity increased and tonic activity persisted even in the expiratory phase. Airway vibration at 10 Hz caused rapid breathing and the phrenic nerve discharged tonically during both the inspiratory phase and the expiratory phase. Tonic activity was also recorded from vagus afferent nerves and the short bursts were synchronized with the 10 Hz oscillations. Airway vibration at 100 Hz may dominantly facilitate pulmonary stretch receptors, and at 10 Hz it may stimulate rapid adapting receptors.

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PHASE RESPONSE CURVES OBTAINED FROM THE PHRENIC ACTIVITIES BY THE SENSORY AFFERENTS STIMULATION. KITANO, S., KOIZUMI, A., KOMATSU, A. Dept. of Anesthesiology and Dept. of Physiology, Tokyo Women's Medical College, Shinjuku, Tokyo 162

In anaesthetized, paralyzed and vagotomized rabbits, short electrical stimulus trains (250 Hz, 2-21 pulses) were applied to the middle external intercostal, vagal and superior laryngeal nerves at various phases in respiratory cycle. Phrenic discharges were recorded as an indicator of the respiratory cycle. Phase response curves were obtained from the phase shift in phrenic responses. At any period of inspiratory phase, application of optimal stimuli produced marked phase advance, which consisted of I-off switching and shortening of the following expiratory phase. In expiratory phase, the stimuli produced prolongation of expiration and thus resulted in the phase delay. These results indicate that these sensory afferent nerves send inputs to I-off switching mechanisms of medullary respiratory neurons.

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EFFECTS OF HYPERCAPNIA ON INSPIRATORY AND EXPIRATORY NEURONS IN NUCLEUS RETROAMBIGUALIS OF CAT. ARITA, H., OHTA, Y., YAMABAYASHI, H., TAMURA, K., AND TAKAHIRA, H. Dept. Med. and Dept. Physiol., Tokai Univ., Isehara, Kanagawa 259-11

Previous results (JAPYA-1983) indicated that hypercapnia depressed internal (expiratory) intercostal muscle activity with an increase in diaphragm activity. The present study tested the CO2 responses of expiratory (E) and inspiratory (I) units in the nucleus retroambigualis in midcollicular decerebrate, spontaneously breathing cats. We obtained intracellular recordings from I-units with slow depolarizing potentials (SDP); its spike activity had an augmenting pattern. Hypercapnia caused an increase in SDP and spike frequency of the I-units. In contrast, E-units with an augmenting firing pattern showed no significant changes in the firing frequency with a small decrease in number of spikes per breath in response to hypercapnia. We conclude that hypercapnia evokes a distinct excitation of I-units, but E-units are not directly affected by CO2 drive. Thus, reciprocal inhibition of I-unit appears to play a key role in integration of E-motoneurons at segmental level.

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RELATIONSHIP BETWEEN STABILITY OF RESPIRATORY UNIT ACTIVITY AND ITS FIRING PHASE IN THE BRAIN STEM OF RABBITS. KAZUO TAKANO, NAOFUMI KIMURA, HARUMI KITAGAWA AND TAKEHIKO HUKUHARA. Dept. Pharmacol. II, Jikei Univ. Sch. Med., Minato-ku, TOKYO, 105.

Experiments were performed in paralyzed and vagotomized rabbits being maintained by artificial ventilation under monitoring end-tidal O_2 and CO_2 levels. Degree of the stability of the periodicity of reticular respiratory units and phrenic nerve activity was quantified by an indicator, coefficient of variation (CV) for the period of respiratory volley. The value of the CVs measured in respect to the initial or ceasing point of time of volleys varied widely unit to unit. CV of unit activities whose firing initiated or ceased close to the starting or ceasing point of time of the phrenic nerve activity (group A; mean $\pm$ SD, 3.62$\pm$ 1.98%, N=49) was significantly ($p<0.001$) smaller than that of the activities whose discharge initiated or ceased in the midway of the inspiratory or expiratory phase of the phrenic nerve activity (group B; 6.86 $\pm$ 3.67%, N=43). The magnitude of CVs in the group A was comparable to that of phrenic nerve activity (3.11 $\pm$ 2,12%, N=43), but that in the group B was significantly ($p<0.01$) larger than that of the phrenic nerve activity. These results suggest that the initiation and ceasing of periodic discharges of the units in the group A depend more strongly on the activity of the bulbar respiratory rhythm generating mechanisms as compared with those of units in the group B.

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FLUCTUATION OF RESPIRATORY VOLLEYS IN THE SYMPATHETIC AND CRANIAL NERVE ACTIVITIES. KIMURA, N., HATTANMARU, Y., TAKANO, K. AND HUKUHARA, T., Jr., Dept. Pharmacol. II, Jikei Univ. Sch. Med., Minato-ku, Tokyo 105.

Experiments were performed on 37 rabbits being anesthetized with ether, immobilized and ventilated artificially under monitoring end-tidal CO_2 level. Bilateral vagus, depressor and sinus nerves were cut in the neck. The delay time from the ordinate to the first pericentral peak in the cross-correlogram between the phrenic nerve activity and the renal sympathetic, hypoglossal or facial nerve activity was defined as a phase difference. When the phrenic respiratory period was prolonged from 1,200 to 2,550 msec in response to the elevation of end-tidal CO_2 level (2.1 to 5.8%), the phase difference from the phrenic respiratory volley to the renal sympathetic respiratory volley varied widely from 780 to -330 msec (mean $\pm$ SD : 430 $\pm$ 220 msec, N=27) and a negative significant correlation ($r=-0.63$, $p<0.001$, N=27) was proved between the phase difference and the phrenic respiratory period. The phase difference from the phrenic respiratory volley to the hypoglossal respiratory volley fluctuated between -200 and 0 msec (-80 $\pm$ 50 msec, N=30) and the phase difference to the facial respiratory volley ranged from -210 to 60 msec (-80 $\pm$ 70 msec, N=30). These results suggest that the central neuronal organization responsible for sympathetic respiratory volley coupled more flexibly with the respiratory rhythm generator than that responsible for hypoglossal or facial respiratory volley.

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TEMPORAL DIFFERENCES IN THE ONSET OF INSPIRATORY ACTIVITY AMONG SUPERIOR LARYNGEAL, HYPOGLOSSAL AND PHRENIC NERVES OF THE VAGOTOMIZED RAT. FUKUDA, Y. Dept. Physiol. Sch. Med. Chiba Univ., Inohana, Chiba 280

Inspiratory discharges of the superior laryngeal (Xsl), hypoglossal (XII) and phrenic (Phr) nerves were recorded in the vagotomized artificially ventilated rat. Effects of changes in chemical stimuli on the temporal difference in the onset of inspiratory activity among these respiratory nerves were analyzed. The inspiratory activity started slowly but much earlier in the Xsl or XII nerve than in the Phr nerve. This slowly developing inspiratory 'readiness' activity (IRA) of the Xsl or XII nerve was followed by subsequent inspiratory burst activity synchronizing with the Phr discharge (inspiratory synchronized activity, ISA). The IRA became small in hyperoxic hypocapnia (minimum chemical stimuli) and it was extremely exaggerated by progressive hypercapnia and hypoxia due to asphyxia (maximum stimuli). At the terminal stage of asphyxia, the IRA slowed down its rate of rise leading to the cessation of respiratory rhythm although the peak amplitude of integrated ISA was well maintained. Because of almost linear correlation between the rate of rise of integrated IRA and respiratory frequency, the IRA was assumed to be an important factor of controlling the respiratory rhythmicity.

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DEPRESSION OF THE INSPIRATORY ACTIVITY BY CEASING THE BLOOD FLOW TO THE UPPER PART OF THE BRAIN STEM. NATSUI,T. AND KUWANA,S. Dept. of Physiol., Sch., of Med., Teikyo Univ., Tokyo 173.

The effects of occlusion of the Art. basilaris and the Art. carotis externa on the inspiratory activity were studied in anesthetized and paralyzed cats. The vagosympathetic nerves were bilaterally sectioned. The arterial pressure in the Art. carotis externa was measured by inserting a catheter into the Art. lingualis. The integrated phrenic nerve activity (PNA) was used as an index of the inspiratory activity. Occlusion of the Art. basilaris at the rostral end of the medulla did not alter the PNA. However, subsequent bilateral occlusions of the Art. carotis externa suppressed markedly the PNA which finally disappeared within one minute (45 out of 50 occlusions in 6 cats). The blood pressure in the Art. carotis externa was 16.6 $\pm$ 5.7 (mean $\pm$ S.D.) during occlusions. These effects of the occlusion on the PNA were also observed in hypercapnia or hypoxia although they were not so notable as that in normocapnia or normoxia. The depression of the inspiratory activity would be due to an ischemia. The results indicate that the upper part of the brain stem has an intrinsic role on the inspiratory activity.

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Suppression of respiration caused by muscular thin-fiber afferents in the decerebrated cat. Eguchi, K., Tadaki, E. and Kumazawa, T. Dept. of Nervous and Sensory Function, Res. Inst. of Environ. Med., Nagoya Univ., Chikusa-ku, Nagoya 464., Dept. of Physiol., Sch. of Dent., Aichi-Gakuin Univ., Chikusa-ku, Nagoya 464

In chloralose-urethane anesthetized, paralyzed, artificially ventilated, and with vagal and sinus nerves bilaterally denervated cats, electrical stimulation of thin-fiber afferents from the gastrocnemius muscle caused two types of suppression in phrenic nervous activity. The one appeared limitedly in the stimulating period; presumably is originated in the spinal cord. The other was naloxone reversible suppression observed within a few minutes after the end of stimulation; presumably opiate systems participate in this respiratory suppression. Whether endogenous opiate systems in the higher nervous system such as hypothalamus are involved was tested by decerebration at the intercollicular level. Similar naloxone reversible respiratory suppression was obtained in 16 out of 17 decerebrated cats without an obvious alteration in "CO2-response". This suppression of respiration might be mediated by endogenous opiate in the caudal brainstem.

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EFFECTS OF VIBRATION ON THE PATTERN OF BREATHING IN RABBITS. SASAKI,K., KUDOH,S. AND TAKAKU,F. School of Med., Tokyo Univ., Hongo Tokyo 113

To test the hypothesis that somatic sensation changes the regulation of breathing, we applied the vibration sense on the knee joint of anesthetized rabbits* for 30 second and measured time of inspiration (Ti,sec.), Time of expiration(Te,sec.), respiratory frequency(F; 60/(Ti+Te), rates/min) and the electrical integration of action potentials of the right phrenicus nerve ($\bar{H}$,percent changes;%). These variables in baseline were .74 $\pm$ .03, 1.07 $\pm$ .07, 32.8 $\pm$ .8, and 100 for Ti,Te,F and $\bar{H}$ (mean $\pm$ SE),respectively,and during vibration were .74$\pm$.03 (Ti,NS), 1.02$\pm$ .07 (Te, $p< .02$), 33.3 $\pm$ .8 (F,$p<.05$) and 80.4 $\pm$ 3.3 ($\bar{H}$,$p<0.001$) with vagi intact. On the otherhand, the values with vagi cut were .98 $\pm$ .03 (in baseline) vs .97 $\pm$ .03 (during vibration, NS), for Ti, 1.01$\pm$ .03 vs .95$\pm$.03 ($p< .001$) for Te, 30.2$\pm$ .7 vs 31.4 $\pm$ .7 ($p< .001$) for F and 100 vs 72.6 $\pm$ 6.4 ($p< .001$) for $\bar{H}$. * n=6

These results indicate that vibration suppreses the neural output of respiration, and increases the respiratory frequency by shortening of expiration with vagi intact or cut.

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EFFECT OF INJECTION OF HIGH CO_2 BLOOD OR HIGH H^+ BLOOD INTO THE VERTEBRAL ARTERY ON THE PHRENIC NERVE ACTIVITY. KUWANA, S. and NATSUI, T. Dept. of Physiol., Teikyo Univ. School of Med., Itabashi-ku, Tokyo 173.

Integrated phrenic nerve discharges were recorded as an output of respiratory activity in anesthetized and artificially ventilated cats. The vagus and carotid sinus nerve were sectioned. Injection of the blood equilibrated with 80% CO_2 + 20% O_2 (high CO_2 blood, pH=6.910, pCO_2 > 500mmHg) into the vertebral artery produced an increase in the phrenic nerve activity. This stimulating effect on the nerve activity was mostly disappeared by the bilateral occlusions of the anterior inferior cerebellar arteries (AICA). On the other hand, injection of the blood containing 150 mM phosphate buffer (high H^+ blood, pH=6.976, pCO_2=43.9mmHg) into the vertebral artery produced a transient inhibition of the nerve activity, followed by a stimulation of it. When the AICA was occluded, the transient inhibitory effect by the injection of high H^+ blood abolished but the stimulating effect maintained. Furthermore, the phrenic nerve activity was increased not only by the injection of high CO_2 blood into the AICA but also by the bilateral occlusion of the AICA alone. These results indicate that the CO_2-stimulating and H^+-inhibiting structures to respiration are perfused by the blood of the AICA but H^+-stimulating structures are not.

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NEURAL MECHANISMS FOR EXPIRATORY EFFECTS OF LUNG INFLATION REFLEX IN THE RABBIT. SAKAI, YUJI. Dept. of Pharmacol., Saitama Med. Sch., Moroyama-Machi, Saitama 350-04

The reflex effects of slowly-adapting pulmonary stretch receptors(PSRs), those evoked by a selective phasic activation of Aβ-group vagal afferent fibers, on both expiratory and inspiratory times(Te and Ti) as well as on both expiratory recurrent laryngeal and intercostal motoneurons(ELMs and EICMs) were investigated in vagotomized, paralyzed and artificially respirated rabbits which were recovered from ether anesthesia. The results were interpreted by a comparison with the discharge pattern of both phasic and tonic PSRs recorded in lightly anesthetized, eupneic animals. Expiratory vagal stimulation repeated at 40 Hz or lower decreased both Te and EICM discharge, followed by a reduced Ti. However, stimulation repeated at 100 Hz or higher resulted in the opposite effects: it increased both Te and EICM activity, followed by an increased Ti. ELMs were always excited by stimulation at each rate between 10-200 Hz. Inspiratory stimulation always decreased both Ti and phrenic activity, followed by a decrease in both Te and expiratory motor discharges. These results support the idea that the basic role of both phasic and tonic PSRs might be rather in the maintenance of rapid shallow breathing, at least, in the rabbit. The finding of an expiratory neuron among the "dorsal respiratory group" which could be driven with a latency of 7-9 msec by vagal afferent volleys, and which might be possibly inhibited by a relay neuron was added to the proof.

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RESPIRATORY DRIVE FROM CARDIOVASCULAR SYSTEM IN EXERCISE HYPERPNEA. TAKAHASHI,E, YAMAMOTO,K,* and MIKAMI,T. Biomed.Controls, Res.Inst.Appl. Elect., Hokkaido Univ., Sapporo, Hokkaido 060

Feasibility of the cardiac output ($\dot{Q}$) increase as a feedforward drive for ventilation was evaluated in spontaneously breathing dogs anesthetized with urethane and α-chloralose. $\dot{V}co_2$ was increased with and without increase in cardiac output by isoproterenol injection and by venous CO_2 loading with an artificial lung, respectively. The result suggested that the exercise hyperpnea requires the increase in the cardiac output since the former showed transient hypo/isocapnic hyperpnea whereas the latter did hypercapnic hyperpnea. Secondary, we undertook the experiment to test the contribution of various parameters of the cardiovascular system such as $\dot{Q}$, Pv, P_{RA}, P_{RV} and P_{PA} to the formation of exercise hyperpnea using balloon catheters inserted into the IVC and the pulmonary artery. Resultant changes in above parameters from the balloon inflation resulted in hypocapnic hyperpnea where ventilation no longer correlated with $\dot{V}co_2$ and $\dot{Q}$. This may suggests the involvement of the information derived from the right heart system, probably via cardiac receptors, in the exercise hyperpnea.

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THE PARTICIPATION OF PULMONARY INFLATION REFLEX IN HEART RATE RESPONSE TO HYPOXIA.
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In general, Hering-Breuer reflex in control of respiration is known to be less potent in humans than in animals. To investigate whether vagal reflex (pulmonary inflation reflex) participates in heart rate(HR) response to hypoxia, we examined 12 healthy subjects. Progressive hypoxia tests were conducted under two conditions, tidal volume (V_T) unrestricted and voluntarily controlled at a given level. Mean value of V_T was 0.84 ± 0.24 L (Mean ± SD) under the controlled ventilation, whereas mean V_T changed from 0.75 ± 0.20 (P_{ETO_2} at 95 torr) to 1.42 ± 0.41 L (P_{ETO_2} at 40 torr) under the unrestricted ventilation. By controlling V_T constant, HR responses to Sa_{O_2} ($\Delta HR/\Delta Sa_{O_2}$) decreased in 9 cases, and changed from -0.712 ± 0.193 to -0.664 ± 0.130 bpm/%. On the other hand, occlusion pressure (P.2) responses($\Delta P.2/\Delta Sa_{O_2}$) changed from -0.153 ± 0.149 to -o.180 ± 0.135 cmH_2O/%. Both changes, however, were not statistically significant. These data indicate that pulmonary inflation reflex is less likely participated in hypoxic HR response from air-breathing to 40 torr P_{ETO_2}, although the influence of behavioral control can not be completely neglected in increasing occlusion pressure response. We conclude that pulmonary inflation reflex in humans may play little role in accelating HR response as in the case of respiratory frequency response.

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RESPIRO-CIRCULATORY RESPONSES TO HYPOXIA AND HYPERCAPNIA IN HIGH SCHOOL JUDO ATHLETES.
OHYABU,Y.,SAKAKIBARA,Y.,HONDA,Y.,MIYAMOTO,Y.*,NAKAZONO,Y.*, ABE,N.*,SATO,N.*AND TAKAHASHI,K.** Dept. Med. Chiba uni. Chiba 280 •Dept. Biomed. Hokkaido uni. ••Dept. Physical Education Tokai uni.•••Dept. Physical Education Kogakuin uni.

Ventilatory as well as cadiac responses to isocapnic progressive hypoxia and progressive hypercapnia(Read'method) were similtaneously examined in 21 high school judo athletes.

Cardiac output was continuously determined by the impedance signals using a computer-based on-line system . Ventilatory and cardiac output responses to progressive hypoxia were similarly curvilinear. In hypercapnia, cardiac output was also curvilinear, but ventilation increased linearly.

No significant relationships were seen between the responses of ventilation and of cardiac output in progressive hypoxia or in hypercapnia.

We concluded that regulation of ventilation and of circulation in both hypoxia and hypercapnia is relatively independent each other.

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EFFECT OF CECAL VOLUME CHANGE ON THE GASTRIC MOTILITY.
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In rats food intake and fecal evacuation increase after cecectomy, while gastrointestinal transit time delays in germ-free rats which have a large cecum. The effect of cecal volume change on the gastric motility was studied in 24 hrs fasted rats anesthetized with urethane(0.8g/kg, i.p.). Increase of cecal volume from 0 to 10 ml(1 ml step) produced a decrease of basal tone of the stomach. Maximal response was obtained above 7 ml of cecal volume. An inhibitory response in the stomach decreased after sympathectomy and was abolished after successive vagotomy. Gastric inhibition by cecal volume increase was also elicited via vago-sympathetic pathway. This response was still induced after decerebration at midcollicular level. Ceco-gastric inhibitory response mediated by sympathetic pathway was abolished after guanethidine(3-5mg/kg, i.v.) but the response mediated by vagal pathway was resistant to atropine(0.2mg/kg) and guanethidine.

It is concluded that an increase in cecal volume induces gastric relaxation via vagal and sympathetic pathways, the efferents of which are non-adrenergic inhibitory and adrenergic nerves, and, therefore, retardation of the gastric contents may occur in rats having a large cecum.

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EFFECTS OF CCK-8 AND RELATED PEPTIDES ON THE CANINE GASTRIC MOTILITY.
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The effects of cholecystokinin-octapeptide(CCK-8) and related peptides on the canine gastric motility was studied in anesthetized dogs using the strain-gauge force transducer. The intra-arterial administration of CCK-8 and related peptides(pGlu-CCK-8, Glt-CCK-8, Suc_1-$MePhe_8$-CCK-7)caused a dose dependent(1-20 ng) increase in gastric motility. Hexamethonium(5 mg/Kg) failed to block the effect of CCK-8 and related peptides. However, administration of atropine(100-200 μg/Kg) sufficient to block the action of 1 μg acetylcholine inhibited both the effect of CCK-8 and pentagastrin. Repetitive injection of the pentagastrin(20 ng), but not of CCK-8, caused a tachyphylaxis. Administration of CCK-8 after the tachyphylaxis still evoked a gastric contraction. On a molar basis, CCK-8 was more effective than pentagastrin. These results suggest that CCK-8 and related peptides stimulate the post-ganglionic cholinergic neurons responsible for gastric contraction.

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VAGAL CONTROL OF PERIODIC GALLBLADDER CONTRACTION IN FASTING DOGS.
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The role of vagi on the gallbladder contraction associated with the periodic interdigestive motility of the upper gastrointestinal tract was studied in concious dogs, each with a gastric fistula, a Heidenhain pouch and a biliary fistula. Bile pressure increased spontaneously in phase with the motility of the stomach, the pouch and the duodenum, which reccurred every 100 min. The peak pressure was 18±1 (mean ± S.E.) cm of H_2O, which preceeded the duodenal peaks by 30 min. Bilateral cervical vagal block with local anesthetics abolished the periodic increase of bile pressure. Gut motility was also reduced but the pouch and duodenal peaks remained unchanged. The periodic activities of the gallbladder and the gut were no longer seen following atropine or pentolinium. Prompt gallbladder contraction which followed pentagastrin could be prevented by both bilateral vagal block and ganglionic blockade. Both, however, did not prevent the action of cholecystokinin. It is concluded that the interdigestive periodic contraction of the gallbladder is mainly dependent on the vagus. The orthodox view that the gallbladder evacuation is primarily controlled by humoral mechanisms needs revaluation.

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NEURAL AND HORMONAL CONTROLS ON THE GALLBLADDER MOTILITY. YAMASATO,T. AND NAKAYAMA,S. Dept of Physiol. Okayama Univ. Med. Sch., Okayama 700

Previously, it is wellknown that vagal stimulation induces contraction of the gallbladder and sympathetic stimulation causes the relaxation and contraction, and also CCK_8 causes contraction of the gallbladder.

In this study, vagal stimulation caused the contraction and relaxation on the neck, body and fundus of the gallbladder same as sympathetic(celiac) stimulation. Vagal contractile response was inhibited by subthreshold celiac stimulation(SCS) but increased by infusion of CCK_8. Celiac contractile response did not alter by subthreshold vagus stimulation(SVS) and CCK_8. Vagal relaxant response did not alter on each part of the gallbladder by SCS, while celiac relaxant response was reduced on the neck and augmented on the body and fundus by SVS. Although basal tone was increased, CCK_8 reduced vagal relaxant response on the neck and fundus. But it was ineffective on the relaxant response in the body. Relaxation by celiac stimulation did not alter after CCK_8 in fusion in the neck, body and fundus.

It is concluded that an activity of vagal excitatory nerve inhibited at its ending by sympathetic nerve, while sympathetic activity is modulated at its ending by vagus nerve, and CCK_8 has an action to potentiate the vagal excitatory effect but no action on the sympathetic effect.

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STUDIES ON PENETRATION PHENOMENA THROUGH INTESTINAL MEMBRANE — EFFECT OF UBIQUINON 10 (Q_{10}) AND MAGNETIC FORCE (3). NAKANO,S., MIYOSHI,M., NAGAMI,K. AND YOSHIOKA,T. Dept. of Physiol., The Tokai Univ. Sch. of Med. 259-11, Isehara, Kanagawa

In previous studies, by use our devised conventional circulating apparatus, we have investigated intestinal penetration of insulin and L-tryptophane as well as activetransport of glucose. On this occasion, we have used Q_{10} of the resperatory chain substances, to study the effect it exerts both in-vivo and in-vitro. Furthermore, the action of the line of magnetic force on the intestinal walls. The results of these studies are as follows: 1) When Q_{10} was applied under in-vitro conditions to the mucosal side of this apparatus, it was found that Q_{10} was able to penetrate slightly at an addition rate of 12.5mg, while the associated insulin and L-tryptophane penetration showed a clear increase. However, when the Q_{10} adition was doubled to 25mg, it was found that, despite the resulting increased Q_{10} penetration, the penetration of above chemicals both tended to be rather suppressed. 2) In identical in-vivo experiments conducted on the intestines of normal rat to Q_{10} intraperitonial injection within a time span of 3-40 days, it was found that the insulin and L-tryptophane penetration rose by approximately 30-40%, with virtually no difference observable according to the period of administration. 3) When intestine was irradiated from serosa to mucosa by 4000-7500 Oe magnetic force, the penetration of these substances were obviously inhibited.

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FFFECT OF ISOPROTERENOL ON CHOLESTEROL METABOLISM IN SALIVARY GLANDS OF MICE. YOSHIDA,M.,KURAHASHI,M.,NAKAMURA,H. AND INOMATA,K* Dept. Oral Physiol. and Physiol.* Higashi-Nippon-Gakuen Univ.Sch.Dent., Ishikari-Tobetsu,Hokkaido 061-02

Effect of isoproterenol (IP) on cholesterol metabolism was investigated in salivary glands of mice. Male ICR-JCL mice were injected with IP(0.3mg/mouse/day). 1. IP injection for 7 days increased the weight and cholesterol biosynthesis of submandibular glands and decreased total cholesterol level in submandibular glands. 2. IP injection for 45 days increased the weight and total cholesterol level of submandibular glands and decreased the uptake of ^{3}H-cholesterol in submandibular glands, while IP injection was without effect on cholesterol biosynthesis. These results suggest that submandibular gland enlargement is reached to maximum after IP injection for 45 days. 3. 21 days subsequent to the withdrawal of IP after IP injection for 24 days, the weight and cholesterol biosynthesis of salivary glands(submandibular plus sublingual) returued to normal values, while total cholesterol level in salivary glands remained to be increased. These results suggest that cholesterol metabolism do not return to normal condition 21 days after IP injection. 4. The uptake of ^{14}C or ^{3}H-cholesterol decreased in submandibular glands after IP injection for 7 or 12 days. The uptake of cholesterol in microsome fraction of submandibular glands increased after IP injection for 12 days. The present study indicates that IP influences the cholesterol metabolism in salivary glands of mice.

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Ursodeoxycholate (UDCA) induced choleresis is compatible with a "secretory choleresis" hypothesis.

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In order to characterize the ursodeoxycholate induced choleresis previously reported by the authors (Life Sci 31:1973, 1982), the bile flow and bile salt excretion rate for one or two hours and the bile salt concentration in the liver at the end of the experiments were examined in control rats, taurine supplemented rats and taurine deprived (oral administration of β-alanine) rats under the intravenous infusion of ursodeoxycholate (1.2 μmol/min/100 g body weight). Among these three groups, the bile flow and bile salt excretion rate during the first hour and 2 hour total periods were negatively correlated, while a significant positive correlation was observed between the bile flow rate for 1 or 2 hours and bile salt concentration in the liver examined after a 1 or 2-hour bile salt infusion. These results seem most easily explained by the thesis that at least part of the bile is produced not by the excretion of bile salt into the bile canaliculus but by its presence inside the hepatocyte stimulating the secretion of other electrolyte(s) into the bile ("secretory choleresis").

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INHIBITORY EFFECTS OF PULLULAN AND/OR ARGININE ON BLOOD GLUCOSE LEVELS AFTER ORAL SUCROSE ADMINISTRATION IN RATS.

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Increases of blood glucose levels after oral sucrose administration in rats were depressed by adding of non-absorbable polysaccaride, pullulan. The maximum suppressive effect was found at an adequate additive ratio to sucrose, not at rather higher one, while not at all of glucose administration. Although this suggests that pullulan affects nothing of insulin release but the sucrase activity on the intestinal brush border, the inhibitory effect on the sucrose absorption was observed, neither in the circulating perfusion through the small intestine in vivo, nor in the experiment of the transepitherial potential difference in vitro.

Arginine, independent of glucose on the absorptive mechanism, was also added to sucrose. Blood glucose levels fell by rising of the arginine concentration, resulting from insulin release. But too much of addition caused no fall of blood glucose level, as both insulin and glucagon might be released. Addition of both pullulan and arginine will show the decrement of blood glucose level, but details are now under studying.

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Epithelial transport of dicarboxylic amino acids by guinea pig ileum.

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The influxes of L-glutamate and L-asparatate across the intestinal brush border membrane were saturable processes that were subjected to competitive inhibition and were absolutely dependent on the presence of Na^+. The addition of acidic amino acid into the mucosal solution induced a sudden and sustained increase in potential difference (ΔPD) which was also absolutely dependent on Na^+. The ΔPD induced by L-glutamate or L-asparatate conformed to Michaelis-Menten kinetics against the concentration of each organic substrate. On the other hand, the relationship between the change in short circuit current evoked by L-glutamate at a fixed (1 mM) concentration and the concentration of Na^+ in the bathing media did not conform to Michaelis-Menten kinetics but sigmoid. Similar relationship was also observed between L-glutamate influx and the concentration of Na^+. The ΔPD was not altered by the total replacement of K^+ by Na^+ in the mucosal solution and showed the optimum pH at around 7 within the range pH 5.4 to 8.4. The transfer of L-glutamate from mucosal to serosal appearance was examined by everted sac of ileum. Significant increase in serosal solution was observed for L-glutamate and L-alanine after the incubation with L-glutamate. The serosal appearance of L-glutamate appeared to be unaffected by the presence or absence of HCO_3^-.

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The stimulatory effect of intraduodenal infusion of Na_2 EDTA on pancreatic secretion and its mechanism in the rat.

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The effects of intraduodenal instillation of Na_2 EDTA and Ca EDTA on pancreatic secretion were determined in concious rats. Rats were prepared with cannulae, draining pure pancreatic juice and bile, separately, and two duodenal cannulae. When a solution of Na_2 EDTA, 25mM, was infused intraduodenally at a rate of 1 ml/hr, pancreatic juice flow, bicarbonate and protein outputs were all significantly increased while bile-pancreatic juice was returned to the duodenum. After a 6-10 hour diversion of bile-pancreatic juice, Na_2 EDTA still increased pancreatic juice flow and bicarbonate output, but the increase of protein output was only transient. In contrast, the infusion of Ca EDTA did not cause these stimulatory effects on pancreas. Intraduodenal Na_2 EDTA produced an increase in secretin level in portal venous blood. It is suggested, therefore, that Ca ion in the duodenum plays an important role in regulating pancreatic secretion in rats.

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PANCREATIC SECRETION REFLEX FOLLOWING STIMULATION OF THE MUCOUS MEMBRANE OF THE CANINE GASTRIC BODY.

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The effect of mucosal stimulation of the gastric body was studied in chloralose anesthetized or decerebrate dogs, in which the gastric body was separated from the antrum with double mucous sutures and the pyloric sphincter was occluded. In experiments on 21 dogs average rate of pancreatic secretion was 20 µl/5 minutes. After 40-60 ml infusion of $CuSO_4$ solutions into the gastric body the secretion increased in every dogs an average of 138 µl/5 minutes. This was accompanied by increased protein and bicarbonate production of 860% and 2500% respectively. Gastrin concentration in the serum was 69.8 pg/ml in control, and it was increased to an average of 247 pg/ml 30 minutes after the infusion of $CuSO_4$ solutions. The pancreatic responses abolished immediately after i. v. injection of atropine sulphate (0.5 mg/kg) in spite of continuation of an increased gastrin concentration. In 4 of 5 additional experiments after transthoracic vagotomy the pancreatic response to the infusion abolished completely. These studies provide evidence for the existence of the oxyntomucosal pancreatic reflex that is developed via the vago-vagal pathways.

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INFLUENCE OF EXTRACELLULAR Na^+ AND Ca^{++} ON EXOCRINE SECRETORY RESPONSES OF RAT PANCREAS.

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Influence of extracellular Na^+ and Ca^{++} on cholecystokinin octapeptide (CCK-8)-induced secretory responses was examined in the isolated perfused rat pancreas. Continuous stimulation with 50 pM CCK-8 induced sustaining secretory responses in the standard ionic environment, whereas, in Na-defficient environment, it induced smaller and transient responses. A dose-response relation was observed between extracellular Ca^{++} concentration and the CCK-8-induced responses in Na-defficient environment. The smaller and transient responses to continuous CCK-8 stimulation in Ca-defficient environment was almost abolished when extracellular NaCl was replaced by equimolar LiCl, indicating that the responses initiated by release of Ca^{++} from intracellular Ca store may require Na^+ influx.

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OESOPHAGO-PANCREATIC SECRETION REFLEX IN DOGS. FURUKAWA, N. AND OKADA, H. Dept. of Physiology, Kawasaki Medical School, Kurashiki 701-01

In dogs provided with the occluded pyloric sphincter and acute cannulation of the main pancreatic duct, the effect of graded thoracic oesophageal distension with a balloon on pancreatic exocrine secretion was studied. In both 24 chloralose anesthetized dogs and 15 decerebrate dogs oesophageal distension (10-90 ml) produced either increase (in 75 tests) or decrease (in 69 tests) in pancreatic secretion. An average increase of secretion rate was 73%, although the graded distension caused usually graded rise of secretion rate. This was accompanied by increased protein output. In the inhibitory response average decrease of secretion rate was 23.0%, which was accompanied by decreased protein output. The increased response of secretion abolished after transthoracic truncal vagotomy or i.v. injection of atropine sulphate (0.5 mg/kg), but decreased response did not. This decreased response completely disappeared after additional splanchnotomy. These results provide evidence for the existence of the oesophago-pancreatic acceleratory and inhibitory reflexes. The afferent pathway in these reflexes involves in the vagal nerves, and the efferent pathways are the vagal and splanchnic nerves.

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EFFECT OF INTRAVENTRICULAR ADMINISTRATION OF COMP. 48/80 ON HYPOTHALAMO-PITUITARY-ADRENAL AXIS IN DOGS. MATSUMOTO, I. AND AIKAWA, T. Dept. of Physiol., Nagasaki Univ., Sch. of Med., Nagasaki 852

Adrenocortical secretion in response to the intraventricular administration of compound 48/80, histamine releaser from mast cells, was examined in dogs. Agents dissolved in saline were infused into the third ventricle at the rate of 15.6 μg/min under pentobarbital anesthesia. Adrenal venous blood sample was collected and the secretory rate of cortisol was measured as an index of ACTH secretion. The secretory rate of cortisol increased significantly after the infusion of compound 48/80 (7.5 μg/kg/min for 5 min), with a concomitant increase in the arterial plasma levels of immunoreactive ACTH. No significant changes in blood pressure and arterial plasma histamine concentration were observed after the infusion of compound 48/80. The simultaneous intraventricular administration of pyrilamine maleate (0.5 μg/kg/min for 10 min), H_1 antagonist, inhibited significantly the secretory response of cortisol to compound 48/80, but that of metiamide (50 μg/kg/min for 10 min), H_2 antagonist, did not inhibit the response. These results suggest a possibility that endogenous histamine which is liberated from brain mast cells stimulates hypothalamo-pituitary-adrenal axis in dogs.

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DUAL EFFECTS OF METHYLXANTHINES ON THE ELECTRICAL ACTIVITY INDUCED BY D-GLUCOSE IN PANCREATIC B-CELLS. SAKAMOTO, Y. AND IMANAGA, I. Dept. of Physiology., Sch. of Medicine., Fukuoka University., Fukuoka., 814-01

In order to get a further information about the characteristics of the slow waves induced by D-glucose(11.2mM), the effects of methylxanthines, Ca ionophore, A23187, DNP, adrenaline and Ca ion on the glucose-induced slow waves were examined. Methylxanthines initialy reduced the duration of slow wave and then prolonged it resulting in sustained depolarization which induced continuous discharges. These effects were marked in IBMX(10^{-3}M) application. In contrast to IBMX, theophyline (10^{-2}M) blocked the glucose-induced electrical activity, but removal of drug enhanced it. Enhanced spike discharges caused by methylxanthines removal were decreased by extracellular high Ca(10mM), adrenaline(10^{-6}M), A23187(10^{-5}g/ml) and DNP(10^{-4}M). On the other hands, in the presence of methylxanthines an antagonistic action of above drugs were reduced.

It was suggested that methylxanthines, besides their action on intracellular C-AMP level, could also modulate the permeability of Ca channels in the plasma membrane of B-cells.

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METABOLIC AND FUNCTIONAL ABNORMALITY OF PLATELETS WITH EXPERIMENTAL STREPTOZOTOCIN(STZ) DIABETES OF GUINEA PIGS. IKESAWA, K., OKAMOTO, S., YAMASHITA, T. AND MATSUOKA, A. Dept. of Clin. Path., and Clin.Lab., Hyogo College of Med., Nishinomiya, Hyogo 663

Blood platelets contain extremely high concentration of ascorbic acid(VC); the function of this acid is quite obscure however. The present study on experimental diabetes aimes to offer a novel knowledge of the functional and metabolic abnormalities of platelets with STZ diabetes of guinea pigs, in which VC is essential vitamin.

STZ diabetes was produced in guinea pigs according to the method of Brosky et al. Animals showed the citotoxic injury of beta cells and hyperglycemia (ca 191~245mg/dl) continued for 6 months. Aggregation of platelets with 0.75μM ADP was slightly increased when compared at 4 min (control:46.5±28.0%, STZ:76.4±8.5%). The aggregation with collagen (2.5μg/ml) was only weakly enhanced. However, the most noteworthy finding was the remarkable decrease of VC in platelets, i.e. 68±14μg/10^{10} normal platelets, decreased to 34±13μg/10^{10} diabetic platelets, suggesting that the underlying metabolic abnormality of platelets VC in diabetic patients from the view point to screening the risk factors for vascular complications, is to be more significant than ever expected. In other woreds, VC contents in platelets determined would be most sensitive index for clinical pathology of diabetic patients, and the decrease of VC contents in platelets seems to be not due to the disturbance of VC uptake through the platelet membrane.

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DYNAMIC ANALYSIS OF PLASMA GLUCOSE AND INSULIN CURVES DURING INTRAVENOUS GLUCOSE TOLERANCE TEST IN MAN— SIMULATION OF IVGTT CURVES BY A MATHEMATICAL MODEL—.

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Parameters based on a linear differential equations model were estimated from each average of the plasma glucose and insulin of patients with diabetes mellitus(DM) and with other non-diabetic diseases. The IVGTT curves of DM were characterized by its retardation of the settling time in both glucose and insulin, its depression and delay of the peak of the plasma insulin and its slowing the initial fall in the glucose curve. Then, how the separate change of each parameter value affected the dynamic pattern of curves was examined. From these simulation under a standardized condition, it was proved that the distinctive pattern in DM depended chiefly on the decrease in values of such parameters representing the rate constant of insulin release, of insulin independent glucose uptake and of insulin dependent glucose uptake.

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POOR MYELIN-FORMATION IN THE hyt CEREBRUM.

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2',3'-Cyclic nucleotide 3'-phosphohydrolase activity in the cerebrum of the inherited primary hypothyroid mouse (hyt/hyt) is reduced in comparison with the normal heterozygote (hyt/+). With regard to DNA and RNA contents, and the RNA/DNA ratio, no differences were observed.

The results of this study indicate that hypomyelination in the hyt mouse is restricted to the cerebrum, and is not related to arrested glial proliferation.

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TRANSPLACENTAL EFFECTS OF DIMIT (ANALOGUE OF L-T_3) ON DEVELOPMENT OF FETAL RAT BRAINS.

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The possible effects of 3,5-Dimethyl-3'-Isopropyl-L-Thyronine (DIMIT) on developing fetal rat brains have been examined. DIMIT has thyromimetic activities and can pass through the placenta. When pregnant Wistar strain rats were administered with DIMIT (10 ng/g body weight/day) between 3 day of gestation and delivery, their infants showed signs of hyperthyroidism with suppressed body weight, tachycardia and tremor. The contents of protein, RNA and DNA (per tissue and per gr tissue) did not changed significantly in the cerebrum, hypothalamus and cerebellum from the infants of DIMIT-treated mothers compared with the control group. Whole protein contents (per whole tissue) were significantly increased in the cerebrum and cerebellum, but intracellular contents of protein (per DNA) were not changed significantly in the three brain areas from the infants of DIMIT-treated mothers. Although tubulin contents per protein were not changed significantly, intracellular contents of tubulin (per DNA) were significantly raised in the three brain areas from the infants of the DIMIT-treated mothers. Since the tubulin is one of major proteins in brain, the increased level of the intracellular tubulin contents might reflect the accelerated development of the nervous tissues in the DIMIT-induced hyperthyroidism.

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ROLE OF POLYAMINES IN THE FORMATION OF EXPERIMENTAL GOITER OF RATS. MATSUZAKI, S. and SUZUKI, M. Dept. of Physiology, Inst. of Endocrinology, Gunma University, Maebashi 371

It has been repeatedly shown that polyamines putrescine, spermidine and spermine play an important role in cell proliferation. The treatment of rats with goitrogens such as methylthiouracil (MTU) and propylthiouracil resulted in a drastic increase in circulating thyrotropin (TSH), the activity of polyamine biosynthetic enzymes and thyroid weight and DNA. The polyamine contents in the thyroid increased greatly during goiter formation and decreased soon after MTU withdrawal. The activity of thyroid ornithine decarboxylase (ODC), the rate-limiting enzyme of polyamine biosynthesis, fluctuated greatly in response to changes in serum TSH levels. The treatment of rats with α-difluoromethylornithine (DFMO), a suicide inhibitor of ODC, resulted in a slight decrease in thyroid weight and polyamine contents. The treatment with excess KI resulted in a marked decrease in thyroid ODC activity in MTU-treated rats and in a decrease in thyroid weight and polyamine contents. These results suggest that the increase in thyroid polyamine contents is essential for the goiter formation in the rat.

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LOCALIZATION OF THE NEURONS CONCERNING WITH TSH SECRETION IN RATS. ISHIKAWA, K. and SUZUKI, M. Dept. of Physiology, Inst. of Endocrinology, Gunma University, Maebashi 371

TRH-containing neurons have been immunohistochemically shown to be widely distributed in rat brain, including the paraventricular nucleus (PVN). Bilateral electrical lesions of the PVN completely inhibited TSH secretory response to cold and iv administration of clonidine (an 2-agonist). After clonidine (with phenoxybenzamine) was injected directly into the hypothalamic nucleus, plasma TSH levels gradually, but significantly rose. In addition, in rats with hypothalamic knife cuts which severed the projections into the median eminence, TSH response to exogenous TRH (100ng/rat, iv) was strikingly magnified. This enhanced response to TRH was observed within 3 days after the knife cut. These results are compatible with the inference that TRH neurons within the PVN are of importance in the secretion of TSH from the pituitary.

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THE HYPOTHALAMIC SOMATOSTATIN (SRIF) CONTENT REGULATED BY THYROID HORMONE. SUZUKI, M. and KATO, M. Dept. of Physiology, Inst. of Endocrinology, Gunma University, Maebashi 371

Thyroidectomized rats failed to release GH as the ultradian burst. Whereas thyroxine (T4) supplement recovered the spontaneous burst secretion and also the responsiveness of the hormone release to electrical stimulation on the hypothalamic ventromedial nucleus. However, T4 restored only partially pituitary responsiveness for GH release to exogenous GRF infusion. Thus we aimed to measure the change by T4 in hypothalamic SRIF content. Normal (I), thyroidectomized 3 weeks prior to experiments (II) and 24 hr after T4 supplement (0.2 mg/kg) (III) rats were used. The hypothalami were cut into 3 parts with a refrigerated microtome and a razor blade, viz., the medial basal (A), anterior (B) and dorsoposterior (C) hypothalamus. Thyroidectomy reduced I-A (275 ± 5.0 ng, n:8) by 48.2% (II-A) ($p<0.02$), I-C (172.2 ± 4.2, n:7) by 25.3% ($p<0.001$) and total I (ABC) by 29.2% ($p<0.001$) respectively. Inversely T4 supplement restored II-A and -ABC as much as 82.3% ($p<0.001$) and 87.1% ($p<0.05$) of each control (I) level respectively.

Then we made immunohistochemical observation on those hypothalamic areas with the use of highly specific anti-SRIF serum and PAP technique. The width of immunostained outer zone of the median eminence was thiner in II than those of I and III.

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PARVOCELLULAR NEUROSECRETORY CELLS IN THE PARAVENTRICULAR NUCLEUS: ELECTROPHYSIOGICAL IDENTIFICATION AND NOXIOUS INPUTS. HAMAMURA, M., SHIBUKI, K., and YAGI. K. Dept. of Physiol., Jichi Med. Sch., Tochigi 329-04.

The present study aimed to identify electrophysiologically the parvocellular (PC) neurosecretory cells in the hypothalamic paraventricular nucleus (PVN) of anaesthetised rats. Certain PVN units showed antidromic spike after median eminence (ME) stimulation but did not respond to posterior pituitary stimulation at the stimulus intensity which evoked antidromic population potentials of maximum amplitude as recorded on the surface of the pituitary stalk. Histological examinations revealed that 26 of these 38 cells were located within the PC region of the PVN. Apparent conduction velocity estimated for antidromic spike after ME stimulation was 0.27 ± 0.1 m/s (mean ± S.D.) and significantly slower than that (0.38 ± 0.07 m/s, n=18) estimated for antidromic spike evoked in the magnocellular PVN units by posterior pituitary stimulation ($P < 0.01$, Mann-Whitney U test). Tail pinching evoked an transient excitation in 3 of the 11 PC units tested. Saphenous nerve stimulation produced excitatory synaptic inputs in 6 of the 13 PC units tested. These data were discussed in relation to CRF neurones and noxious inputs to them.

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REGULATION OF VASOPRESSIN RELEASE BY PLASMA-BORNE ANGIOTENSIN IN CONSCIOUS RATS. YAMAGUCHI, K., *KOIKE, M. AND *HAMA, H. Dept. of Physiol., Niigata Univ. Sch. Med., Niigata City 951 and *Kido Hospital, Niigata City 950

To evaluate a possible role of peripherally generated angiotensin II (AII) in the regulation of vasopressin (ADH) release, the effects on plasma AII and ADH of AII injected intraperitoneally together with various solutions were examined in conscious rats. Plasma AII and ADH were measured by sensitive and specific radioimmunoassay by utilizing the trunk blood collected after decapitation. The injections of 150 mM NaCl containing AII (6, 12 or 24 μg/2 ml/100g body weight) increased plasma AII at 15 and 30 min in a dose-related manner, while plasma ADH was unaltered. However, when given together with 600 mM NaCl, AII (8 μg/2 ml/100g) significantly potentiated the plasma ADH response to the vehicle at 15, 30 and 60 min without affecting those of plasma osmolality and hematocrit. The elevations in plasma AII and osmolality induced by the treatment were comparable to those observed under water deprivation for 46 h. AII administered together with hypertonic sucrose or mannitol solution was without effect on plasma ADH, though these saccharide solutions were equipotent to 600 mM NaCl in augmenting plasma ADH and osmolality. From these results, we concluded that plasma-borne AII may physiologically participate in ADH release by potentiating its response to the hyperosmolality arising from increases in sodium salts.

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FACILITATION OF NOXIOUS INPUTS TO SUPRAOPTIC VASOPRESSIN NEURONES DURING HYPOVOLEMIA IN RATS. SHIBUKI, K., HAMAMURA, M. and YAGI, K. Dept. of Physiol., Jichi Med. Sch., Tochigi 329-04

Effects of saphenous nerve stimulation were studied on spike activity of supraoptic neurosecretory cells in anaesthetised rats. Of forty neurosecretory units tested, twenty-four were identified as vasopressin-secreting cells by their phasic discharge pattern and eighteen of the twenty-four cells (75 %) were excited after the nerve stimulation (3 mA and 1 ms pulses). Of the sixteen units which exhibited non-phasic discharge pattern, eleven (69 %) were excited by the stimulation. Threshold for evoking the excitation was nearly equal to that for simultaneously evoked flexor reflex. Intravenously injected gallamine (50 mg/kg) bloked flexor reflex completely but did not alter the excitatory response in each tested cell. Amplitude of the excitatory response was greater after haemorrhage (5 ml/kg) than that before extraction or after retransfusion of the shed blood. These data indicate that vasopressin-secreting neurones are excited by noxious stimuli and the excitation is potentiated in hypovolemic states.

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POSSIBLE OCCURRENCE OF THE INTERNAL Na-DEPENDENT Ca INFLUX MECHANISM ASSOCIATED WITH VASOPRESSIN RELEASE IN THE POSTERIOR PITUITARY. SORIMACHI, M. Dept. Physiol., Sch. Med., Kagoshima Univ., Kagoshima 890.

The isolated posterior pituitary of the rat was incubated with a medium lacking Ca and the amount of arginine-vasopressin(AVP) released into the medium was measured by the radioimmunoassay. Exposure of the gland to a medium containing 2 mM Ca caused a significant increase of AVP secretion. Treatment of the gland with a medium either containing ouabain(0.1 mM) or lacking K for 40 min markedly increased this response. The responses after ouabain treatment were dependent on the concentration of Ca and were strongly inhibited by Ca channel blockers such as D 600(50 uM) and Co(2 mM). Prior exposure of the gland to a medium containing a low concentration of Na(20 mM) markedly reduced the response to Ca. On the other hand, the response to Ca during high K(60 mM) depolarization was little affected by a low Na medium, ruling out the possibility that ouabain potentiates the response via its depolarizing action.

These results are essentially similar to those obtained with the adrenal chromaffin cells and therefore suggest that the internal Na-dependent Ca influx mechanism is present in the posterior pituitary.

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A STUDY ON DETECTORS OF PLASMA OSMOLALITY FOR OXYTOCIN RELEASE. NEGORO, H., HIGUCHI, T., HONDA, K. AND FUKUOKA, T. Dept. Physiol., Fukui Medical School, Matsuoka, Fukui 910-11

Two experiments were conducted to test whether the Verney's osmoreceptor concept is applicable to the mechanism for oxytocin release. In experiment I, 28 male rats were infused with Locke solution (LS), 0.6 M NaCl dissolved in LS, 1.2 M mannitol in LS or 1.2 M urea in LS via an atrial cannula at the rate of 1 μl/sec under unanesthetized condition. Plasma oxytocin measured by radioimmunoassay were increased significantly ($p < 0.001$) after NaCl or mannitol infusion but not after urea or Locke solution infusion, whereas plasma osmolality increase significantly ($p < 0.05$) after the infusion of each solution except after the infusion of Locke solution. In experiment II 34 rats were injected artificial cerebrospinal fluid (CSF) or artificial CSF to which either NaCl, mannitol or urea was added into the third ventricle. The injection of NaCl CSF or mannitol CSF also increased plasma oxytocin significantly ($p < 0.01$) but that of urea CSF or artificial CSF alone was without effect. These results indicated that detectors of rise in plasma osmolality for oxytocin release are osmoreceptors rather than sodium detectors.

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BIOSYNTHETIC REGURATION OF PROTEINASE F IN MOUSE SUBMANDIBULAR GLAND. UEHA, T., HOSOI, K., ISHII, Y. AND NEMOTO, I. Dept. Oral Physiol., Josai Dent., Univ., Sakado, Saitama, 350-02

We recently found a new esteroproteinase, called proteinase F from the mouse submandibular gland (Biochim. Biophys. Acta 756, 163-170, 1983). In contrast to known proteinases such as proteinase A, D or P-esterase, proteinase F is rich in female mice than in male. The biosynthesis of proteinase F in vivo was inhibited by androgen in males but not in females. From several parameters of hormone treatments using castrated adult and newborn mice, the inhibition of proteinase F biosynthesis by androgen in adult male seemed to require the presence of thyroid gland, which may secrete some inhibitory factor in response to androgen, and also the lack of response to androgen in adult females seemed to be due to unknown factor(s) which may be secreted from the testis of newborn mice. The pituitary gland may secrete a certain substance which stimulates the proteinase F biosynthesis. The androgen stimulated the biosynthesis of proteinase F in the hypophysectomized mice which are also hypothyroidism.

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A SIMPLE AND USEFUL SCREENING TEST FOR PSYCHOGENIC IMPOTENCE. SENO,H., DAINOSON,K.* AND KARACAN,I.** Dept. Physiol., Sch. Med., Tokushima Univ., Tokushima 770. *Dept. Neuropsychiat., Sch. Med., Kurume Univ., Kurume 830. **Baylor Coll. Med., Houston, Texas 77030

The stamp-ring technique (Barry, J.M. et al., 1980) with USA 1 cent postage stamp was investigated on the validity for the occurrence of nocturnal penile tumescence (NPT). After answering the sexual function questionnaire and getting interview and genital examination, 69 potent men and 103 impotent men slept 3 consecutive nights and obtained polygraphic recordings (EEG, EOG, EMG, EKG, Respiration, etc.) all night long. They also had a stamp-ring and a mercury strain gauge at their penis (Karacan, I., 1969) to detect NPT for the evaluation of impotence. When NPT occurred, a technician checked the breakage of the stamp-ring, measured penile rigidity, buckle rating and penile circumference change, took a picture and estimated his and patient's own evaluations of the erection.

The stamp-ring technique detected sufficient NPTs for vaginal penetration in 64 potent men and insufficient ones in 83 impotent men (Chi-square 92.38, $P<0.001$). The results indicated that a sufficient erection for vaginal penetration, not in a full erection, was required before the stamp-ring breakage occurred. However, it does not provide the investigator with the number and duration of NPT and with its coincidence with REM sleep. Therefore, the stamp-ring test seems to be most useful for patient's evaluation before the clinic visit.

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ESTROGEN REVERSED LUTEINIZING HORMONE RESPONSE TO IMMOBILIZATION STRESS. HIGUCHI,T., HONDA, K., FUKUOKA, T. AND NEGORO, H. Dept. of Physiol. Fukui Medical School, Fukui 910-11

The effects of estradiol benzoate (EB) on stress-induced luteinizing hormone (LH) response were examined. Serum LH concentrations were determined by radioimmunoassay just before, 10, 30 and 60 min after the start of immobilization stress. In gonadectomized rats without EB treatment, the stress induced a rapid decrease in serum LH levels while pituitary responsiveness to LHRH was unaltered. Two days after EB (100 ug/kg) injection, basal LH levels in serum before stress were decreased and raised significantly (P 0.01) by immobilization. A similar increase of LH in response to the stress was observed in ovareictomized rats treated with noradrenaline synthesis inhibitor (FLA-63) that reduced basal LH levels. It is suggested that stress inhibited LHRH release in gonadectomized rats without estrogen treatment but stimulated it after estrgen injection and that this difference in LH responsiveness may be dependent on the basal activity of LHRH neurons or on noradrenergic influence on LHRH neurons prior to the stress.

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CENTRAL EFFECT OF THYROTROPIN-RELEASING HORMONE (TRH) ON THE ELECTROMYOGRAM OF RAT UTERUS. Sugawara, M.* and Tonoue, T. Dept. Physiol.II, Sch. Vet. Med., Azabu Univ., Sagamihara, Kanagawa 229

The central regulation by the neuropeptide of the reproductive organs through the autonomic nervous system has been less extensively investigated as compared with other organs such as the gastrointestinal tract. The existence of direct neural connections between the CNS and ovary has been suggested during the past few years. In this study, adult virgin female rats were anesthetized with nembutal and the electromyogram of uterine horn was conducted through a suction electrode. The electromyogram before and after intracerebroventricular injection of TRH (2.5 μg/100 g) was recorded. TRH suppressed the generation of spike burst which was expected to occur at a regular interval in the proestrus, estrus and metestrus rat. Thus, the interval between the burst before and after TRH injection was prolonged by 3 fold of the control. This response was not produced in the diestrus rat. In the estrus rat, the bilateral vagotomy did but cord-transection did not abolish the TRH-effect on the uterus. The results suggested that TRH in the brain may affect the uterine activity through the vagus nerve.

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ESTROGEN-SENSITIVE NEURONS IN THE FEMALE RAT HYPOTHALAMUS PROJECT TO THE MIDBRAIN CENTRAL GRAY VIA THE SUPRAOPTIC COMMISSURE.
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Electrophysiologic dissection of efferent path of the ventromedial hypothalamus (VMH) to the midbrain central gray (CG) was accompliahed in urethane-anesthetized ovariectomized female rats with acute knife cut in either the periventricular system (PVS) or the supraoptic commissure (SOC). The two preparations differed in the frequency distribution of antidromic spike latency after CG stimulation ($p<.05$). The responses in the PVS-cut animals (n=80) were fewer at 14-16 msec than in the females without knife cut (n=167); those in the SOC-cut animals (n=78) responded less frequently at 17-19 msec (both at $p<.05$). CG-evoked potential collided with those induced from the dorsal longitudinal fascicle or the perifornical region. Estrogen supplement to the animal decreased antidromic activation threshold in VMH neurons descending in the SOC ($p<.001$) but not those in the PVS. The estrogen-sensitive VMH cells descending in the SOC may be involved in the control of estrogen-dependent behavior such as lordosis.

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EFFECTS OF THE MEDIAL PREOPTIC AREA STIMULATION ON THE MIDBRAIN CENTRAL GRAY REGULATING TO LORDOSIS IN FEMALE RATS. NABEKURA, J.*, YAMABE, K.*, AND OOMURA, Y. Dept. Physiol., Fac. Med., Kyushu Univ., Fukuoka 812

To investigate the functional relationship between the medial preoptic area (MPOA) and the midbrain central gray (MCG) on lordosis behavior, single neuron responses of the MCG were examined in anesthetized oophorectomized estrogen primed female rats, to electrical stimulation of the MPOA and for iontophoretic application of LH-RH. Tri-phasic field potentials evoked in the MCG by electrical stimulation of MPOA, did not change after the roof-cut of MPOA. Out of the 91 MCG neurons tested, MPOA stimulation caused orthodromic excitation in 62%, inhibition in 32%, and antidromic activation in 5%. Monosynaptic neurons with short and long latencies showed increased EPSPs, but their latencies remained constant even after graded increase in the stimulus intensities, as shown by intracelluar recording. Polysynaptic orthodromic excitation showed long latency and variable EPSP, while inhibition showed IPSP. LH-RH applied to 110 MCG neurons, facilitated 36%, inhibited 22% and did not affect 42%. The MCG neurons which were facilitated by LH-RH received excitatory input from the MPOA. Neurons which showed long latent monosynaptic excitation were facilitated by LH-RH application.
We conclude that LH-RH may be an excitatory transmitter or modulator in the fiber pathways from the MPOA to MCG neurons that is related to the lordosis reflex.

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SITES AND MECHANISMS OF β-ENDORPHIN ACTING ON THE ESTROGEN-ACTIVATED SEXUAL RECEPTIVITY IN THE FEMALE RATS. TORII, M and KUBO, K. Dept. of Physiol., Inst. of Constitutional Med., Kumamoto Univ., Kumamoto 862

The sites and mechanisms of β-endorphin (EP) to facilitate lordosis response by acting at the initial stages of estrogen (E) feedback on the brain were studied in the spayed rats primed with E and progesterone. EP, enkephalins, naloxone, or anti-EP antiserum were injected at E-priming into the third ventricle or the discrete brain areas. The lordosis response after steroid primings was facilitated by intraventricular EP, and inhibited by Met- and Leu-enkephalin, naloxone, and the antiserum. Thus, it is inferred that the endogenous EP, but not the enkephalins, may primarily contribute to the development of E activation of the behavioral receptivity. Lordosis response was inhibited by microinjection of either the antiserum or naloxone into the mesencephalic central grey, facilitated by it into the medial septo-preoptic regions, but not affected by it into the ventromedial hypothalamus. Previous study indicated that EP-facilitation of lordosis response was closely related with the E actions on the septo-preoptic regions. Thus, EP may act at the central grey to modify E actions on the forebrain influences on lordosis reflex.

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EFFECT OF OPIOID PEPTIDES ON SEX STEROIDAL MODIFICATION OF SLEEP CIRCADIAN RHYTHM IN FEMALE RATS. YAMAOKA, S., Dept. of Physiol., Saitama Medical School, Moroyama, Irumagun, Saitama, 350-04,

It is known that the opioid peptides treatment results in the inhibition of LH release. And we have reported that the changes in circadian paradoxical sleep(PS) rhythm reflect the central feedback of sex steroids.In this study, we examine the role of opioid peptides in the steroidal modification of sleep circadian rhythm in female animals. In cycling rats, opioid peptides(β-endorphine:β-EP, met-enkephaline: mEK, leu-enkephaline: lEK) disrupted the night PS decrease on proestrous night and the ovulation. While, naloxone(NAL) blocked their effects. In ovariectomized(OVX) rats,β-EP, mEK and lEK disturbed the estradiol(E2) induced night PS decrease. While, the NAL treatment also disturbed this effect. The progesteron(P) treatment alone 72hr after E2 and opioid peptides or NAL showed the night PS decrease. But P with opioid peptides or NAL blocked the night PS decrease. The block of E2 induced night PS decrease by NAL was disturbed by DDC(a catecholamine synthesis inhibitor), but P with NAL and DDC did not show night PS decrease. On the other hand, in OVX guinea pigs, opioid peptides and NAL did not affect on steroidal modification of PS circadian rhythm. From these results, it is suggested that the opioid peptide differently affect on the central mechanism of steroidal feedback to PS circadian rhythm among the cycling rats, OVX rats and OVX guinea pigs.

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EFFECT OF ESTROGEN IMPLANTATION INTO THE FOREBRAIN AND BASAL HYPOTHALAMUS ON PULSATILE LH SECRETION IN OVARIECTOMISED RATS. TADOKORO, Y., AKEMA, T. AND KIMURA, F. Dept. Physiol., Yokohama City Univ., Yokohama, Kanagawa 232

Pulsatile secretion of luteinizing hormone (LH) was evaluated before and after local implantation of crystalline estradiol benzoate (EB) into various brain regions in ovariectomized rats. In rats in which EB was implanted in the medial basal hypothalamus (MBH), the amplitude of existing LH pulses significantly decreased while the frequency did not chenge for 3 h after implantation. In rats with EB implanted in the preoptic suprachiasmatic area (POSC) or the nucleus of the tractus diagonalis and the diagonal band of Broca (DBB), mean LH pulse frequency decreased rapidly while the pulse amplitude did not chenge for 3 h after implantation. Rats with the EB implant in the bed nucleus of the stria terminalis, the medial preoptic area, the medial septal nucleus or the anterior hypothalamic area showed unchenged amplitude and frequency of pulsatile LH secretion. Implantation of progesterone into the POSC of ovariectomized rats produced no significant chenge in LH secretory profiles. It appears that the sites of estrogen action in modulating pulsatile LH secretion are not wide-spread but rather restricted within a small part of the brain, including the POSC and DBB in frequency modulation and the MBH and/or the pituitary in amplitude modulation.

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FAILURE OF ESTRADIOL TO ANTAGONIZE THE INHIBITORY ACTION OF DOPAMINE ON PROLACTIN SECRETION IN RATS WITH MEDIAL BASAL HYPOTHALAMIC LESIONS. ARITA, J. AND PORTER, J.C. Dept. Physiol., Yokohama City Univ., Yokohama, Kanagawa 232

Intravenous infusion of dopamine (DA) inhibited prolactin (PRL) release induced by treatment with alpha-methyl-tyrosine (aMT) in ovariectomized rats. The DA infusion was ineffective in ihhibiting the PRL release after aMT in estradiol benzoate (EB)-treated rats. The ability of EB to antagonize the inhibitory action of DA on PRL secretion was lost in rats with medial basal hypothalamic (MBH) lesions. This failure of EB to antagonize the inhibitory action of DA on PRL secretion in MBH-lesioned rats was not the result of changes in DA receptors in the pituitary gland. However, it was found that EB treatment reduced the amount of DA incorporated into subcellular particles cosedimented with PRL secretory granules in aMT-treated rats but not in MBH-lesioned rats. It is suggested that an alteration in the process of compartmentalization of DA in mammotrophs is responsible for the loss of the ability of estradiol to antagonize the inhibitory action of DA on PRL secretion in MBH-lesioned rats.

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A STUDY ON THE ANALYSIS OF POSTURE BY THE DISTRIBUTION OF FOOT-PRESSURE AND THE CURVE OF PARIETAL REGION. ITOW, H., SAKAMOTO, M*., OSADA, Y., AND RI, R*., Body Reformatory School., Meguro, Tokyo 153

We investigated more minutely on the posture by the distribution of foot-pressure and the curve of parietal region. We used the measuremental apparatus of foot-pressure and the drawing machine of the curve of parietal region. In addition, the subjects was 22 the students of Waseda University. Thus we recognized formerly the existence of 6 patterns for the standard of posture, but considering from the two measuremental results we recognized the existence of increased 16 patterns.

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THE ERROR IN FOREARM POSITION SENSE TEST IN NORMAL SUBJECTS. MIYAMOTO, S., SAWADA, Y., AOKI, M.* and FUJITO, Y.* School of Allied Health Professions and * Dept. of Physiology, Sapporo Medical College, Sapporo 060.

The accuracy of forearm position sense was assessed in 33 normal men and women. For this, a specially devised semicircular plastic protractor, vertically held in front of the subject along the midline, was utilized. The subject was asked to perform finger pointing tasks extending the arms without visual guidance. The error magnitudes were estimated in terms of angular displacements from the zero degree (horizontal level of either arm in one hand task) and between the two indicated fingers in two hands' task. The error direction was expressed as plus or minus depending on the directions of indicated finger points above or below the zero degree, or reference degree of either arm. The dominant hand pointed significantly below the zero degree in one hand task; $-1.9^{\circ}\pm2.7^{\circ}$ (M$\pm$SD), $P<0.005$ in men and $-2.8^{\circ}\pm3.4^{\circ}$, $P<0.025$ in women. On the other hand, nondominant hand pointed above or below the zero degree; $-0.9^{\circ}\pm3.0^{\circ}$, $P>0.05$ in men and $+0.1^{\circ}\pm2.2^{\circ}$ in women $P>0.05$. In both hand's task; dominant hand pointed significantly below the zero degree; $-2.1^{\circ}\pm1.4^{\circ}$, $P<0.005$ in women, but $-0.6^{\circ}\pm3.7^{\circ}$, $P>0.05$ not significant in men. In women, the dominant hand pointed significantly lower than the non dominant hand: $-2.1^{\circ}\pm1.4^{\circ}$, $P<0.005$.

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CENTRAL CONTROL OF HUMAN GAIT SHOWN BY AUTOREGRESSIVE ACTIVITY OF LOWER LIMB. YAMASAKI, Y., SASAKI, T., TSUZUKI, S., AND TORII, M. Dept. Physiol., Inst. Constit. Med., Kumamoto Univ., Kumamoto 862

The present study was attempted to demonstrate the characteristics of central control on human gait from autoregressive activity of lower limb during treadmill walking in the course of counting each step by oneself by repeating a set of 3 to 5. The obtained experimental conclusions were as follows: 1) Step length can be divided into two components, step duration and step speed. The power spectrum of step speed revealed the peak at a periodicity of the number of steps in the set as well as that of step length, whereas in the power spectrum of step duration the peak diminished. 2) When the number of steps in a set was abruptly changed into another, a dominant peak at a periodicity of the number of steps in the set appeared in the respective power spectra. 3) The power spectrum in counting each step silently was compared with those in counting aloud and in synchronizing to the rhythmical beat of metronome. Although the highest percentage of power at a periodicity of the number of steps in the set occurred in using a metronome, it seemed that there was hardly any change in peak of power at the periodicity.

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VENTILATORY DYNAMICS DURING THE EXERCISE ONSET OF CYCLING LOADS.
SHIMOSHIKIRYO, K., NAGATA, A. AND MURO,M. Bio-Dynamics , Toho University School of Medicine , Tokyo 143

The time course of ventilatory and circulatory responses to cycling loads by the bicycle type ergometer (iso-power ergometer) was studied about 6 healthy adult men. This exercise load was produced quantitatively and continuously by the computerized method of the electromagnetic power brake. Exercise loads used in this study were composed from the constant step-mode (square-type load with 100 watts intensity and 5 min. duration) and the gradual ramp-mode (time-progress-type load with 100 watts/min. slope). Trials of subjects were twice times at 2 days. Pulmonary ventilation ($\dot{V}E$), breathing pattern (tidal volume and respiratory frequency), oxygen uptake ($\dot{V}O2$), CO2 output ($\dot{V}CO2$) gas exchange (R), heart rate (HR) and integrated electromyogram of the vastus lateralis (IEMG) were measured in the time course of this exercise-mode, symetrically.

Following results were obtained: 1) Comparing the first and the second exercise trials, the transient phase at three parameters changes of $\dot{V}O2$, $\dot{V}CO2$ and $\dot{V}E$ were decreased in these half time and duration, significantly. 2) At the 1st trial, IEMG during the ramp load exercise was observed to be overshot temporally at following 40 sec after the load onset. 3) Significant relationship was appeared between the time constant and exercise intensity from $\dot{V}O2$ parameters.

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EFFECTS OF CONTINUOUS TRAINING UPON THE DEVELOPMENT OF CARDIORESPIRATORY FUNCTION IN BOYS.
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For the purpose of elucidating the interrelationship between growth and effects of continuous physcial training on adolescents under the age of peak height velocity (PHV), aerobic power and cardiorespiratory function of 8 high school boys (15 yrs), who had engaged in Judo training during the low-teen ages, were measured using a noninvasive method of Wasserman. The subjects performed a bicycle exercise, in which work load was increased by a 10 W step each 30 sec. The exercise was continued until the subject was exhausted, or a plateau of $\dot{V}O_2$ was attained. A significant increase in $\dot{V}O_2$max,anaerobic threshold(AT), cardiac output and stroke volume (SV) and a significant bradycardia were observed in trained boys in comparison with untrained boys of the same age. However, the differences were not significant except for heart rate if normalized for body weight. A positive correlation was observed between $\dot{V}O_2$max and either of peak SV or body weight.

It has been concluded from the above results that (1) training during the PHV age intensifies primarily the muscle mass growth,(2) cardiorespiratory function increases to match the O_2 consumption required by the increased muacle, and (3) heart volume plays a dominant role to determine the aerobic work capacity.

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CHANGES OF HUMAN TYMPANIC MEMBRANE TEMPERATURE PRODUCED BY HEAD COOLING DURING LOCOMOTIVE EXERCISE.
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1. Subjects were long distance runners, walking racers and swimmers. Tympanic membrane temperature(Tty), rectal temperature, skin temperature, heart rate and O consumption with head cooling or not were recorded simultaneously during exercise. Two of exercise were used by Monark supine ergometer (100 watt 20 min) and bicycle ergometer (150 watt 20 min). Tty in long distance runners and walking racers decreased always greater in the case of head cooling during exercise. But the influence of head cooling on Tty was not observed in swimmers during exercise.
2. Locomotive exercise performance increased in physical teachers by head cooling during maximal bicycle ergometer exercise.

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RENAL FUNCTION DURING PHYSICAL EXERCISE. IKAWA,S., SUZUKI,M., SHIOTA,M., KWAKAMI,[1] K. AND THOMOSETT,[2] C.R.* Dept. Lab. Med., Tokyo Jikei-Kai Univ., Sch. of Med., Minatoku, Tokyo, 105 1) Dept. of Radio isotope, 2) Lyon Univ.

In our prior study, decrease of urinary osmolality and increase of urine volume were observed at 30 min after exhaustive exercise. To investigate mechanism of changes in urinary osmolality and urine volume after exhaustive exercise, five healthy male volunteers were used in this study. On saline loading experiment(SE), they ran on the treadmill for 10 to 12 min at 30 min after saline solution(0.9% NaCl) intake. On control experiment(CE), they ran without any solution loading. Increase in aldosterone and ADH concentration in plasma after run were slightly inhibited on SE. Urine volume, Na, Cl, and K excretion in urine on SE were higher than on control experiment, but the pattern of urinary osmolality and urine volume changes was as same as on CE. The marked increase in Na/Cl ratio in urine(2-7) was also observed at 30 min after exercise. The fall in urinary osmolality and increased urine volume at 30 min after exhaustive exercise were accompanied by increase in aldosterone and decrease in ADH concentrations in plasma, and kallikrein excretion in urine increased at 30 min after exercise both in the SE and CE.

Remarkable rise in Na/Cl ratio may contribute to maintain ion balance in acidemia during exhaustive exercise.

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EFFECTS OF INTERMITTENT LONG-TERM HYPOBARIC ENVIRONMENT AND SWIMMING TRAINING ON ENZYME ACTIVITIES OF SKELETAL MUSCLES IN RATS. TAGUCHI, S., HATA, Y., ITOH, K. AND ITOH, M. College of Liberal Arts, Kyoto University, Kyoto 606

The male Sprague-Dawley rats were divided into three treatment groups. They consisted of : (A) untreated control rats born and raised at sea level(n=6), (B) sea level rats with swimming training(n=4), (C) rats with swimming training under intermittent hypobaric pressure in terms of 2 wk interval for 14 weeks(n=5). C group showed significantly greater values in Hb and Hct than those of B and A group, and also greater in plasma protein than A group. These blood parameters would indicate that C group had normally polycythemia cosequent to hypobaric adaptation. In muscle glycogen, C group had greater content in m. tibialis anterior and superficial portion of vastus lateralis(SVL) than those of group A. In deep portion of vastus lateralis (DVL), group B showed higher values than that of group A. The SVL was the only muscle that showed a significant difference of PFK in response to hypobaria and training. There were no statistically significant differences in succinate dehydrogenase among groups. The C group had the longest swimming time on performance test, followed by group B and A. In comparison with previous studies, it seems probable that during postnatal development, environmental oxygen tension with physical exercise is an important factor that determine the activity of respiratory enzyme after birth.

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Effect of physical training on β-adrenergic receptors of rat fat cells. Izawa, T., Koshimizu, E., *Komabayashi, T. AND *Tsuboi, M. Dept. of Physical Educa. AND *Dept. of Pharmacol., Tokyo coll. of pharma., Hachioji, Tokyo 192-03

Effect of physical training on β-adrenergic receptors in rat fat cells was investigated. (-)[^{3}H]Dihydroalprenolol (DHA) binding to intact fat cells was not satulated at high dose radio ligand. This result suggests receptors turnover of cell surface (e.g. internalization). In this study, to prevent receptors turnover of cell surface, fat cells were treated with 10 μM valinomycin. DHA binding to valinomycin-treated fat cells was saturated at 70-80 nM radio ligand. Scatchard plots of steady state binding data from valinomycin-treated fat cells display the existance of at least two populations of binding sites which is high affinity binding site and low one. Binding capacity of high affinity binding site was similar in trained, paired-fed and control group. Whereas binding capacity of low one was increased about 1.5-hold in trained (0.746±0.119 p mol/10^6 cells, Bmax S.E., respectively) compared with control (0.515 ±0.022 p mol/10^6 cells). Moreover, this capacity per cell surface area was significantly increased in trained compared with control.

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EFFECT OF GLYCOGEN DEPLETION ON O_2 DEBT AND BLOOD LACTATE. NARUSAWA, M., TERAO, T., NAGAMI, K. and NAKANO, S. Dept. of Physiol., Tokai Univ. Sch. of Med., Isehara, Kanagawa 259-11

To study a relationship between O_2 debt and blood lactate after exercise, five male subjects exercised on bicycle ergometer at 80% of $\dot{V}O_2$ max. work loads five min for each state, in normal glycogen(NG), glycogen-depleted(GD) and glycogen-depleted with administrated glucose(GDG) states. In the GD state, each subject performed ergometer work eliciting 65% Of $\dot{V}O_2$ max. until exhaustion and after the depletion protocol, subjects abstained from caloric intake until after 24 hrs. In the GDG state, after the glycogen-depleted state, each subject was given orally glucose 100g before one hr the experimental test.

The results were as follows: (1) The postexercise $\dot{V}O_2$ curve was unaffected by either state. And O_2 debt was not significantly difference between three states. (2) The postexercise max. Δblood lactate in the GD and GDG states were significantly reduced than in the NG state. But the lactic O_2 debt was not significantly difference in three states.

These results suggested that blood lactate concentrations during exercise did not affect to lactic O_2 debt.

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CHANGES IN FATTY ACID COMPOSITION WITH TRAINING. IWAGAKI,S.Dept. of Physiol., Sch.of Physical Education, Tokai univ., Kitakaname, Hiratsuka 259-12

To clarify direct evidences of exercise and rest on changes in fatty acid composition with training, interval swimming was loaded to the rats. Immediately after the swimming, oleic and linoleic acid compositions of heart muscle triglyceride(TG) decreased and re -markable increase of stearic acid and decrease of oleic acid compositions of skeletal TG were observed. Linoleic acid composition of cardiolipin decreased in heart muscle and increased in skeletal mucles. Stearic acid composition of phosphatidylethanolamine(PE) increased in both muscles, but in fatty acid composition of phosphatidylcholine(PC) dec -rease of stearic acid and increase of oleic acid compositions were observed in heart muscle but in skeletal muscles. At the 4th day after the swimming, there were differenc -es in fatty acid composition of heart muscle between fast and slow recovery groups; pa -lmitic acid composition of TG,PE and PC were decreased in slow recovery group, but the -re were not differnces of the composition in other lipids of skeletal muscles. Linoleic acid composition of cardiolipin in heart and skeletal muscles significantly increased during the rest and especially in skeletal muscle the composition increased over the va -lues observed after the swimming. Besides, there were differences in the composition between fast and slow recovery groups and the composition of slow recovery group was hi -gher than that of fast recovery group.

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CARDIOVASCULAR LIPIDS IN EXERCISED RATS. FURUI, H., HARA, T., HASHIMOTO, M., TAKAHASHI, K. AND MASUMURA, S. Dept. of Physiol., Shimane Medical Univ., Izumo, Shimane 693

It has been recently reported that physical training increases plasma HDL. This finding suggests that exercise would prevent cardiovascular system from lipid deposit, because plasma HDL deprives vascular wall of cholesterol. We estimated cardiovascular lipids (cholesterol, phospholipid and triglyceride) in trained animals.

In this experiment, young female Wistar rats (4 weeks of age) ran at 7 - 25 m/min with a slope of 10° for 1 hr/day, 6 days/week. After 4 weeks, the same rats were made to run continuously at 25 m/min for 1 hr/day. The entire experiment lasted 12 weeks. The estimated values of these lipids were expressed in terms of mg/g wet weight.

With respect to sedentary rats, lipid contents in cardiac tissue were 1.49 mg/g for cholesterol, 12,2 mg/g for phospholipid and 2.91 mg/g for triglyceride, while those found in the aorta were 1,12 mg/g for cholesterol, 3.03 mg/g for phospholipid and 3,97 mg/g for triglyceride. After forced running, triglyceride in the aorta decreased by 28 % ($p<0,005$), whereas the other cardiovascular lipids unchanged. It is deduced from our findings that running exercise is effective in reducing triglyceride in the circulatory system as compared with cholesterol and phospholipid.

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CHANGES OF URINARY SUBSTANCES AFTER EXERCISES ON A BICYCLE ERGOMETER IN ENDURANCE AND SPRINT RUNNERS. OGO, K. and INOMOTO, T. Dept. Health and Physical Educ.,Fac. Educ. Kumamoto Univ.,Kumamoto 860,Dept.Mor.,Inst. Const. Med., Kumamoto Univ., Kumamoto 862

The relation between the intensity of exercise on a bicycle ergometer and the changes of urinary composition up to 120 min. after exercise was investigated in endurance runners (E group) and sprinters (S group). Urinary volume,Na^+,Cl^-,$K^+$and uric acid decreased evidently in both groups, immediately after exhaustive exercise which was progressive load (60 rpm) for the measure of $\dot{V}O_2$ max. The urinary volume,Na^+ and Cl^-recovered more rapidly in E group than in S group. Though the urinary components of both groups decreased immediately after the rapid (90 rpm) and high intensity exercise, except urinary volume of S group, the urinary volume, creatinine, uric acid and Cl^-recovered more rapidly in S group than in E group. The high intensity exercise which associated with very large oxygen debt, indicated not only oligo uretic effet but also diuretic effect.

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URIC ACID METABOLISM AT EXERCISE (4th report). MIKAMI, T. AND ITO, A. Institute of Health and Sports Science, Tsukuba University, Ibaragi

The purpose of this study was to investigate the influence of exhaustive exercise on renal urate secretion and reabsorption. One subject performed exhaustive running on a treadmill 6 hours after intake of drugs. The administration of drugs was performed as follows: 1) 4g of prazinamide and 0.1g of benzbromarone, 2) 4g of prazinamide and 1g of benecid, 3) 0.1g of benzbromarone. On basis of these results,the following values were calculated. 1) Glomerular filtration rate of urate. 2),3) Rate of urate reabsorbed at proximal and distal tubule. 4) Rate of urate secreted at distal tubule. The results were as follows: 1) Rate of secreted urate became 21% lower than resting value. 2) Rate of urate reabsorbed at distal tubule became 7% higher than resting value. 3)Urinary urate excretion was lowest when urinary lactate excretion increased remarkably. These results suggest that the primary cause of urinary urate excretion decreased by exhaustive exercise is decreased urate secretion,second cause is increased urate reabsorbed at distal tubule and that increase of urinary lactate excretion may dimish urinary urate secreted at distal tubule.

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EXERCISE-INDUCED CHANGES IN BLOOD TRACE METALS AND RELATED FACTORS IN MEN. OHNO, H. Dept. of Physiology, Asahikawa Medical College, Nishikagura, Asahikawa 078-11

Influences of bicycle exercise (about 75 % $\dot{V}o_2max$ for 30 min) on the concentrations of 3 trace metals (Zn, Cu, and Fe) and related factors in red cells and/or plasma were studied on 11 untrained healthy male students. Lower concentrations of total Zn, and of Zn derived from carbonic anhydrase B type (CA-B), and a higher concentration of Zn derived from CA-C in red cells were found immediately after exercise, but after 30-min rest they all returned to their pre-exercise levels. The concentration of Zn other than that derived from the CA did not vary substantially after exercise. The change in total Zn concentration correlated well with that in CA-B level immediately after exercise as well as after 30-min rest. On the other hand, there were significant increases in the plasma concentrations of total Zn, and of α_2-macroglobulin(α_2-MG)-bound Zn immediately after exercise, while no such effect was observed in albumin-bound Zn. A positive correlation was found between the changes in concentrations of total Zn, and of α_2-MG immediately after exercise. The blood concentrations of Cu, and Fe, and related factors showed similar changes immediately after exercise.

These results suggest that physical exercise of short duration induces the movement of trace metals into human plasma.

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OBSERVATION OF HIGH AND LOW FREQUENCY FATIGUED MUSCLES IN THE RAT USING 31PNMR.

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31PNMR spectrum method was applied to investigate conditions in high and low frequency fatigued muscles intact of the rat. The lower extremity muscles (left and right legs) were stimulated to contract for five minutes by the electrical method. The stimlation frequency was used 50 Hz and 20 Hz to generate high-short and low-long terms muscular fatigue, respectively and this pulse-wave duration was 0.1 msec.

After the stimulation, each fatigued muscle was isolated to sample for the preparation on 31PNMR spectrum analysis. Also, ATP in fatigued muscles was measured, compared with fresh (control) muscles, quantitatively. At the high frequency fatigued muscle, the evoked initial tension was maintained for only a few seconds and then was falled down at following 30 seconds until the level between 20 % and 30 % of the initial value. In contrast, at the low frequency fatigued muscle, the tension was decreased more slowly than that of the high frequency muscle. However, ATP substance were shown to be decreased at the high frequency fatigued muscle, while at the high frequency fatigued muscle, this material were not appeared to be reduced predominantly. These data indicate that each fatigued muscle is depended on the difference between high and low frequency stimulation and then muscular performance and its substance should be generated in different levels according to each fatigued method.

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CAUSE OF GENESIS OF RADIAL AND MIRROR SYMMETRIES IN ANIMAL BODY PLAN. TSUKEDA, K. Dept. Med., Tokyo Univ., Hongo, Tokyo 113

Most animal plans are either radial or mirror symmetry. Observations of fossils have revealed that the former appeared and evolved when warm temperature lasted, while the latter evolved when the temperature tended to lower. We can assume that, in the genesis of the earth, elements were produced in increasing order of atmic weight, allowing duplication of lighter elements. Compounds with such structures as C-N-O, P-S-Cl, Se-Br, and I usually have lower melting points, ranging from -200° to 300°C. On the other hand, compounds including Na-Mg, K-Ca, and Mn-Fe have relatively high melting points, ranging from 0° to 1200°C. In the process of evolution, it is considered that animals with radial symmetry were created when organisms adapted to high temperature after forming body structure specifically using compounds with lower melting points, and that animals with mirror symmetry were created when organisms adapted to lower temperature after forming body structure specifically using compounds with relatively high melting points.

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GEOGRAPHIC DISTRIBUTION OF BASAL METABOLIC LEVELS AND BIOLOGICAL EQUATOR SASAKI, T. Dept. Physiol., Kumamoto Univ., Kumamoto 862

The so-called racial difference in basal metabolism is complicated by a variety of differences in ethnic groups, mode of living, food habits and climate. To differentiate them data were collected on 85 indigenous populations of varying races as well as 79 non-indigenous populations, living under diverse climatic conditions after having moved from temperate zones.

Basal metabolism is high in the indigenous groups living in the arctic and highland regions, and low in those living in the tropics. A slight lowering is also noticed in the Orientals. The change resulted mainly from acclimatization to heat and cold. Some insist no racial differences, while others claim different types of adaptation among ethnic groups. The non-indigenous populations in the arctic regions show a slight rise in basal metabolism, and those in the tropics a slight fall.

Distribution of metabolic levels across the latitudes is asymmetric with respect to the geophysical equator. The biological equator of basal metabolism was computed to be at 11°N in the indigenous populations, and at 20°N in the non-indigenous populations.

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RESPONCES ON THE CARDIOVASCULAR FUNCTIONS AND METABOLIC SYSTEMS IN RATS UNDER THE STATIC-ANTIORTHOSTATICS EXPOSURE. KOHNO, M., SUDOH, M., NAKAYA, M., AND SAIKI, H. Space Mecicine Laboratory, The Jikei Univ. School of Med., Minato-ku, Tokyo 105

The deteriorative changes of cardiovascular and metabolic systems in rat during prolonged hypokinetics by suspension technique, and the changes of blood pressure in rat during the short duration exposure of the position of abnormal body axes (-45°, -90°, +45°, +90°) measured by modified Popivic cannuration technique were studied. Results: 1) Resting metabolic rate of anti-orthostatic group was decreased. The physiological effects of hypodynamics are more profound at anti-orthostatics than at orthostatics. 2) The increasing rate of the intrathoracic arterial pressure by anti-orthostatic (20°head-down) suspension for a week was only +7% of control value. 3) The intrathoracic arterial pressure did not change by anti-orthostatics (20°head-down) exposure for 10 minutes, while the central venous pressure was increased. These results seem to indicate that blood volume of the intrathoracic is increased under anti-orthostasis. 4) An increase in the central venous pressure resulted from the head-down tilt (-45°, -90°), suggesting an increase in venous return and central blood volume. The head-up tilt (+45°, +90°) caused decrease in the central venous pressure.

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STUDIES OF SAMPLING AND INJECTION SYSTEM IN CATS UNDER HYPERBARIC CHAMBER WITH TRANSFUSION SYRINGE.
MIZUSHIMA, Y., SEKI, K., SHIDARA, F., TAYA, Y., IWASAKI, M.* AND KUWABARA, N.** Japan Marine Science and Technology Center. Yokosuka, Kanagawa 237. ** Dept. Med., Gifu Univ., Gifu-shi, Gifu 500. ** Life Science Institute, Sophia Univ., Chiyoda-ku, Tokyo.

Previous studies under hyperbaric environment have been limited to blood sampling and drug administration because could not transfuse cats from outside of the hyperbaric chamber. We developed the new apparatus which can be transfused blood and injected drugs. A 30 liters acryl hyperbaric chamber was designed for a working of 11 ATA. It has two systems for direct blood sampling to the outside(1ATA) and alternative dose injection. They tested at 1 ATA air, 6 ATA air and 11 ATA He-02. Male cats were used and polyethylene catheters were inserted into the vein cephalica. The venous catheter was flushed and connected to the trancfusion syringe of this apparatus. After adaptation at hyperbaric chamber with EEG recording blood sampling were taken outside of the chamber. The results this sampling system was satisfied.

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THERMAL INSULATION MECHANISM OF SMALL MAMMALS AT HIGH-ALTITUDE
Yanagidaira, Y. and Ueda, G. Dept. of Adapt. Physiol., Shinshu Univ. School Medicine, Matsumoto, 390

Wild deer mice (n=256) with 14 - 23 g body weight (BW) were captured at three different altitudes, 610 - 1,000 m, 1,900 m and 2,400 m. After raising for about two weeks at the captured sites, the mice were brought back to the 610 m-level and tested in a 0°C-room. As a result, cold adaptation was proved for high-altitude mice. In this connection, the thermal insulation characteristics were investigated. The results were as follows: the pelt/BW decreased as the mean temperatures (ET) of the captured sites increased, the coefficient of correlation r was -0.63, the r for fur/BW vs ET was -0.49, the r for epidermis/BW vs ET was -0.403, the r for the corium with sebum/BW vs ET was -0.49, the r for epidermis with fur/BW vs ET was -0.58, and the under fur appearance rate (%) in examined mice vs ET had a r of -0.71. All were statistically significant. As a whole, all the body-insulating components were playing some roles, increasing the weights in response to the cold. It is interpreted that the fur increase was in part replaced by the increased under fur. The growth of under fur was remarkable in 2,400 m - mice.

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EFFECTS OF CINEPAZIDE ON RATS HIGH ALTITUDE ACCLIMATIZATION.
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Rats (n=72) were raised at 610 m and 2,400 m-altitude levels for 120 days. Both were divided into control and cinepazide given groups. The latter received the drug freely available in the concentration of 100 mg/ℓ of water. In the lower altitude (LA), the ventricular weight was less than that in the high altitude (HA). The right to left ratio was 0.31 ± 0.002 in LA and 0.43 ± 0.011 in HA. The cinepazide-group in HA showed a decrease in the ratio. This agreed well with the fact that the increase of hematocrit in HA was alleviated by the drug intake. Further, 2,3-DPG concentration in HA increased from 25.2 ± 1.00 μM/g-Hb to 29.1 ± 1.68, and the red-cell deformability measured by membrane-pore passage time changed from 5.39 to 4.82 msec, suggesting the possibility of compensation for hematocrit increase. Finally, the survival time under extreme low pressures was increased by the cinepazide intake in LA group, also indicating the decrease of the red-cell passage time. In this series, however, the blood-viscosity values in LA and HA indicated no significant differences.

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EFFECT OF HEAT STRESS AND INSPIRED OXYGEN PRESSURE ON HYPERBARIC BRADYCARDIA
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The heart rate (HR) response to oxygen pressure (PIO_2) was studied in human subjects under various ambient pressures to elucidate the factor(s) in modifying the development of hyperbaric bradycardia. Neither 31 atmospheres absolute (ATA) with nearly normal oxygen pressure ($He-O_2$, gas density (ρ)=5.6 $kg \cdot m^{-3}$) nor oxygen inhalation at 1 ATA (ρ=1.33) developed significant slowing of HR. Air compressed 4 ATA (PIO_2=638 mmHg, ρ=4.55) developed a significant bradycardia. By breathing oxygen at 1.6 ATA (PIO_2 =1200) or at 1.3 ATA (PIO_2=980), a significant slowing of HR was observed at resting state. Hyperbaric bradycardia was observed in the environment that PIO_2 was higher than 980, or in a combined environment which had a gas density of more than 4.55 and PIO_2 of higher than 638. The hyperbaric bradycardia is explained as the result of the action of the cardioinhibitory center. The HR increased in proportion to the raise in esophageal temperature (Tes) during the heat loading at 4 ATA, and the increase rate ($\Delta HR/\Delta Tes$) was identical to that at 1 ATA, however, HR corresponding to a given Tes was less at the hyperbaric environment. The increase in heart rate during heat stress is mainly attributed to the increased plasma concentration of norepinephrine, however, the hyperbaric bradycardia was not masked by the increased sympathetic activity.

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CHANGES IN WATER AND ELECTROLYTE METABOLISM AND RELATED HORMONES DURING COMPRESSION FROM 1 TO 31 ATA IN MAN.
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To study the effect of compression on physiological functions, changes in water and electrolyte metabolism and related hormones were examined in 4 men during compression (Comp) from 1 to 31 ATA and subsequent initial high pressure (HP) period. Comp started at 9h and finished at 21h, with PO_2 rise to 0.3 atm and with room temperature rise from 27 to 31.5°C. Urine flow increased at the night following Comp, while elevated plasma (p-) ADH was observed during late Comp and on the morning following Comp, suggesting that ADH action may be suppressed under hyperbaric heliox environment. Elevated s-K, urinary (u-) K, s-Pi, s-Ca and reduced u-Ca were observed during Comp and/or HP. Rises in s-K and u-Pi may be explained by respiratory acidosis. Levels of s-aldosterone (Ald) and cortisol and plasma renin activity (PRA) at 22h were elevated, suggesting increased Ald secretion probably induced by both PRA and ACTH may have affected electrolyte metabolism. U-17-OHCS, adrenaline and noradrenaline rose during Comp and their rates at night exceeded those in the previous day. Since most of above changes were observed during subsequent 31 ATA period, it was difficult to discriminate effects of compression from those of high pressure.

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CHANGE BY ASCOCHLORIN OF DIABETIC SYMPTOM OF GENETICAL OBESE MOUSE C57BL/6J-ob/ob.
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A fungi's product ASCOCHLORIN(AS)-6 was administered to ob/ob and thin litter mate for one week. The mouse showed some decreases in water intake, urine volume, urine glucose, serum glucose, serum triglyceride, serum nonesterified fatty acids and serum immunoreactive insulin. They also showed a reaction resembling partially to that of normal mice in glucose tolerance test and insulin tolerance test, suggesting that AS-6 repaires insulin sensitivity in periferal tissues.

A derivative AS-103 was also administered to ob/ob mouse intra-orally. Greater decreases than by AS-6 was observed in urine glucose, serum glucose, serum cholesterol and serum immunoreactive insulin, but the changes in water intake, urine volume, serum triglyceride, serum nonesterified fatty acids was not observed.

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SOME EVIDENCES OF EARLY HYPOXIC ACCLIMATION IN MAN DURING SHORT-TERM DAILY EXPOSURE TO SIMULATED HIGH-ALTITUDE.
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Heart rate (HR) and cardiac output (CO) (impedance method) in rest and exercise during 2-4 hours daily exposure to simulated altitudes of 4,000m (0.61ATA)-7,000m (0.41) were determined in 3 subjects on 5 successive days. Two of 3 subjects were veteran mountaineers and the other was not. In the former two, the slope of HR increase in response to a given, stepwise increasing workload by a bicycle ergometer became less steeper day after day, at the high altitudes. Such decline of the slope under high altitude may be one of features of hypoxic acclimation since it has been observed in another group after actual mountaineering. The CO in rest and exercise began to decrease after the 4th day. Another characteristic feature of the acclimation could be seen in this CO decrease, where it depended largely on the decrease in stroke volume instead of HR as had been reported for mountaineering.

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EFFECTS OF CENTRIFUGAL ACCELERATION UPON THE ELECTRICAL ACTIVITY OF BRAIN IN HAMSTERS.
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Changes of EEG during centrifugal acceleration which have been reported are mostly concerned with +Z axis of human and also of many animal species. This paper is dealt with -Gz (from tail to head) in hamsters. Twenty-five hamsters were used. Magnitudes of G were 4,6,8 and 10G. EEG was recorded from the visual cortex by silver ball electrodes implanted. VEP and photic driving were recorded the same recording electrode. Results were summarized as follows: 1) The EEG was unchanged by loaded G from 4G to 10G. 2) In a condition shown relatively fast wave state, the photic driving showed a small amplitude response for 2 - 3 sec. after stimulation. In power spectra of EEG during photic stimulation, the peaks could be recognized in the frequency of photic stimulation and its second harmonics. 3) Under the condition of slow wave state, the photic driving was revealed a higher amplitude response. The peaks of power spectra were recognized in the stimulation frequency and its subharmonic. 4) The amplitude of P_1-N_1 in VEP was decreased during 8G and 10G loading. 5) The peak latency of P_1 in VEP was unchanged during G loading, but after cessation, the peak latency of P_1 was delayed. From these results, it was suggested that the centrifugal acceleration, especially in higher G, affected on the cerebral circulation, such as brain congestion.

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CHANGES OF STROKE VOLUME DURING CENTRIFUGAL ACCELERATION IN HAMSTERS.
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The present paper was intended to investigate whether effects of centrifugal acceleration (G) upon cardiac output were different by the direction of animal body axis loaded, and also whether it could be find some differences between intact and labyrinthectomized animals. The hamster was lightly anaesthetized with Nembutal. G was applied in -Gx, -Gz and +Gz on a hamster. G were 2, 4, 6, 8 and 10G. Stroke volume was estimated by impedance plethysmograph on the chest. In -Gx, the stroke volume of control (intact) group was almost unchanged in 2 and 4G, but decreased from 6 to 10G. The stroke volume of the labyrinthectomized group was increased by loading from 4 to 10G. On the other hand, the stroke volume in -Gz was decreased in both groups. The relationship between the rate of decrease of stroke volume and the magnitude of G was almost linear in both -Gx and -Gz. Changes of stroke volume in the control group were smaller than labyrinthectomized. In +Gz, the stroke volume showed a sudden decrease during increasing G, and in some cases the ischemic block was revealed in ECG. From these results, the decrease of stroke volume in -Gz may be explained by the mechanical forces due to G, resulting the decrease of venous return as well as the increase of peripheral vascular resistance. In +Gz, it is supposed that blood is stored in peripheral organs, particularly in lower extremity.

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TIME SERIES ANALYSIS OF THE EFFECT OF PROTEIN FEED ON THE OXYGEN UPTAKE IN MICE。
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The oxygen uptake in mice was continuously recorded by gravimetric method with a electronic balance (Sartorius, 1205 MP)and a printer (Sartorius, 7808) using a microcomputer。 The gravimetric method depends on the chemical reaction of soda lime and carbon dioxide expired to measure oxygen uptake。 The sample of soda lime was spread over the bottom of the calorimeter box。 Oxygen uptake by a mouse is associated with a drop in the gas pressre of the calorimeter box and followed with inflow of the air。 This is the only cause of the increase in weight of the box。 The cyclical components of time series of oxygen uptake in mice were observed. Time series analysis was applied to the data using the BASIC programs. The trend function y= 1。200X - 4。098 , where y is the trend value for a given time.

The least square spectrum function, F(t)=1。20 + 0.45 X COS(40。00°t - 3.44) was computed。 In order to get periodicity in protein feed mice we require further investigations。

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THE STUDIES ON THE LOW PROTEIN ADAPTATION ON RAT IN THE SUCCESSIVE GENERATION.
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In an attempt to verify the fact of adaptation to low protein diet in successive generations, we have been feeding the rat on 7% casein diet. It was found that the growth rate decreased and sexual maturation delayed successively as the generation proceeded, though a slight recovery was seen after the second generation, which was the only sign of adaptation until the fourth generation. The concentration of plasma total protein especially albumin decreased, but globulin did not. The number of red blood cell and Ht decreased in protein deficiency, though a tendency of recovering seemed to be appeared in the fourth generation.

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EFFECT OF KAPPEN-TEA ON ALCOHOL ADMINISTRATED MICE.
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They say the Kappen-tea have a good effect on hangover. Then we studied an influence of the Kappen-tea for acute dysfunction of liver,blood alcohol concentrations on alcohol administrated mice.

We examined effects of the Kappen-tea for weight. Mice drank an undulluted,quintaple,and decuple solution of the Kappen-tea,free. So,the result of this examination,we didn't find the conscious difference,increasing weight.

We report these liver functions of groups of alcohol administrated mice drank these solutions drawn Kappen-tea free,groups of alcohol duluted by solutions drawn the Kappen-tea administrated mice,these blood alcohol concentrations of alcohol administrated mice drank water,Geen-tea,and Kappen-tea.

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Effects of metabolic inhibitors on the release of amylase and sialic acid and oxygen uptake induced by pilocarpine and isoproterenol in dog submandibular gland slices.

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Effects of metabolic inhibitors on the secretion and oxygen uptake stimulated by pilocarpine (PI) and isoproterenol (IPR) were investigated. The secretion and oxygen uptake were stimulated by PI AND IPR. Both PI and IPR-stimulated secretion and respiration were significantly blocked by oxidative phosphorylation antagonists, oligomycin, antimycin, azide and malonate. 2,4-Dinitrophenol also significantly increased the oxygen uptake, but inhibited the secretion induced by PI and IPR. Iodoacetic acid inhibited the secretion and oxygen uptake induced by PI and IPR. In addition, ouabain significantly inhibited the secretion and oxygen uptake by PI, on the other hand, the oxygen uptake by IPR was inhibited by ouabain, but the secretion was not inhibited.

These results suggest that the secretion from submandibular glands requires continuous supply of energy.

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THE RELATIONSHIP BETWEEN CIRCADIAN VARIATION IN BODY TEMPERATURE AND COMFORTABLE TEMPERATURE OF WATER BATH. TERAI, Y., OGAWA, T., ASAYAMA, M., SUGENOYA, J. and MIYAGAWA, T. Dept. of Physiol., Aichi Med. Univ., Nagakute, Aichi.

Changes in comfortable water temperature during water bathing were examined in association with circadian variation in body temperature, and the results were compared with those in air reported previously. The subject was immersed up to his neck in a water bath. The temperature of water circulating the bath was adjusted to the most comfortable level by the subject himself operating a remote thermostat. Preferred water temperature (Tw-pref) ranged 35-36°C and was significantly higher in the rising phase than in the falling phase ($P<0.02$). The rate of changes in Tre and that in Tes had positive correlations with Tw-pref ($P<0.01$, 0.001, resp.), and negative correlations with $\bar{T}s$ minus Tw-pref ($P<0.01$, 0.001, resp.). Tw-pref varied within a more limited range and was adjusted more accuratly than Ta-pref, and the difference in Tw-pref between the rising phase and the falling phase was more highly significant than that in Ta-pref. It was considered that the subjects had a higher thermal sensitivity to water than to air, because of a higher heat conductivity of the former. These results indicate that comfortable environmental temperature can be affected by circadian variations in body temperature, and reveal that behavioral temperature regulation can be driven by the biological clock.

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THE EFFECT OF PHENTOLAMINE ON DYNAMIC PROPERTY OF THE THERMOREGULATORY SYSTEM IN RATS. ASAKI, Y. AND * IRIKI, M. (Dept. of Physiol., Tokyo Metropol. Inst. Gerontol., Itabashi-ku, Tokyo 173, * Dept. of Physiol., Med. Univ. of Yamanashi, Nakakoma-gun, Yamanashi 409-38)

The influence of phentolamine i.p. injection (10 - 80 μg/kg) on the effect of extraneous disturbances on thermal homeostasis of rats in a hot environment was studied. The ambient temperature of the chamber (Ta), in which the rats freely moved, was controlled to fluctuate in a sine wave. Its cyclic interval was one hour and its amplitude was ±5 °C. The mean Ta during a 2-hour experiment was 30 °C. Continuously recorded were the rectal temperature (Tr), the tail skin temperature (Ts) and Ta. The amplitudes of the one-hour cyclic interval and 2-hour mean levels for Tr and Ts were evaluated and used for further analyses.

After administration of 40 and 80 μg/kg phentolamine, the following significant changes were observed: 1. The ratio of the amplitude of Ts to that of Ta decreased, and 2. that of Tr to Ta increased. 3. The mean Ts increased, and 4. the mean Tr decreased. These results demonstrate that the functional efficiency of the thermoregulatory system of rats in a hot environment is decreased by i.p. injection of phentolamine at doses more than 40 μg/kg.

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ESTIMATION OF CHANGES IN BODY HEAT DISTRIBUTIONS DURING HEAT LOAD UNDER A SIMULATED ALTITUDE OF 5,600 M IN MAN. KONDA,N., MIKI,K., SHIRAKI,K., SAGAWA,S. Dept. Physiol., Sch. Med., Univ. Occup. Environ. Hlth., Kitakyushu 807

Esophageal (Tes) and mean skin ($\bar{T}sk$) temperatures, metabolic heat production, evaporative and non-evaporative heat exchanges were measured to estimate the change in body heat distributions in healthy males during transient heat loads (28-38°C) at sea level (SL) and a simulated altitude of 5,600 m (HA). Dynamic changes of heat distributions of the core and the skin were estimated by diving the body into 3 compartments (core, skin, and blood) and by applying equations (1) Mean body temperature ($\bar{T}b$) = aTes + (1-a)$\bar{T}sk$, (2) Body Heat Content = Body Weight x Specific Heat x $\bar{T}b$. Then, the equation (3) $a = 1 - (\beta - \gamma\ msk/mc) / \alpha$ is derived, where msk/mc is the ratio of blood distibution of the skin to the core and β, γ, α are heat capacities of the skin, the core and the whole body, respectively. Thus, the value of a becomes bigger when vasodilation occurs. The calculated values of a at 28°C were 0.90 and 0.94 at SL and HA, respectively, and they increased to 0.96 during the heat loads at both atmospheric pressures. These evidences may suggest that the vasodilation is not related to the regulation of body temperature but is induced by HA per se. At the altitude a man is in a state of difficulty in controlling body temperature because the proportion of blood flow into the skin does not increase any more by heat loads.

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HEMODYNAMIC RESPONSES TO TRANSIENT HEAT EXPOSURE IN MAN AT A SIMULATED ALTITUDE OF 5,600 M. SAGAWA,S., MIKI,K., SHIRAKI,K., KONDA,N. Dept. Physiol., Sch. Med., Univ. Occup. Environ. Hlth., Kitakyushu 807

Cardiac output (CO), total peripheral resistance, skin blood flow (SkBF), and esophageal (Tes) and skin (Tsk) temperature were measured in 6 male subjects to estimate hemodynamic responses to a transient heat loading (28-38 °C) at a simulated high altitude of 5,600 m (1/2 ATA). Mean Tsk ($\bar{T}$sk) elevated significantly at the equilibrium state in thermoneutrality at 1/2 ATA, whereas Tes did not. A significant increase in forearm SkBF was observed at 1/2 ATA without any change in finger SkBF. Cardiac functions did not change at 1/2 ATA except for a significant increase in the heart rate. A faster raise of Tes and a retarded raise of $\bar{T}$sk were observed by the heat stress at 1/2 ATA. The different attitude toward the temperature response between the two atmospheric pressures will be explained by the difference in the response of SkBF; the pre-existence of vasodilation at thermoneutrality in 1/2 ATA may prevent a further increase in SkBF during the heat loading. The increase in finger Tsk and SkBF during the heat loading was in a less extent at 1/2 ATA in comparison to sea level. Above results will suggest that the high altitude makes a alteration in hemodynamic characteristics of distal parts of the body. Thus, the role of the distal part of the body on the temperature regulation may be less predominant at high altitude.

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ELECTROLYTE AND FLUID BALANCE UNDER THERMAL DEHYDRATION IN THE RAT: ANALYSIS OF VOLUNTARY DEHYDRATION. NOSE, H., MORIMOTO, T., AND YAWATA, T. Dept. Physiol., Kyoto Pref. Univ. Med., Kamigyo-ku Kyoto 602

The mechanism of "voluntary dehydration" after thermal dehydration was analyzed. Adult male rats were exposed to a hot, dry environment (D.B.T.: 36°C; R.H.: 20%) over 6 to 8 hrs, and dehydration amounting to 7-9% of body weight was induced. During the period of dehydration, the rats excreted Na (204 μEq/100g b.w.) and K (225 μEq/100g b.w.) in urine and saliva. About 70% of the excreted Na came from interstitial space of skin, and 92% of K came from intracellular space of muscle. After the dehydration, the rats were divided into 3 groups to which distilled water, 0.45% or 0.9% NaCl sol. was given ad libitum. The amount of water intake reached to an equilibrium in 6 to 12 hrs: the distilled water group regained only 50% of the lost water, while the other two groups regained almost all of the lost water. As to electrolytes,the latter two groups gained Na by about 500 μEq/100g b.w., while the distilled water group gained none. Thus total amount of Na regained by 0.45% or 0.9% NaCl sol. group was almost equivalent to the amount of the cation (Na + K) excreted in urine and saliva during the dehydration. These results suggest that "voluntary dehydration" is caused by the losses of electrolytes which occur with the loss of water during thermal dehydration.

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Effect of methylatropine on thermal salivation of unanesthetized-and ketaral-anesthetized rats. FURUYAMA, F. AND OHARA, K. Dept. Physiol., Nagoya City Univ. Med. Sch., Kawasumi-cho, Mizuho-ku, Nagoya 467

Heat tolerant rats were able to clump their core temperature near 40.0°C by remarkable increase in thermal salivation induced by a small increase in ambient temperature(37.5-40.0°C). Thermal salivation of heat tolerant rats were more resistant to atropines than the other strains. It took longer time for atropines-injected rats to initiate thermal salivation. Ketaral-anesthetized heat tolerant rats secreted the most amount of saliva at ambient temperature of 40°C in 6 different anesthetics-injected rats. Ketaral abolished the atropine resistant thermal salivation. These findings suggest that the atropine resistant thermal salivation in heat tolerant rats is not controlled by autonomic nervous system but by some other system, which is blocked by ketaral in the central nervous system.

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EFFECT OF VENOUS BLOOD POOLING ON SKIN TEMPERATURE OF THE FINGER. NUNOMURA, T., HIRATA, K. AND NAGASAKA, T. Dept. of Physiol., School of Med., Kanazawa Univ., Kanazawa 920

The effect of venous blood pooling on the relationship between skin blood flow (BF) and skin temperature (T_{sk}) was studied in human finger at rest and during exercise. The venous blood pooling was made by inflating a pneumatic cuff wrapped around the wrist (20 mmHg). T_{sk} of the index finger was measured by a thermistor. BF of the middle finger was measured by venous occlusion plethysmography using a Whitney strain gauge. At rest, 5 min of venous pooling was repeated at 5 min intervals at an ambient temperature of 25°C. BF varied between 30 and 150 ml/100ml·min and T_{sk} varied between 30 and 34°C. Venous pooling did not affect the relationship between BF and T_{sk}. Exercise was performed by bicycle ergometer for 30 min at 45% $\dot{V}O_2$ max at 20°C. Venous pooling was made for one hand continuously from 15 min before exercise. BF and T_{sk} of fingers in both hands were measured. BF and T_{sk} increased gradually during exercise. The slope of the relationship between BF and T_{sk} was steeper in the finger with venous pooling than in the finger without venous pooling.

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EFFECTS OF SELECTIVE THERMAL STIMULATION OF THORACIC AND LUMBOSACRAL CORDS ON CARDIOVASCULAR FUNCTIONS IN RATS. TSUCHIYA, K. Dept. of Environmental Physiol. Inst. for Tropical Medicine. Nagasaki Univ. Sakamoto-machi, Nagasaki, 852.

Changes in heart rate (HR), arterial blood pressure (BP) and in the tail vasomotor activity during selective thermal stimulation of the thoracic (Th) and lumbosacral (LS) cords were investigated in rats anesthetized with urethane (lg/kg, i.p.). For spinal thermal stimulation, U-shaped polyethylene tubes inserted in the peridural spaces were perfused alternatively with cold and warm water in each for 5 minutes. In normal (CNS-intact) rats an increase in HR as well as BP was induced by selective cooling of the Th cord but, on the contrary, a decrease in HR by LS cooling. An antagonistic change in HR was also observed after acute decerebration at the mid-collicular level, but any change in tail skin temperature did not occur because of low skin temperature due to sustained vasoconstriction. This antagonistic change in HR in the present experiment vanished after acute spinalization at the cervical level. From these results, it was suggested that the lower part of brain stem may play an important role for the antagonistic change in HR during selective cooling of the Th and LS cords.

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THERMOGENIC RESPONSES AND BLOOD GASES IN RATS AND HAMSTERS DURING HYPOTHERMIA. DOI, K., SHIBUYA, I. AND UCHIDA, K. Dept. of Physiol., Yamagata Univ. Sch. of Med., Yamagata 990-23

The present study was undertaken to determine what changes occurred and what was possible causative factors limiting survival in the later stage of hypothermia in rats and hamsters. Hypothermic induction to the colonic temperature (Tc) of 18 °C by combination of cold and light restrain was rapid (3 hr) in fur-clipped rats (male, 193–256 g) and slow (6 hr) in so treated hamsters (male, 85–102 g). With progressive decreases in Tc after their stable periods, there were parallel falls in oxygen consumption, heart rate and respiratory frequency. In rats, arterial blood pH was significantly lowered from 7.462 at 38 °C to 7.315 at 18 °C. This drop may result from a respiratory acidosis due to a reduction in ventilation. This concept was supported by the findings of increased arterial blood Pco_2 and $[HCO_3^-]$, decreased Po_2 and no change of plasma lactic acid level. On the other hand, pH was maintained constant over a wide range of Tc in hamsters. There were observed marked decline of blood glucose (20 mg%) and severe depletion of hepatic glycogen in hamsters, while such results were not obtained in rats. It is concluded that the decline of pH is deteriorate for rats and the depletion of carbohydrate energy supply for hamsters in the deep hypothermia (18 °C).

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ULTRADIAN RHYTHM OF OXYGEN CONSUMPTION IN RATS DURING EXPOSURE TO COLD.
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It has been previously reported that basal oxygen consumption ($\dot{V}o_2$) in rats showed relatively regular ultradian rhythm and its rhythm was amplified at the dark period (Doi,1980). To find out whether ultradian rhythm still appear and become evident at the state of enhanced energy metabolism, $\dot{V}o_2$ was continuously measured before and during exposure to cold ($3^{\circ}C$) for totally 4 hrs. There was observed the amplified ultradian rhythm, but no fluctuations in tail and colonic temperature, and heat loss. There was found small periodical fluctuations in shivering, but shivering and locomotor activity did not synchronize with ultradian rhythm. When non-shivering thermogenesis(NST) was somewhat reduced due to chronic administration of 6-hydroxy dopamine, ultradian rhythm was not modified. Struggling and concomitant enhanced respiration showed a good synchronization with ultradian rhythm. These results suggest that ultradian rhythm of $\dot{V}o_2$ during exposure to cold results from struggling movement of animals other than heat loss, locomotor activity, shivering and NST.

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AUTONOMIC THERMOREGURATORY RESPONSE TO SELECTIVE TAIL COOLING IN RATS.
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Oxygen consumption ($\dot{V}O_2$), aortic temperature (T_{ao}) and back skin temperature ($T_{sk,back}$) were measured during selective tail colling ($25 \rightarrow 20 \rightarrow 15 \rightarrow 10 \rightarrow 6^{\circ}C$) in rats in cold ($15^{\circ}C$) and warm ($26^{\circ}C$) environments. At a tail skin temperature of $25^{\circ}C$, $\dot{V}O_2$ was higher and $T_{sk,back}$ was lower in cold environment than in warm environment. In cold environment selective tail cooling produced no significant changes in $\dot{V}O_2$, T_{ao} or $T_{sk,back}$. In warm environment, although selective tail cooling did not produce significant changes in $\dot{V}O_2$ or T_{ao}, it caused a progressive decrease in $T_{sk,back}$. It may be suggested that selective tail cooling especially in warm environment would produce vasoconstriction in the back skin of rats. However, a decrease in tail skin temperature during cold exposure may not contribute to cold-induced increase in heat production or vasoconstriction in back skin of rats.

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THE CHARACTERISTICS OF PERIPHERAL FINGER S TEMPERATURE AFTER COOLING OF HAND BY ICE-WATER.
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The skin temperature of peripferal fingers is apt to external enviroment. In accodance with the load of cooling of hand, peripheral blood vessels are spastic with the excitment of the sympathetic nerve and the circulatry systems are ischaemic. After the load, the results are follow. The skin temperature of fingers rises steadily at first, at about $20^{\circ}c$, becomes dull for about ten sec. and then rises steadily again. Simultanously, the hormons in blood serum are determined quantitatively. As the result of determination, only Vasopressin is responsed by the cooling of ice water.

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DIGITAL VASCULAR REACTION TO COLD IN AIR AND IN WATER. IHZUKA, H., WATANUKI, M. AND HORI, S. Dept. of Physiol., Hyogo Coll. of Med., Nishinomiya, Hyogo 663

Male university students were selected as subjects. Each subject in sitting-resting position inserted his left hand with palm downward and with fingers extended slightly into the cold chamber of -10 °C for 30 min. in the chamber of 27 °C and 70 % relative humidity. On the other day, each subject dipped his left hand in stirring water of 5 °C. Both series of experiments were carried out in four seasons. In both series of experiments, the skin temperature on the center of the dorsal surface of the distal phalanx of the left middle finger was recorded continuously by copper-constantan thermocouple during periods of 5 min. before cold exposure, cold exposure and 20 min. of recovery. In both series of experiments, the time for first temperature rise and recovery time became shorter as the skin temperature of finger before cold exposure was higher, the temperature of first rise and the mean skin temperature during cold exposure became higher with rise in skin temperature before cold exposure. Digital vascular reaction to cold in air showed greater individual differences than that in cold water in all seasons. The mean skin temperature of each subject in air was well correlated with that in water. Frequency of cold pains experienced by subjects in air was much smaller than that in water. The greatest digital vascular reaction to cold was observed in summer in both series of experiments.

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CONTINUOUS RECORDINGS OF SWEATING RATE BY USE OF A NEW RESISTANCE HYGROMETER. 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, economical apparatus for continuous recordings of sweating rate utilizing a new hygrometer (Chichibu Cement Co. Ltd. CGS-H11) which changes resistance with changes in vapor tension of the ambient air. The sensor contains a thin hygrotropic film of a aluminum phophate. An acryl skin capsule, 20 mm in inner diameter and 4 mm in height, was equipped with the resistance hygrometer on the internal surface of the roof. A thermometer using a silicon transister of microdisc type was placed on the skin area covered with the capsule. Dry N_2 gas was passed through the lumen of the capsule via a gas inlet and outlet tubes at a fixed flow rate in order to remove the vapor generated by insensible perspiration. A change in water content of the air in the capsule was thus dependent upon the sweating rate from the skin area. A linear relationship between the amount of relative humidity in the capsule and the output voltage (10V/100 % RH) was obtained within a range of relative humidity from 30 to 90 %. A few results of its preliminary application to human beings were also demonstrated. Thus, mental calculation and mechanical vibratory stimulation to the hand caused a significant increase of the sweating rate from the palmer area.

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REGIONAL DIFFERENCES AND SEASONAL DIFFERENCES IN SWEATING REACTION DURING EXERCISE IN A HOT ENVIRONMENT. TSUJITA, J., TOHORI, M., HORI, S., KIMURA, M.* AND ARAKI, T.* Dept. of Physiol., Hyogo Coll. of Med., Nishinomiya, Hyogo 663, *Dept. of Health Science, Hyogo Univ. of Teacher Education, Kato, Hyogo 673-14

Observation of regional relationship of amount of sweat during exercise were made on 7 young male subjects in summer and in winter. Exercise was performed on a bicycle ergometer at a constant work load at a cycling rate of 50 rpm for 60 min. in a room at 30 °C with 60 % relative humidity. Work intensity was kept at a level corresponding to 30, 40, 50, 60, 70, 80 % of the maximal oxygen uptake determined by bicycle ergometer exercise. Local sweat samples were collected at 19 cutaneous sites successively 10 min. intervals by the filter paper method. Sweat rate were greater in summer than in winter. Sweat rate was closely related to metabolic rate. Great individual variations in patterns of local sweat distribution were observed in both seasons. Local sweat rates in the trunk tended to be greater than those in extremity irrespective of work load. Local sweat rate on the chest and thigh in comparison with those on other cutaneous sites became more profusely with increase in work load.

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MECHANISM OF FACILITATION OF GENERALIZED SWEATING CAUSED BY CO_2 INHALATION. SUGENOYA, J., OGAWA, T., ASAYAMA, M., MIYAGAWA, T. and TERAI, Y. Dept. of Physiology, Aichi Medical University, Nagakute-cho, Aichi 480-11

Frequency of sweat expulsions (Fsw) during CO_2 inhalation was analyzed in 3 subjects. Each subject, thermally equilibrated at a specified level of Ta (35-45°C; rh, 40%), inhaled gas mixture containing 5.5-6% of CO_2 and 20.9% of O_2 for 15 min. Local sweat rates were continuously recorded from bilateral forearm areas by capacitance hygrometry; synchronous expulsions were counted and Fsw was calculated. Fsw increased markedly during CO_2 inhalation; the regression line of Fsw against aTcore+$b\bar{T}s$ (used as an indicator of thermal inputs; a and b, determined for the individual subject) shifted to the left during CO_2 inhalation. The increase in local sweat rate with the increase in Fsw was greater during CO_2 inhalation than before. It was considered that hypercapnia may cause to increase the central sudomotor neural activity probably by affecting the cutaneous and/or central thermosensitivity and/or the central processing of thermal inputs, and further that it may exert an effect on the release of transmitter substance at the neuroglandular junction and/or secretory activity of the sweat gland.

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NICOTINE SENSITIVITY OF SUDOMOTOR NERVE IN THE VOLAR SKIN OF PRIMATES — A COMPARATIVE STUDY. AOKI, T. AND IZUMI, H. Dept. of Physiol., Tohoku Univ. Sch. of Dent., Sendai

The nicotine sensitivity of peripheral sudomotor nerve, judged from axon reflex sweating response, was examined in the finger tip of pes or manus in 17 species of primate including Human. Among these, all of macaques (Japanese, Formosan, Rhesus, Crab-eating, Red-faced, Pig-tailed, Bonnet and Assam monkey) and Baboon were outstandingly insensitive to nicotine. However, they could respond, though not remarkably, to DMPP, indicating the presence of nicotinic receptor in their nerves. These nicotine-insensitive nerves were histochemically confirmed to contain not only acetylcholinesterase (AChE) but also butyrylcholinesterase (BuChE), whereas nicotine-sensitive nerves such as in Human, Chimpanzee, Gibbon, Spider monkey, Slow loris, Galago and Lemur contained only AChE. These led us to assume that BuChE might be associated, directly or indirectly, with the low nicotine sensitivity. However, selective inhibition of BuChE by iso-OMPA, a specific BuChE inhibitor, failed to substantially affect the nicotine responsiveness. It may be that the site of nicotinic receptor in these nerves is provided with some diffusion barrier to nicotine, in which BuChE is casually localized, though the possibility that the undevelopment of nicotinic receptor is partly responsible for the low nicotine sensitivity can not be entirely ruled out. Definite elucidation should await further investigation.

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INHIBITORY EFFECT OF EXERCISE ON SHIVERING THERMOGENESIS. NOMOTO,S., NOMOTO-KOZAWA,E. and IRIKI,M.* Dept. Physiol., Tokyo Metropol. Inst. Gerontol., Itabashiku, Tokyo-173, *Dept. Physiol., Med. Univ. Yamanashi, Nakakomagun, Yamanashi-409-38

Shivering can be induced during exercise by spinal cord cooling in the pigeon. In this study, we investigated relationships between the induced shivering and treadmill exercise in the same species.

Six domestic pigeons with chronically implanted spinal thermodes were exercised on a treadmill for 15 min at a speed of 0.6 km/h at neutral ambient temperature. Oxygen consumption ($\dot{V}O_2$) was measured as an indicator of shivering thermogenesis in the experiments. The spinal cord was cooled to various temperature levels for 5 min both during exercise and rest.

During rest, $\dot{V}O_2$ (=shivering) was linearly increased with decreasing spinal temperature from 0 °C to 7 °C lower than the pre-cooling spinal temperature. But during exercise, spinal cooling till about 2 °C had no effect on inducing shivering. Cooler stimuli more than ca. 2 °C elicited shivering linearly with decreasing spinal temperature till about 7 °C. At the same level of spinal cooling, the amount of induced shivering during exercise was less than that during rest.

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Cold adaptability of a lasting consistency in rats reared at 5°C for successive generations. MORIYA, K., YAHATA, T. AND KUROSHIMA, A. Laboratory of Health Education, Faculty of Education, Hokkaido Univ., Sapporo 060 and Dept. of Physiology, Asahikawa Medical College, Nishikagura, Asahikawa 078-11

Cold tolerance and nonshivering thermogenesis were examined in Wistar male rats reared at 25°C for 3 generations after they had been reared at 5°C for 29 generations (Deadapted 3G rats). When Deadapted 3G rats (fasted for 18 hours) were exposed to -5°C for 120 minutes, the rectal temperature remained unchanged as well as 5°C-adapted rats for 5 weeks (C_{5W} rats), while it decreased rapidly in 25°C-adapted control rats. When norepinephrine (NE) was intravenously infused (2 μg/min for 30 min) under hexobarbital anesthesia, the rectal temperature of Deadapted 3G rats increased 0.49°C, while that of control rats did not increase. Oxygen consumption and carbon dioxide output were measured in unanesthetized and unrestrained rats. Both variables did not differ after 0.9% saline injection between Deadapted 3G and the control rats. By NE injection (40 μg/100 g B.Wt., i.p.), oxygen consumption as well as carbon dioxide output increased more greatly in Deadapted 3G than the control rats. Interscaplar and cervical brown adipose tissue weight of Deadapted 3G rats was heavier than that of the control rats, being similar to the weight of C_{5W} rats.

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CROSS-ADAPTIVE EFFECT OF CHRONIC STRESS ON COLD AND ITS MECHANISM. KUROSHIMA, A., HABARA, Y., UEHARA, A., MURAZUMI, K., YAHATA, T. AND OHNO, T. Dept. of Physiol., Asahikawa Medical College, Nishikagura, Asahikawa 078-11, and Division of Nutrition Physiol., Hokkaido Educational College, Hokumoncho 9, Asahikawa 070

Three-hour immobilization stress was forced on the male adult rats by means of restraining them on the board 6 days a week for 1 to 8 weeks. These stressed animals exhibited an improved cold tolerance as shown by no significant fall of colonic temperature in the cold at -5 ℃ for 300 min, while the colonic temperature of the controls fall progressively during this period. Nonshivering thermogenesis as assessed by noradrenaline-induced increase in oxygen consumption was significantly potentiated in the stressed rats. The weight and protein content of the interscapular brown adipose tissue increased and the mitochondria of this tissue developed more in the stressed rats. Plasma insulin, insulin/glucagon molar ratio and thyroxine were lowered in the stressed rats, while plasma triiodothyronine remained unchanged. Extirpation of interscapular brown adipose tissue resulted in the loss of improved cold tolerance and a significant decrease of nonshivering thermogenesis in the stressed rats.

These results indicate that the long-term stress may induce cross-adaptation between stress and cold through an enhanced capacity of nonshivering thermogenesis mediated, at least in part, via stimulation of the function of brown adipose tissue.

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STUDIES ON REGULATION OF CULTURED BROWN ADIPOSE TISSUE. YAHATA, Y., AND KUROSHIMA, A. Dept. of Physiol., Asahikawa Medical College, Nishikagura, Asahikawa 078-11

In order to clarify the mechanisms of differential and functional development of brown adipocytes, the effect of insulin (40 nM)(I), glucagon (400 nM)(G), noradrenaline (6 mM)(NA) and nerve growth factor (2 to 10 nM)(NGF) were directly investigated with brown adipose tissue from neonatal rats cultured in 10 % fetal bovine serum-medium 199 for 1-2 weeks under 95 % air - 5 % CO_2 atmosphere at 33 ℃. I stimulated lipid accumulation and enlarged cell size of outgrown adipocyte. Mitochondria were smaller than that of control and their cristae were less distinct. I+G enlarged nucleus and cytoplasm but suppressed the lipid accumulation induced by I. Mitochondria in I+G -treated cells were larger and their cristae became more prominent than those of I-treated ones. G caused similar ultrastructural changes to those by I+G. I+NA induced similar effects on adipocytes to I+G,but their mitochondria size was not differ from that of I-treated cells. NGF also suppressed the lipid accumulation by I, while it caused marked development of mitochondria, RER and Golgi bodies. These results seem to indicate that multiple humoral factors such as glucagon, insulin, NA and possibly NGF promote differentiation and functional development of brown adipocyte through the enhancement of protein and lipid metabolism.

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INCREASE IN INSULIN BINDING CAPACITY OF ADIPOCYTE BY COLD ACCLIMATION. HABARA, Y., UEHARA, A. AND KUROSHIMA, A. Dept. of Physiol., Asahikawa Medical College, Nishi-kagura, Asahikawa 078-11.

We have recently reported that both insulin and glucagon contents of brown adipose tissue were increased in cold-acclimated rat and that the number of insulin receptors of brown adipocyte also increased during cold acclimation. From these studies, we concluded that insulin may be closely related to nonshivering thermogenesis of brown adipocyte through its lipogenic action. In the present experiment we investigate the changes in insulin binding capacity of white adipocyte using [125]I-insulin in order to obtain further information about the contribution of insulin during cold acclimation. Wistar strain male rats were kept at 5°C for 4 to 5 weeks and isolated adipocytes were prepared by the method of Rodbell. The insulin binding capacity of adipocyte of cold acclimated rat increased twice as much as that of warm controls (25°C).

The present finding suggests that total number of insulin binding sites per adipocyte increase in cold acclimation and this cellular strategy may compensate for the depletion of stored metabolic fuels, especially lipids, utilization of which is enhanced by noradrenaline and glucagon during cold acclimation.

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EFFECTS OF EXERCISE ON BAT THERMOGENESIS IN RATS. SHIBATA, H. AND NAGASAKA, T. Dept. of Physiol., School of Med., Kanazawa Univ., Kanazawa 920.

The effect of exercise on brown adipose tissue (BAT) thermogenesis was studied in rats at an ambient temperature of 24°C. They were forced to run on a treadmill at 13 - 20 m/min for 10 - 30 min while measuring colonic (T_{col}) and interscapular BAT (T_{bat}) temperatures. In the naive rats, forced running for 10 min raised both T_{bat} and T_{col}. The rise in T_{bat} was greater than that in T_{col} for the 10 min exercise period. The rats were then subjected to an exercise adaptation regimen (10 - 30 min exercise a day for at least two weeks) using the same treadmill. Thereafter, the rats were subjected to forced running at 20 m/min for 30 min. Although both T_{bat} and T_{col} were raised by the forced running, the increase in T_{bat} was almost the same as that in T_{col}. Since the difference between T_{bat} and T_{col} reflects the extent of heat production of the interscapular BAT, it was concluded that exercise caused BAT thermogenesis in the naive rats but not in the rats adapted to exercise.

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SEXUALLY DIFFERENTIATED BRAINS PRESENT DIFFERENT FEBRILE RESPONSE IN RATS. ONO,T., MORIMOTO,A., YANASE,M. AND MURAKAMI,N. Dept. of Physiol., Yamaguchi Univ. School of Med., Kogushi, Ube, Yamaguchi 755

Although it has been generally believed that normal rats did not respond to lipopolysaccharide, when endogenous pyrogen (EP) was i.v. injected normal rats did produce a biphasic fever, suggesting the presence of the physiological mechanism for fever development. However, the magnitude in febrile response is very different between male and female; there are sexual differences of febrile response. The differences are probably caused by the ability to produce EP and/or the susceptibility of the central mechanism for thermoregulation to EP. In the present report the latter was intensively investigated. 1) Sexual differences of EP-induced fever did not depend on the activity of the gonads from castration experiments in both sexes. 2) EP-induced fever in genetic female, to which testosterone was administered within the critical period after the birth and whose brain was sexually masculinized in turn, was not different from that in control male. These results indicate that sexually differentiated brain involves the central mechanism for fever development and the autonomic function is also influenced by the dimorphism in CNS organization.

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THE EFFECT OF FASTING ON ENDOTOXIN-INDUCED HEAT PRODUCTION. JAVADY SHOJOONY, M., HAYASHI, M., NAGASAKA, T. Dept. of Physiol., School of Med., Kanazawa Univ., Kanazawa 920

The calorigenic response of conscious adult fed control (C) and 60-hrs fasted (F) guinea pigs to E. Coli endotoxin injection (5 $\mu g \cdot kg^{-1}$) was tested at T_a=25°C. The temperatures of carotid artery (T_{ao}), interscapular brown adipose tissue (T_{BAT}) and ear skin (T_s); the shivering activity (EMG), and the rate of heat production (M), dry ($R + C + K$) and evaporative (E) heat loss were continuously monitored. During the febrile response, in contrast to C group, F group showed a significantly low thermogenic activity. In C group a typical biphasic elevation of T_{ao}, T_{BAT} and M were observed, which were significantly more than those in F group. In C group the thermogenic activity of BAT ($T_{BAT} - T_{ao}$) increased and was significantly higher than that in F group. There was no significant difference between shivering activity of two groups. The suppression of endotoxin-induced thermogenesis in fasted guinea pigs may be prompted by the decreased of heat production response. The decreased heat production response may be caused by suppression of thermogenic activity of BAT in febrile fasted guniea pigs.

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EFFECTS OF PYROGEN AND ANTIPYRETIC ON THERMOREGULATORY EFFECTOR FUNCTIONS IN RABBITS. KOSAKA, M., OHWATARI, N., IWAMOTO, J., FUJIWARA, M., TSUCHIYA, K., FAN, Y.* AND SOELIADI, Hw.* Dept. of Environment. Physiol. and Epidemiol., Inst. for Tropical Medicine, Nagasaki Univ., Sakamoto-machi, Nagasaki, 852.

It is particularly important to determine the possible effect of hypothalamic blood flow changes on fluctuations in hypothalamic temperature. The blood flow was increased or decreased with similar shifts of biphasic character in hypothalamic temperature during LPS-induced fever in rabbits. In the present investigation, effect of sulpyrine antipyretic on thermoregulatory effector functions was studied on conscious febrile rabbits. In LPS-pyrogen induced febrile rabbits, receiving sulpyrine by intravenous injection, there occurred a rapid fall in hypothalamic temperature differed significantly from the fall in retal temperature. And this dissociation of temperatures between the PO/AH and rectum was succeeded not only by various evaporative heat loss such as thermal panting and peripheral cutaneous vasodilation, but metabolic heat production. None of the intravenous doses of sulpyrine caused any change in rectal temperature as well as in oxygen consumption in control rabbits. This opposing, crossed thermoregulatory responses might be induced by the additive interaction between hypothalamic and extrahypothalamic temperature signals both being further modified in afferent cold-warm pathway, by local hypothalamic temperature as well as by chemical action between pyretic and antipyretic in CNS.

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AN ANALYSIS OF THE PATTERN OF FEVER INDUCED BY LEUKOCYTIC PYROGEN. FUJIWARA, M., IWAMOTO, J., OHWATARI, N., FAN, Y*., SOELIADI, Hw*., KOSAKA, M. Dept. Epidemiol. Environmental Physiol., Inst. for Tropical Med., Nagasaki Univ. Nagasaki 852.

Leukocytic pyrogen (LP) was obtained from granurocyte-free leukocytes (lymphocytes and monocytes) which had been separated on Ficoll-conray gradients from fresh blood of rabbits, activated by LPS in RPMI 1640 solution for two hours at 37°C, with or without salicylates. These crude pyrogens were applied to young male rabbits for measuring rectal temperature (Tr), ear temperature (Te), respiratory rate, oxygen consumption, which were compared with LPS-administered cases. Fever induced by LP was composed of two phases like LPS-fever, however the latency of LPS-fever was usually more than 30 min, and that of LP-fever was within 10 min. An initial fall of Te in LPS-fever was gradual and after one hour Te was fixed at low stable temperature level for several hours. But the initial fall of Te in LP-fever was rapid and low Te level, which induced short phase of slight fever on Tr, was persisted not more than 30 min. LP produced by mixed cells incubated with salicylate induced same pattern of fever, however the duration of first low Te level was shortened. From these findings, it was assumed that the initial fall of Te elicited from extrinsic PGE originally produced in incubating tubes and the later phase was the real fever induced by LP.

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CUTANEOUS AND RENAL VASCULAR RESPONSES ELICITED BY SPINAL CORD WARMING IN PCPA- AND 5,7-DHT-TREATED RABBITS. NAGAI, M. AND IRIKI, M. Dept. Physiol., Med. Univ. Yamanashi, Tamaho, Nakakoma, Yamanashi 409-38

The effect of spinal cord warming on ear skin temperature and renal blood flow were investigated in the rabbit pretreated with PCPA and 5,7-DHT.

PCPA(150 mg/kg and 300 mg/kg) was intraperitoneally injected 72 and 48 hr prior to the experiments. PCPA content in the CNS was decreased to 20% of the control value after this treatment. NA and DA contents were not significantly changed with PCPA. In PCPA-treated animals, ear skin vasodilatation and renal vasoconstriction in response to spinal cord warming were both enhanced. The amplitude of pressor responses to the unilateral carotid occlusion were not different between control and PCPA-treated animals.

5,7-DHT(300 mg/kg) was injected intracisternally. Ear skin and renal vascular responses to spinal cord warming were inhibited 1 day and 7 days after the injection. The amplitude of the pressor response to the unilateral carotid occlusion was reduced in the animal 1 day after the injection, and recovered 7 days after the injection.

These data show that the 5-HT system is involved in the vascular responses elicited by temperature signals of the spinal cord origin.

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BAROREFLEXIVE SUPPRESSION OF HEAT PRODUCTION AND FALL IN BODY TEMPERATURE FOLLOWING PERIPHERAL ADMINISTRATION OF VASOPRESSIN IN RATS. SHIDO, O., KIFUNE, A.* AND NAGASAKA, T. Dept. of Physiol., School of Med., Kanazawa Univ., Kanazawa 920

Intravenous injection of vasopressin (lysin-8-vasopressin: LVP) in unanesthetized rats at 18°C caused a decrease in heat production (M) and a fall in colonic temperature (T_{col}) with an elevation of aortic blood pressure (BP) and bradycardia. These changes were related to the dosage of LVP administered. Temperature of interscapular brown adipose tissue (BAT) fell after LVP injection. Therefore, the decrease in M by peripheral LVP is largely due to suppression of BAT thermogenesis. After sinoaortic deafferentation, the changes were greatly reduced except for the elevation in BP. LVP of 0.25 μg, more than a sufficient dose to cause a significant elevation of BP when injected peripherally, had no effect on M and T_{col}. From these results, we concluded that the hypothermic effect of LVP injected peripherally is largely attributed to the baroreflexive suppression of nonshivering thermogenesis, and is not due to the action on the temperature regulatory centers.

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EFFECTS OF β-ENDORPHIN ON METABOLIC THERMOREGULATION. KUBO, K., TSUZUKI, S. and SASAKI, T. Dept. of Physiol., Kumamoto Univ., Kumamoto 862.

The effects of β-endorphin (EP) on rectal temperature (Tre) and oxygen consumption ($\dot{V}O_2$) under acute exposure to various ambient temperatures (Ta) were studied in the warm-acclimated (Ta 23°C) rats. $\dot{V}O_2$ was measured with an open-circuit method. The rats first placed in a warm environment (Ta 22.5-24.5°C) were exposed to Ta 22.5, 16.5, 10.5 or 0.5°C. After 30 min EP or saline was injected into the third ventricle. In the control (saline) rats, a negative correlation between Ta and $\dot{V}O_2$ was observed. The lower Ta caused the higher $\dot{V}O_2$, and Tre was maintained at any Ta. EP induced a rise in Tre with an increase of $\dot{V}O_2$ at Ta above 16.5°C, but a fall in Tre with a decrease of $\dot{V}O_2$ below 10.5°C. "Critical Ta" differentiating the rising and lowering responses was estimated between 16.5°C and 10.5°C. Mean $\dot{V}O_2$ during 30 min period of either hyperthermic or hypothermic response in each Ta was equivalent. The data suggest that EP may act to suppress both mechanisms of inhibitory control of heat production above the critical Ta and an additional heat production below it, and to set the metabolism of the organism at a neutral level of heat production independently to Ta.

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CHANGE IN THRESHOLD CORE TEMPERATURE FOR SHIVERING AFTER LPS INJECTION IN INDOMETHACIN TREATED RABBITS HASHIMOTO,M., BANDO,T. and IRIKI,M. Dept. Physiol., Yamanashi Med. Col., Tamaho, Nakakoma, Yamanashi 409-38.

The threshold core temperature for shivering (ST) was not changed in a plateau phase of a febrile state induced by LPS injection, while ST increased by injection of prostaglandin (PG) E2. To test further the hypothesis that the effect of LPS on ST was not mediated by PGE2, similar experiments were done in rabbits pretreated by an inhibitor of biosynthesis of PGs, indomethacin (IND).

Unanesthetized rabbits under the ambient temperature of 30°C were used. IND was injected intraperitoneally at 30 min before intracerebroventricular injection of LPS. ST was determined by using internal cooling before and after LPS injection. Core- and ear skin temperature, activities of back and thigh muscles and respiratory rate were recorded continuously. The level of PGE2 in the cerebrospinal fluid (CSF) was measured before and after the injection of IND and LPS by radioimmunoassay.

Results were as follows: 1) Increase in core temperature after LPS injection was inhibited by IND treatment. 2) No prominent increase in ST was not found by LPS injection after IND treatment. 3) Increase in PGE2 level in CSF caused by LPS injection was inhibited by IND treatment.

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CHANGES IN RECTAL TEMPERATURE AFTER THE IMPLANTATION OF ANDROGEN INTO THE BRAIN. MOHRI, M. Dept. of Physiol. Sch. of Med. Kanazawa Univ. Kanazawa, Ishikawa 920

Wistar male and female rats were implanted or administered subcutaneously, testosterone propionate(TP) or dehydroepiandrosterone(DHA) and the rectal temperature(Trec), heart rate(HR) and heat production(M) were measured. The implantation was done according to the Pellegrino's brain atlas, and small amount of TP or DHA was implanted to medial preoptic hypothalamus 3 weeks after the operation. Subcutaneously TP was administered 1 mg/100 g body weight, and DHA 6 mg/100 g body weight. Trec and M of TP implanted rats raised just after the implantation and 0.5 - 3.5 hours reached 38.6 - 39.2 °C (Trec 1.2 - 1.8 °C). Trec of DHA implanted rats showed no significant change and M of DHA implanted rats lowered slightly. Trec, HR or M of TP or DHA administered rats lowered slightly but showed no significant change. From these results it can be concluded that there is a difference between the action of TP, testicular androgen, and that of DHA, adrenal androgen, upon the thermoregulatory mechanism is still to be elucidated.

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RESPONSES OF THE CUTANEOUS VASOCONSTRICTION AND RISE IN COLONIC TEMPERATURE ELICITED BY MICROSTIMULATION OF THE MEDULLA OBLONGATA IN RATS. OHNUKI, Y.*, NAKAYAMA, T. AND ISHIKAWA, Y. Dept. of Physiol., Osaka Univ. Med. Sch., Kita-Ku, Osaka 530

Local warming of the preoptic and anterior hypothalamus (POAH) induces tail vasodilation in rats. It is assumed that parts of efferent signals arising from the POAH thermo-sensitive neurons in response to local warming bring about the vasodilatory response via the medullary vasomotor areas. Medullary structures responsible to cutaneous vasodilation were medial and dorsolateral parts. In this study, the sites at which microstimulation (MS) induced cutaneous vasoconstrictory responses were explored in the medulla oblongata of urethanized rats. MS (1ms, 20Hz, 100μA, 10min) was given at 659 sites with unipolar microelectrode, the tip diameter of which was 5μm. Tail, forepaw and hindpaw skin temperatures were recorded at a room temperature of 28.5°C. MS of the medulla oblongata induced vasoconstrictory response of the tail, hindpaw and forepaw. Most frequently observed was vasoconstriction in the tail, and least in the forepaw. Medullary sites, the MS of which produced cutaneous vasoconstriction, are mainly concentrated in the medial parts including the N. reticularis gigantocellularis and the nearby areas. The cutaneous vasoconstriction was accompanied by a rise in colonic temperature, blood pressure, heart rate and respiratory rate.

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EFFECT OF ARGININE VASOPRESSIN ON RAT PREOPTIC AND SEPTAL THERMOSENSITIVE NEURONS.
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Single unit activities were recorded with multibarrelled microelectrodes from medial preoptic area (MPO) and lateral septum of urethane anaesthetized male rats. Thermosensitivity of neurons recorded was examined by changing the local hypothalamic temperature through an implanted water-perfusing thermode. Subsequently, arginine vasopressin (AVP) and its antagonist, Deamino-Dicarba-AVP (D-AVP), were applied iontophoretically to the recording cells.

A total 102 MPO neurons were studied for their responses to thermal and pharmacological stimulation. AVP induced excitation in 13 (36.1%) of 36 warm-units and inhibition in 15 (41.7%) of 36 warm-units. AVP-induced excitation was closely related to the properties of thermal response pattern of warm-units. Excitatory responses were most frequently found in the warm-units exhibiting thermosensitivity only in the hypothermic range (34-38 °C), while the inhibitory responses were observed in another types of warm-units. AVP-induced inhibition were reversibly blocked by simultaneous application of D-AVP. Of 10 cold-units, 8 units were inhibited and 2 were not affected by AVP. In 43 of 56 thermally insensitive neurons, AVP had no effect. In a total of 30 lateral septal units, almost all (8 of 9) thermosensitive units were inhibited by AVP.

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ACTIVITY OF PREOPTIC NEURONS DURING THERMOREGULATORY AND FEEDING OPERANT BEHAVIOR IN THE MONKEY.
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Unit activities of 71 preoptic area (POA) neurons were studied in a monkey during high fixed ratio (=30) bar pressing (BP) thermoregulatory cooling task (CT) or feeding task (FT). The monkey performed CT under guidance of a visual cue during exposure to high ambient temperature (Ta, 35-42 C) and was rewarded with a fall in Ta. FT was guided by a different visual cue. Nineteen units showed a sustained increase or decrease in activities throughout the BP and cool reward periods in CT. Six of these units showed an overshoot or undershoot response at a certain level in Ta falling phase, which was found to be lower when the core temperature was higher. Four units in another group responded similarly only in the reward period. Some of these 10 units also responded to presentation of food in FT. The firing rate of 6 units were maintained high long before the initiation of BP, decreased drastically at the later stage of BP and remained low for a long time. A majority of 10 units which responded to Ta changes were more or less influenced by the performance of CT, and some also even by FT action. Responses of some units to sensory stimuli (cue light and tone, Ta changes and sight of food) tended to differ depending on the level of animal's core temperature or hunger-satiation state.

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CONVERGENCE OF PERIPHERAL AND CENTRAL THERMAL SIGNALS ON THE SULCAL PREFRONTAL CORTEX IN THE RAT.
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We have already reported the evidence of the sulcal prefrontal cortical (S-PFC) involvement in thermoregulation. S-PFC neurons were examined for their responsiveness to thermal stimulation of scrotal skin (Tsc) and hypothalamus (Thyp) in urethane-anesthetized rats. Of the 145 neurons tested, 86% (N=124) of the units responded to Tsc changes and 71% (N=103) to Thyp changes. Tsc-responsive units were classified into 4 types. Simple-warm units (n=44) increased and decreased their firing rates (FR) with a rising and falling Tsc in a 32-42°C range. Simple-cold units (n=36) showed an opposite direction in their FR responses to Tsc changes. Complex-cold units (n=20) decreased their FR when Tsc rose over a 32-34°C range, but FR suddenly increased when Tsc fell by 0.5-1.5 °C from any given peak once FR had been suppressed. Complex-warm units (n=24) exhibited a mirror image of responses to Tsc changes. Majority of Tsc simple-warm units increased their FR with a rise in Thyp (warm type), and that of Tsc simple-cold and Tsc complex-cold units decreased their FR with a rise in Thyp (cold type). Responses of Tsc complex-warm units were equally divided into the Thyp warm and cold type. The present results give further support to the assumption that the S-PFC exerts its influence on the activity of hypothalamic thermosensitive neurons and thermoregulatory responses.

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RESPONSES OF THE HYPOTHALAMIC TEMPERATURE-RESPONSIVE NEURONS IN RATS TO INTRAVASCULARLY INJECTED ENDOGENOUS PYROGEN OR PROSTAGLANDIN E_2.

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In rats, which have been regarded for a long time as not to produce a fever, the presence of physiological mechanism to produce a fever was recently revealed in our laboratory. Thus it was examined whether the hypothalamic temperature-responsive neuron (TR neuron) responded to the endogenous pyrogen (EP) in the same manner as those in dog, rabbit and cat.
1) With systemic administration of EP two third of the TR neurons responded well with the same direction as those reported in other animals.
2) The intravascular injection of PGE_2, which has been supposed to be a final mediator for fever development, caused significantly differrent behavior in the warm cells from those with EP injection.
3) The firing rate of the TR neurons showed the characteristic alteration prior to or following to the onset of fever development with a transient change by PGE_2 or a sustained change by EP.
4) These results mean that PGE_2 is not only a final mediator, if any, but other factors may participate for fever development in rats.

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THRESHOLD TEMPERATURES OF THALAMIC AND HYPOTHALAMIC NEURONS RESPONDING TO SCROTAL TEMPERATURE CHANGE.

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Neurons in the rat's thalamus and hypothalamus abruptly change their firing rates from minimum to maximum, or vise versa, with increase in scrotal temperature of 1°C or less (switching response). When two of these neurons are simultaneously recorded and the scrotum is ramp-wise warmed, the neurons show switching responses at about the same threshold scrotal temperature. In this study, to test whether the responses take place at the same threshold temperature also in static conditions, the threshold temperature was compared in 21 pairs of neurons simulataneously recorded from the right and left thalamus and 10 pairs from the right thalamus and left hypothalamus by changing scrotal temperature in step-wise fashion. For all pairs investigated, the two neurons simultaneously changed their activities in response to a step increase in temperature near their threshold. In no case did we secceed in setting scrotal temperature in such a way that one neuron of a pair remained in subthreshold state while the other had attained the suprathreshold state. Scrotal warming produced desynchronization of cortical EEG. The change in EEG occurred at about the same scrotal temperature at which the switching responses of the diencephalic neurons were elicited.

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EFFECTS OF ENDOGENOUS PYROGEN AND PROSTAGLANDIN E_2 ON THERMOSENSITIVE NEURONS IN SLICE PREPARATION OF RAT HYPOTHALAMUS.

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Using slice preparations, we investigated the effect of endogenous pyrogen on the activities of thermosensitive neurons. Bath-applied crude EP facilitated the activities of warm-sensitive neurons although heat-inactivated crude EP also facilitated those. To remove many substances other than EP, outside the range of molecular weight 10000-50000, crude EP was partially purified with the ultrafiltration. Such partially purified EP had no effect on warm-sensitive neurons when applied in close proximity to the neurons through the micropipet attached to the recording electrode. Bath-applied partially purified EP had also no effect on the most of the neurons, but EP facilitated 6 of 14 warm-sensitive neurons. And bath-applied prostaglandin E_2 (PGE_2) had the same effects on the activities of warm-sensitive neurons as those of bath-applied partially purified EP. Therefore, these results means that partially purified EP acts on the slices and then, releases newly synthesized PGE_2 which in turn changes the activities of thermosensitive neurons.

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HYPOTHALAMIC AND TAIL SKIN TEMPERATURES DURING INTRACRANIAL SELF-STIMULATION IN RATS.
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During intracranial self-stimulation, rats can get reward by pressing a lever against a weight load of 100g, so that the animals have to dissipate extra heat which is produced by the work for lever press. In this study autonomic and behabioral thermoregulatory responses were observed under such conflicting conditions between the reward and thermoregulation. A stimulus electrode was implanted into the medial forebrain bundle. For each lever press (work:0.02J), the rats received a 0.5 sec train of 60Hz sinusoidal current. Rats were placed in a chamber with ambient temperature (T_a) of 22°C or 36°C. At a T_a of 22°C, with the onset of lever press hypothalamic temperature (T_{hy}) began to rise at a constant rate. When T_{hy} reached a certain level, tail skin temperature (T_t) suddenly increased (vasodilation) and then T_{hy} remained constant. The greater the intensity of stimulating current, the higher the threshold T_{hy} for tail vasodilation. The rate of T_{hy} increase was greater at T_a of 36°C than at 22°C. When T_{hy} reached a certain level, grooming or body extension appeared and lever press was interupted. The threshold T_{hy} for body extension increased with increase in the intensity of stimulating current.

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INVOLVEMENT OF THE VENTROMEDIAL AND ANTERIOR HYPOTHALAMUS IN THE CONTROL OF BROWN ADIPOSE TISSUE THERMOGENESIS.
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The effects of local anesthesia of the ventromedial hypothalamus (VMH), anterior hypothalamus (AH) or lateral hypothalamus (LH) were observed on the brown adipose tissue (BAT) thermogenesis. Rats were chronically implanted with thermodes, guide cannulae in the brain and polyethylene tubes beneath the interscapular BAT. BAT temperature (Tbat) decreased when lidocaine was injected bilaterally into VMH during PO cooling. The mean maximum decrease of Tbat (n=5) was 0.5°C which occurred 6 minutes after lidocaine injection. Tbat increased when lidocaine was injected into AH at the ambient temperature (Ta) of about 28°C. The mean maximum increase of Tbat (n=5) was 1.0°C which occurred 7 minutes after lidocaine injection. This increase was attenuated by pretreatment of propranolol. Lidocaine into LH had no effect on Tbat when Ta was about 28°C. Tbat was not influenced by similar injection of saline into VMH, AH or LH. These results suggest that VMH facilitates BAT thermogenesis and AH inhibites it.

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BEHAVIORAL AND AUTONOMIC THERMOREGULATION DURING FEVER AT HEAT LOAD IN GUINEA PIG.
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Guinea pigs were trained to receive 5 °C air for 5 sec following a bar-pressing at ambient temperature load (T_{load}) of 35 and 38 °C. Animals were given endotoxin 10-15 μg/kg i.m.. The ear (T_{ear}), the rectal (T_{rec}), and the selected ambient ($T_{a.sel}$) temperatures were recorded. Fever curves were divided into following phases; rising, plateau and falling. ($T_{ear} - T_{a.sel}$), the difference between T_{ear} and $T_{a.sel}$, was used to represent the magnitude of heat loss (J. Physiol. Soc. Japan, 45, p593, 1983). During the afebrile stage animals regulated their T_{rec} with both behavioral and autonomic heat loss responses and ($T_{ear} - T_{a.sel}$) was the same under both T_{load}s. During the febrile stage the values of ($T_{ear} - T_{a.sel}$) were reduced in the rising and the plateau phase, were nearly the same in the falling phase as in the afebrile stage at both T_{load}s. The rises of T_{rec} were the same at both T_{load}s. No difference was found between those values in corresponding phases at both T_{load}s. The results further showed that during the rising phase the decrease in heat loss was mainly attributable to autonomic means under T_{load} of 35 °C and to behavioral means under T_{load} of 38 °C. It is suggested that there is the central integration of behavioral and autonomic thermoregulatory system in the preoptic and anterior hypothalamus.

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EFFECTS OF PREOPTIC THERMAL STIMULATION ON ELECTRICAL ACTIVITY OF NEUROSECRETORY CELLS IN SUPRAOPTIC NUCLEUS OF THE HYPOTHALAMUS. MATSUMURA, K. AND NAKAYAMA, T. Dept. of Physiol., Osaka Univ. Med. Sch., Kita-ku, Osaka 530

In anesthetized rats, the effect of preoptic (PO) thermal stimulation was studied on the supraoptic (SO) neurosecretory cells identified antidromically. More than 70 % of the SO neurons responded to the PO thermal stimulation. Thirty-six percents of these thermally responsive neurons increased and decreased the firing rates statically in response to gradual PO warming and cooling, respectively. The remaining 64 % of the neurons altered the firing rate dynamically in response to gradual PO warming and cooling. Most of them showed higher firing rate in the falling phase of PO temperature than in the rising phase. However, when PO temperature was kept constant at several levels for more than 1.5 min, the dynamically responded neurons showed static firing activities depending on the levels of PO temperature. All of them fired at the minimal rate when PO temperature was kept constant between 34 and 36°C, and at the maximal rate between 38 and 42°C. Sometimes PO thermal stimulation evoked fluctuations in arterial blood pressure. However, in the 36 % of the thermally responsive neurons, changes in the firing rate were not accompanied by any fluctuation in the blood pressure. These results suggest that brain temperature influences the SO neuronal activity.

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〔会　報〕

日本生理学会昭和59年度第1回常任幹事会議事録要旨

日　時：昭和59年3月27日(火)　午後2時～6時
会　場：群馬大学医学部会議室
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，真島英信，入沢　宏，富田忠雄，永坂鉄夫，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，嶺巓教三，平尾武久(当番幹事)，鈴木光雄(当番幹事)，内薗耕二(前幹事)，鈴木泰三(前幹事)，菊地錄二(教育委員長)
欠席者：岩間吉也，及川俊彦
議　長：高木貞敬(当番幹事)

Ⅰ．報　告

1. 庶務報告（伊藤庶務幹事）

◦会員数（昭和59年1月～3月）．入会73名，退会70名，会員総数3,225名)，(一般会員2,981，評議員969，特別会員28，名誉会員1，準会員243）である．この間，死亡された円谷　豊，松本政雄，三宅彰英各評議員に対する追悼の辞が述べられた．

◦科学研究助成．昭和58年度内藤記念科学振興賞に高木貞敬氏，昭和59年度山田科学振興財団研究援助に津本忠治，村上元彦，熊沢孝朗の三氏を推薦した．

2. 会計報告（星会計幹事）

◦日本生理学会昭和58年度決算（昭和58年1月1日より昭和58年12月31日まで）．について了承された．(表2)この決算については真島，島津両会計幹事の監査を受け，承認を得た．

◦国際交流基金およびビデオテープ製作販売会計の特別会計決算について了承された．（表3）

3. 日本生理学雑誌編集報告（酒井編集幹事）

◦昭和59年度46巻1～5号についての発刊と編集状況(表4)．なお46巻特別企画として各地区の生理学談話会変遷史執筆が予定されている．余外集，試道集の復刻版が予定されている．

◦昭和59年度編集委員の交代（4月より）について．中浜　博委員は西山明徳氏と交代になる．

4. J.J.P.編集会計報告（中山委員長）

◦昭和58年度決算（58年4月より59年3月まで．）と59年度予算(見込)について了承された（表5)．

◦minireview．当分の間編集委員会による invited paper である．

◦short communication．速報の意味を持たせ毎号5編までは優先する．

◦J.J.P.の紙質の向上．送料が40万円ほど増すので現状のままとする．

◦大会英文抄録．日生誌からJ.J.P.に移す案もあるが現状のままとする．

◦J.J.P.刊行状況．Vol.33，No.6 は刊行部数1,600部で，内訳は表6のとおりである．

5. 教育委員会報告（菊地委員長）

委員会が2回開催された．

6. 選挙管理委員会報告（真島委員長）

委員会は1月と2月に開催され，常任幹事選挙の開票，集計を行った．また，各地区の新常任幹事は表7のとおりである．

7. 評議員選考委員会報告（高木委員長）

昭和59年度の日本生理学会評議員候補者は42名(表8)であり，全員が推薦された．

8. 生理学用語委員会報告（酒井委員長）

生理学用語集は再校中で，英・日索引を作製し，本年度中に出版可能となった．

9. 国際生理科学連合報告（昭和58年9月に就任の伊藤ＩＵＰＳ理事）

◦ＩＵＰＳ大会誘致で1992年には，イギリスと日本が争うことになりそうであるが，ヴァンクーバー大会(1986年）までその後の情報を収集して対処する．

◦分担金．1人当り3ドル，計7,500ドル分担するよう求められている．

◦神経科学国際会議．1987年に開かれる予定であるが，場所は未定である．

10. 生理科学研究連絡委員会報告（本間委員長）

1992年ＩＵＰＳ大会にイギリスが立候補する予定か，

また日本が立候補するかどうかについて情報をよく収集し考慮することが必要である．ＩＵＰＳ大会を４年毎にする案も考慮中である．

11. 第62回（昭和60年）日本生理学会大会の準備状況について（纐纈幹事）

・会期は昭和60年３月28日（木）～30日（土），場所は久留米市，演題の締切りは59年11月10日．１演者１題のみとして，若手研究者を中心とするシンポジウムを行う予定である．

12. 第61回（昭和59年）日本生理学会大会に関する報告（当番幹事）

・演題は当初735題申し込みがあったが，７題の辞退があり，728題が10会場にてすべて口頭で発表されることになった．参加者は約1,400名が見込まれている．外人特別講演(Dr. Hagbarth, Dr. Llinás)，市民講演会，記念撮影も行われる．

13. その他

名誉会員 Eccles 氏の特別講演が４月に東京および岡崎で行われる．

・生理研の報告（内薗前幹事）．生理研には８研究部門，５客員部門があり，設備が整った．

関連事項として「日本生理学会と国立生理学研究所との将来に亘る関係の緊密化について」高木幹事より意見が述べられた．

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59年度予算案について

了承された（表９）．

3. 当番幹事・委員長改選の投票結果

伊藤正男庶務幹事，星　猛会計幹事，酒井敏夫編集幹事，真島英信・島津　浩会計監事，島津　浩会則委員会委員長，中馬一郎教育委員会委員長，本間三郎評議員選考委員会委員長，新島　旭研究費委員会委員長，酒井敏夫用語委員会委員長，真島英信選挙管理委員会委員長，酒井敏夫日本生理学教室史編集委員会委員長が選出された．J. J. P. 編集委員長は編集委員の互選により，現中山昭雄委員長の任期は58年４月１日～60年３月31日である．

なお，当番幹事は今回は選挙権を持たないものとし，この件に関しては会則委員会に検討を依頼する．J. J. P. 編集長の任期は２年間では短かすぎるのではないかとの意見が出され，この件も会則委員会にて検討をする．

4. 評議員推薦について

42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．

5. 名誉会員推薦について

ニューヨーク州立大学特別教授 C. McC. Brooks 博士と NIH の田崎一二博士が推薦され，両氏とも名誉会員候補として承認された．

6. 日本医学会評議員および連絡委員の選出について

日本医学会評議員として真島英信氏，日本医学会連絡委員として酒井敏夫氏が選出された．

7. 地方談話会のあり方について

中山昭雄幹事より地方談話会のあり方を再検討してはどうかとの具体的提案が５項目出されたが，各地区で検討することになった．なお，日生誌では過去の談話会についての変遷史を地区別に執筆掲載する．

8. 第63回（昭和61年度）日本生理学会大会について

当番校を山形大学（望月政司氏ら）に決定した．

・第64回(昭和62年度)は医学会総会が東京で開かれるが，千葉大・本間三郎氏が生理学会大会の当番幹事をつとめ，医学会分科会委員長を兼任することになった．

第61回日本生理学会評議員会・総会議事要旨

日　時：昭和59年３月29日(木)　午後５時～６時40分
会　場：前橋市群馬県民会館大ホール
出席者：約200名
議　長：高木貞敬，三浦光彦，平尾武久，鈴木光雄

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，了承された．

Ⅰ．評議員会・総会報告

以下のことが報告された．

1. 庶務報告（伊藤庶務幹事）

・会員数．昭和58年12月現在，一般会員3,018名，特

別会員28名，名誉会員１名（表１）.

◦物故会員．この１年間に，東　竜太郎，小林庄一，松本政雄，円谷　豊，三宅彰英，ハリー　グルンドフェスト６氏，深く哀悼の意を表します．

◦科学研究助成．昭和58年度内藤記念賞に高木貞敬氏．昭和59年度山田科学振興財団への推薦申し込みは３件であり，３名を推薦した．

◦常任幹事・役員改選．昭和59年３月27日，新旧両幹事の出席のもとで常任幹事会が開催され，議事は新幹事のみで進められ，役員の改選（表７，常任議事録Ⅱ議題３）を行った．

2. 会計報告（星会計幹事）

昭和58年度決算報告（表２）の後，承認された．会計監査（２月６日）にて適正と判断を受けた．

3. 日本生理学雑誌編集報告（酒井編集幹事）

45巻は12月に完了し，46巻３号を刊行．４号は編集完了．同月同号の出版が可能となった．

各誌はうすいが，総説は力作なので内容は充実しつつある．特に第１号は生理学会60周年を記念して特別企画とした．

これからの編集方針：１巻に一つ位特別企画を行う予定であり，46巻では12号に，各地区生理談話会の変遷史を企画する予定である．

生理学の広場：寄稿をお願いしたい．

８，９号：大会英文抄録集．

論文表題集：別冊号として出版を予定している．

その他：余外集・試道集の復刻版を100部刊行を予定している．

4. J.J.P. 編集報告（中山委員長）

◦会計報告．（表５）.

◦編集委員会報告．頁数：58年度は1,140頁，59年度は1,200頁程度の予定である．

論文受理数：105件.

short communication：毎号５編までは優先する．

minireview：34巻から掲載開始，内容は刷上がり10頁位．現在は編集委員会で著者を選定している．

大会の英文抄録：J.J.P. への移行は困難なため見送る．

J.J.P. の改名：結論出ず，引き続き検討する．

委員会構成：内分泌生理担当１名，分子細胞生理担当１名を増員して10名.

委員長任期：現在２年となっているが，もう少し長くしたらどうかとの意見があるので委員会で検討中である．

5. 教育委員会報告（菊地委員長）

15名で構成され，年２回委員会を開催した．今年で３年目となる．

◦初年度．教育講演のビデオを作成し，純益74万円を得た．その中20万円を生理学会に繰り入れた．

◦次年度．教育に関するアンケート調査を２回行った．１回目の結果を45巻７号に発表した．２回目の結果は目下整理中，46巻７号に結果を発表する予定であり，残金も生理学会に繰り込む予定である．

◦委員長の交代．新委員長には中馬一郎氏.

6. 選挙管理委員会報告（真島委員長）

◦常任幹事選挙．評議員963名に投票用紙を発送．

投票総数600で投票率は62.3％であった．

新常任幹事候補者名（表７）.

7. 評議員選考委員会報告（高木委員長）

本間，真島両委員と審議した結果，申し込み者全員（表８）を推薦した．

8. 日本生理学教室史編集委員会報告（酒井委員長）

500部印刷したが，463部売れた．なお上巻の完成に25年かかり，その間，浦本，内山，名取，各氏の大きな努力によった．下巻は新しい方にお願いしたい．

9. 生理学用語委員会報告（酒井委員長）

再校が終ったので夏までにメドを付けたい．

10. 国際生理科学連合会報告（伊藤 IUPS 理事）

昭和58年９月，シドニーで行われＩＵＰＳの general assembly において次の事項が審議された．

◦発展途上国に対する教育事業．日本生理学会が100万円援助し，それに対して謝辞を受けた．

◦次回ＩＵＰＳ大会．1986年にバンクーバーで開催決定.

プログラム委員として久野　宗氏が参画の予定である．

◦1989年ＩＵＰＳ大会．５ヶ国が招請演説を行い，予備投票の結果フィンランドが１位となった．

◦1992年ＩＵＰＳ大会．日本が招請するか，今後大きな問題である．

◦分担金．１人当り２ドルから３ドルへの値上げ案が採択された．

11. 生理科学研究連絡委員会報告（本間委員長）

◦生理研連シンポジウム．本年は生理研内薗氏が世話人となる．

◦ＩＵＰＳの分担金．学術会議に増額を要請する予定である．

◦学術会議の法律制定．これに伴う第13期新会員は来年７月に決まる予定である．

12. 第62回（昭和60年）日本生理学会大会の準備

状況（纐纈幹事）

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．

13. 第61回（昭和59年）日本生理学会大会の報告（当番幹事）

演題総数735題，取り消し8題，大会参加人員約1,400名，グループディナー19.

14. その他

名誉会員 Eccles 教授が3月30日来日し，3週間滞在，その間，7都市にて講演を行う予定である．

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附議し承認された（表9）．

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8）．

3. 名誉会員推薦

C. McC. Brooks，田崎一二，両氏を評議員会が推薦し，総会にて承認された．

4. 日本医学会評議員および連絡委員の選出

日本医学会評議員に真島幹事，日本医学会連絡委員に酒井幹事が承認された．

5. 中部地区常任幹事1名増員

伊藤庶務幹事より会則変更の趣旨説明の後，評議員会の決議を経て，総会にて承認された．（註：この件については4月中に中部地区において増員のための選挙が行われ，江橋節郎評議員が当選した．（表7）

6. 第63回（昭和61年）日本生理学会大会

山形大学で開催予定の旨，承認された．

7. その他

第64回（昭和62年）日本生理学会大会について伊藤庶務幹事より，医学会総会の年にあたるので早く当番校を決めたいとの説明があった．千葉大学で開催予定の旨，承認された．

また，生理学会分科会長に本間氏の併任が承認された．

評議員会総会終了後，前大会当番校を代表して河村洋二郎氏より，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58年12月末現在）

会員	
一般会員	3,018名
特別会員	28名
名誉会員	1名
準会員	
学校図書館	135部(125件)
研究所，書店	118部(73件)
寄贈および交換	
外国	21部
国内	15部
合計	3,336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		

表2 日本生理学会昭和58年度決算報告

（自 昭和58年1月1日 至 昭和58年12月31日）

収入

	58年度予算	58年度決算	増減	備考
① 前年度繰越金	4,958,038円	4,958,038円		
② 昭和58年度収入	23,955,000	32,980,653		
（内訳）				
会費	17,190,000	17,744,000	＋	
購読料	1,800,000	1,819,860	＋	
論文掲載料	1,500,000	1,383,387	－	

広告料	1,100,000	1,024,000	−	11件
会誌分冊売	35,000	54,800	+	
預金利子	130,000	134,641	+	第一勧業銀行
日本医学会奨励金	100,000	200,000	+	2件
印税	350,000	662,465	+	生理学実習書，用語集
論文表題集	1,700,000	1,620,000	−	
雑収入	50,000	200,000	+	教育委員会
生理学教室史上巻	—	8,137,500		(110万未納→繰越し)
①+② 合計	**28,913,038**	**37,938,691**		

支出

(内訳)	58年度予算	58年度決算	増減	備考
会誌印刷代	11,714,000	11,414,652	−	44巻12号～45巻11号
会誌発送代	2,500,000	2,672,132	+	同上
編集会議費	120,000	79,035	−	日本生理学雑誌
校正料	250,000	246,000	−	同上
原稿査読料	45,000	24,000	−	同上
大会援助費	500,000	500,000		当番校
事務室使用料	2,099,040	2,099,040		12ヶ月
事務室光熱費	300,000	287,170	−	同上
通信費	850,000	830,340		
事務費	850,000	684,610		
備品	80,000	84,000	+	カードキャビネット
会合費	350,000	301,883	−	常任幹事会，委員会
旅費	1,300,000	1,429,174	+	同上
人件費	3,000,000	2,923,625	−	アルバイト含む
職員健康保健	125,000	80,586	−	
職員退職金積立	100,000	100,000		
論文表題集	1,700,000	1,379,149	−	
国際交流基金	250,000	250,000		
雑費	100,000	275,620	+	名誉会員記念品他
予備費	2,679,998	200,000	−	教育委員会
生理学教室史上巻		9,154,500		
合計	**28,913,038**	**35,015,516**		
昭和59年度繰越金総額		2,923,175		
合計		**37,938,691**		

表3．昭和58年度特別会計決算報告

Ⅰ．国際交流基金

収入		支出	
① 前年度繰越金	1,062,016円	久野教授シドニー派遣旅費	350,000
② 昭和58年度収入	257,176	振替手数料	800
(内訳)		合計	**350,800**
積立金(一般会計より)	250,000	昭和59年度繰越金総額	**968,392**
利子	7,176		
①+② 合計	**1,319,192**		

Ⅱ．ビデオテープ製作販売会計(教育委員会担当)

	収　　入	
①	前年度繰越金	424,646
②	昭和58年度収入	975,266
	(内　訳)	
	解説講演ビデオテープ代金(40本分)	970,000
	利　　子	5,266
①+②	合　　計	**1,399,912**
	支　　出	
	ビデオ製作費及び郵送料	904,560
	振替手数料	2,400
	学会会計に入金	200,000
	合　　計	**1,106,960**
	昭和59年度繰越金総額	**292,952**

申込状況

1回目(57年7月～) 55本　総額 1,375,000 (完了)

2回目(58年11月～) 20本　総額 470,000

(未入金 250,000)

表4．日本生理学雑誌45巻(58年度)編集報告

45巻1～12号(8・9号合併)発行11回

45巻総頁数 824頁

(内　訳)	編	頁数	44巻総頁数：
原　著	7	73	795頁
短　報	4	14	
総　説	6	97	
研究法	2	26	
解説講演	2	25	
口演演題		262	
学会抄録		96	
会報，その他(目次，索引含)		169	
広　告		62	

尚，業績表題集は別冊として刊行．

表5．JJP昭和58年度決算および59年度予算(見込)

(昭和59年3月5日)

支　出	58年度 予算額	決算額	摘　要	59年度 予算額	摘　要
製作費	12,627,000円	12,437,800円	1148頁	13,139,000円	1200頁
審査・英文校閲料	1,683,000	1,505,900		1,720,000	
旅費・会議費	644,000	748,600		1,076,000	
通信・消耗品費	1,389,000	1,389,000		1,389,000	据置き
人件費	2,060,000	2,060,000		2,060,000	据置き
雑事務費	809,000	809,000		809,000	据置き
送料(外国)	3,489,000	2,541,000		2,920,000	
送料(国内)	2,678,000	2,491,000		2,886,000	
支出合計	**25,379,000**	**23,982,300**		**25,999,000**	
収　入					
国内会員	6,160,000	5,964,100	876名 @7,000→@8,000	7,200,000	876→900名 @8,000
国内機関	1,950,000	1,963,300	124名 @15,000→@20,000	2,600,000	130名 @20,000
外国購読	8,184,000	6,352,500	343名 80$→100$ @200	7,300,000	360名 100$ 50$ @200
別刷バックナンバー	3,414,000	3,620,100		3,729,000	
文部省助成	5,670,000	5,670,000		5,670,000	
繰越金	6,145	6,145		△ 406,155	
収入合計	**25,384,145**	**23,576,145**		**26,092,845**	
差引繰越	5,145	△ 406,155		93,845	

表6．JJP刊行状況 (Vol. 33, No. 6) 刊行部数 1600部

個人購読	876	1020	外国購読	343	357
団体購読	124		外国会員	7	
国内寄贈	10		その他	7	
その他	10				

表 7．各地区常任幹事(29名)

(任期昭和59年～62年改選時)

50音順敬称略

北海道	加藤　正道	広重　力
東北	田崎　京二	西山　明徳
関東	新島　旭	本郷　利憲
	本間　三郎	三浦　光彦
東京	伊藤　正男	酒井　敏夫
	島津　浩	竹内　昭
	塚田　裕三	古河　太郎
	星　猛	真島　英信
中部	入沢　宏	富田　忠雄
	永坂　鉄夫	御手洗玄洋
	江橋　節郎	
	(定員増により4月選出)	
近畿	久野　宗	中馬　一郎
	中山　昭雄	藤本　守
中国四国	岡田　博匡	中山　沃
九州	大村　裕	纐纈　教三
	後藤　昌義	

表 8．59年度新評議員

計42名 (五十音順)

氏名	所属，現職
井上昌次郎	東京医歯大，医材研，教授
井口　敞恵	久留米大，医，講師
伊藤　博澄	琉球大，医，助教授
和泉　博之	東北大，歯，助手
岩元　純	長崎大，熱医研，助手
大久保千代次	国立公衆衛生院，衣住衛生，室長
大森浩一郎	関西医大，講師
大渡　伸	長崎大，熱医研，助手
岡本　治正	東京大，脳研，助手
椛　秀人	高知医大，助手
川越　礼子	順天堂大，医，助手
河　和善	群馬大，医，講師
河野貴美子	日本医大，助手
清末　達人	大分医大，助手
小山　省三	山梨医大，助手
桜永　昌徳	日本医大，講師
笹本　一茂	九州大，歯，講師
佐竹　典子	大阪医大，講師
柴田　裕行	金沢大，医，講師
芝山秀太郎	鹿屋体育大，教授
須藤　正道	東京慈恵医大，宇宙医研，助手
杉崎　哲朗	東邦大，医，助手
高山　清茂	群馬大，医，講師
続　修二	熊本大，体質医研，助手
時政　孝行	久留米大，医，講師
徳田　雅明	香川医大，助手
中江　俊夫	東北大，医，助手
中垣　育子	大阪医大，助手
長井　吉清	東北大，医，助手
西牟田　守	国立栄養研究所，研究員
服部　幸雄	岡山大，医，講師
林　文明	公立長生病院内科
堀江　登	神戸学院大，栄養，助手
松村　裕	大阪医大，講師
村上　政隆	大阪医大，助手
村本健一郎	富山高等工業専門学校，助手
森　博彦	大阪医大，講師
森本　茂	東京慈恵医大，助手
山崎　昌廣	熊本大，体質医研，助手
吉田　明夫	公立長生病院，内科，医長
吉松　博信	九州大，医，助手
吉村　恵	久留米大，医，講師

表 9．日本生理学会昭和59年度予算

(自　昭和59年1月1日
至　昭和59年12月31日)

収　入

①	前年度繰越金	2,923,175円	
②	昭和59年度収入	25,190,000	
	(内訳)		備考
	会費	17,280,000	(会費 6,000円×3,116×0.9 臨時会費含む)
	購読料	1,740,000	(準会費 8,000円×241×0.9)
	論文掲載料	1,500,000	

	広告料	1,100,000	
	会誌分冊売	50,000	
	預金利子	130,000	(第一勧業銀行)
	日本医学会奨励金	100,000	
	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	
	生理学教室史上巻	1,140,000	
①+②	合計	**28,113,175**	

支出

(内訳)			備考
	会誌印刷代	11,500,000	(45巻12号—46巻11号)
	会誌発送代	2,700,000	(同上)
	編集会議費	120,000	(日本生理学雑誌)
	校正料	250,000	(同上)
	原稿査読料	30,000	(同上)
	大会援助費	500,000	
	事務室使用料	2,241,394	(12ヶ月)
	事務室光熱費	300,000	(同上)
	通信費	850,000	(会誌外通信)
	事務費	800,000	(印刷物，事務雑費)
	備品	80,000	
	会合費	350,000	(常任幹事会，委員会)
	旅費	1,500,000	(同上)
	人件費	3,100,000	(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	1,651,781	
	合計	**28,113,175**	

〔お知らせ〕

第31回生理学中部談話会御案内(第2報)

1. 期　日　昭和59年10月18日(木)　13：00より
   19日(金)　17：00まで
2. 会　場　昇仙閣ホテル
3. 口演時間　15分の予定
   口演は1研究機関あたりなるべく1題にお願いします．ポスター発表を第1日(18日)夜に討論時間を充分にとって行う予定ですので御利用下さい．
4. 参加費　2,000円
5. 懇親会　昇仙閣ホテル(夕食を兼ねる)
6. 宿　泊　1泊15,000円
   (税サービス料込，朝食代，夕食代(懇親会兼用)含む．ただし4～5人部屋)

つきましては，次の事項につき貴研究機関関係者の御予定をお知らせ下さい．〆切6月15日

1. 参加予定者数
2. 演題発表予定数
3. 宿泊予定者数

今後の案内予定……上記の返信をいただいた方のみに発送

第3報　演題募集要領　7月上旬

演題〆切　　8月上旬
第4報　予稿集　　9月上旬
昭和59年5月
当番幹事　山梨医科大学
第一生理　入来正躬
第二生理　竹内　亨
問い合せ，連絡先
〒409-38　山梨県中巨摩郡玉穂村下河東1110
山梨医科大学　生理学教室
第31回生理学中部談話会事務局

第68回近畿生理学談話会御案内

第68回近畿生理学談話会を下記の要領で開催致します．多数の御参加をお待ち申し上げます．

1. 日　時：昭和59年10月27日（土）
午前9時30分から（予定）
2. 会　場：日本生命中之島研修所(阪大医学部隣り)
〒530 大阪市北区中之島4-3-43
電話 06-443-3131
3. 演題と予稿集抄録の締切り：8月25日（土）必着
予稿集の原稿は同封の書式にしたがってタイプまたは黒インクで記入して下さい．そのまま印刷に使用致します．口演時間は討論を含め15分間を予定致しております．
4. プログラム及び予稿集：印刷が出来しだい参加お申込みの方宛にお送りする予定です．
5. 会　費：一般会費1,000円，予稿集代1,500円，評議員会昼食代1,000円
6. 評議員会：昼食時（正午～午後1時）
大阪大学医学部2階会議室で開催します．議題は予め御連絡下さい．
7. 参加者および評議員会出席者氏名を教室単位で御記入のうえ，8月25日までに下記宛てお申込み下さい．
なお，会費は内訳を記入のうえ，8月25日までに御送金下さい．
口座番号　大阪-6-26918番
加入者名　近畿生理学談話会
8. 申込み先：〒530 大阪市北区中之島4-3-57
大阪大学医学部高次研生理　津本忠治
電話 06-443-5531（内線 275）

第17回東北生理学談話会御案内

今年の談話会を下記の要領で開催しますので，多くの方々の参加と発表をお待ちいたしております．

記

1. 日　時：昭和59年11月10日(土)，11日(日)
2. 場　所：福島市飯坂温泉「あづま荘」
電話 0245-42-3381
3. 演題および参加申込締切：昭和59年9月10日(月)（必着）
4. 参加費：1,000円（当日）
5. 宿泊費：8,000円（含懇親会費）
宿泊しないで懇親会にのみ出席する場合は5,000円となります．
6. 申込方法：申込用紙に所定の事項を記入し，宿泊予約金（1人3,000円）とともに所属教室(研究室)ごとにとりまとめて現金書留にて下記宛お送り下さい．
〒960 福島市杉妻町5-75
福島医大　第2生理学教室
東北生理談話会　塚原　進
7. 問い合せ，連絡先：〒960 福島市杉妻町5-75
福島医大　第2生理　片平清昭
電話 0245-21-1211（内7132）

――学会事務局より――

59年度会費6,000円未納の方に振替用紙を添付しました．御多忙とは存じますが，御納入下さいますよう御願いいたします．

尚 J. J. P 購読料は窓口が学会事務センター（振替口座 東京9-55247）です．お間違いのないよう御注意下さい．

大会及び地方会で発表予定で非会員の方がおられましたら，事務局にお知らせ下さい．入会申込書，振替用紙をお送りいたします．

「日本生理学教室史上巻」	特別頒価	20,000円
「昭和56年度生理学論文表題集」	定 価	5,000円
「昭和57年度生理学論文表題集」	定 価	5,000円

残部が若干ございますので，はがきでお申込下さい．

新版「生理学用語集」のお薦め

5年の歳月をかけて，内容の改訂，増補を行いました新版「生理学用語集」がこのほど完成いたしました．各研究室におかれましては，この用語集を御採用御使用なさるようにお薦めいたします．

B6判　320頁　定価 3,900円

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〔編集後記〕

日生誌46巻7月号に「校正に思う」という一文を書き，これまで日生誌編集の裏方として校正作業に協力して下さったお二人を紹介し，大部となる大会号の校正には苦労が多かったことであろうと想像した．

第41巻(昭和54年)の大会号からは，英文抄録となり，これをオフ・セット印刷としたので校正の手数が極端に減ったことにも触れた．今思えばこのオフ・セット印刷の決断は，—その効用の国際性は別として—，一つの大きな進歩であった様に思われる．この年度の演題数は461で，合併号だけの頁数は487頁であったが，最近のように出題数の制限が無くなった現在では，例えば第60回大会号(第45巻)では演題数657題，614頁と膨張している．この位の量になると，活字印刷の場合の校正は片手間のアルバイトでは済まされない．この様な理由から，オフ・セット印刷への切換えを行ったことに対して礼賛したのである．

消エネ化の方向に舵を取ったとはいえ，目次，人名索引並びに青ページ等の校正は避けるわけにはいかない．大会号の編集は“当番校の責任”で行われるものであるが，一寸気を抜いたものになると，校正時にその皺寄せが現われる．この時ぐらい，印刷所，校正担当者，並びに編集部の三位一体の妙が発揮される時はない．大会号の作製は，猛暑のさ中に行われていることを御想像下さい．

実をいうと，校正係を失った日生誌編集委員会は，大会号の校正で夏休みを返上しなければならないと覚悟していた．「校正に思う」を書いたお蔭で，編集委員会への同情の声が送られて来たり，生理学者が校正等の労をとるなどもっての外であるとし，校正係専従の方の御紹介を給わったりした．ここに有難くお礼を申し上げます．八方手を尽した結果，前に述べた佐々木，植山両氏のごときベテランの岡田勝巳さんが手を貸して下さるようになった．早速岡田さんは，不馴れな生理学用語に苦労されながら奮闘されている．

(昭和59年8月16日　酒井敏夫)

編集委員

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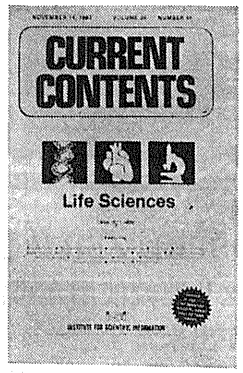
CURRENT CONTENTS

カレントコンテンツは"Winner of the Information Industry Association Hall of Fame Award"を受賞し、研究者の方々に、自分の研究テーマに関する論文が、学術誌に掲載される前に的確に把握する手段として広く利用されております。つまり、本誌は研究者が専門及び関連分野の進展に追随していくことを可能にした包括的サービス誌と言えるでしょう。まさに、カレントコンテンツは効果的・経済的な文献検索、論文選択、情報収集の迅速性といった諸問題に対する一つの解答であります。

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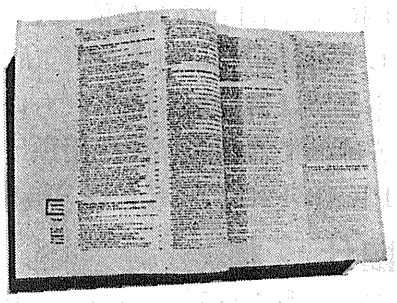
〔対象分野〕

行動科学　生化学　植物　臨床医学　細胞学
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遺伝　老人病　長寿学　血液　栄養　免疫
微生物　分子生物　神経科学　有機化学　生理
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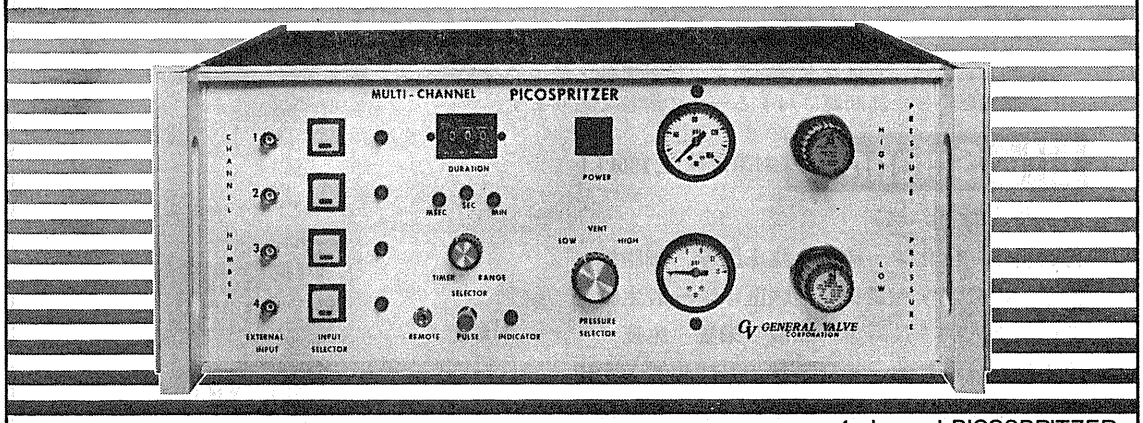
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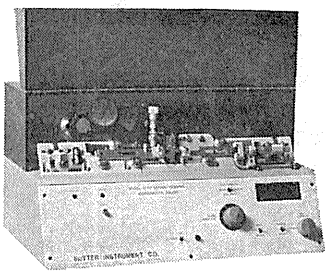
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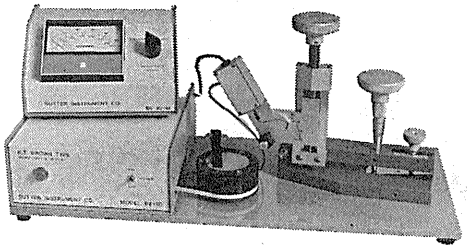
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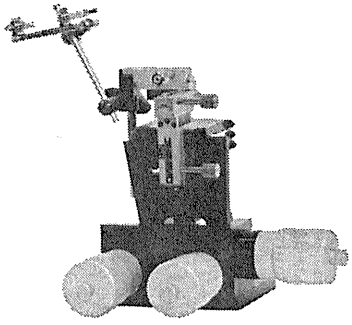


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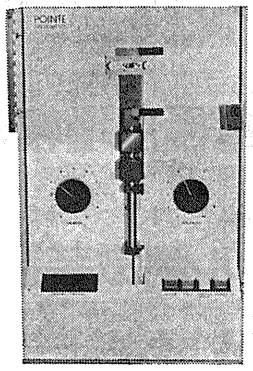
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2点間の電位差をダイレクトに示す

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色素注入も可能な高性能タイプ

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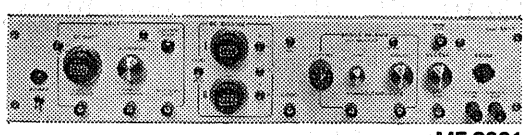
ME-3221 DCシフト 2chDCバッキング 刺激通電機構つき

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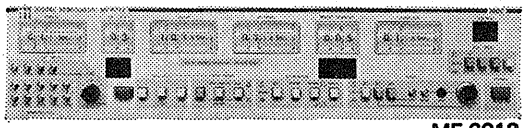
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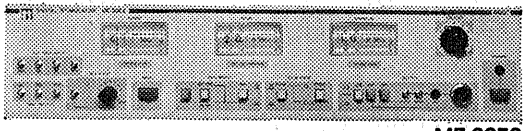
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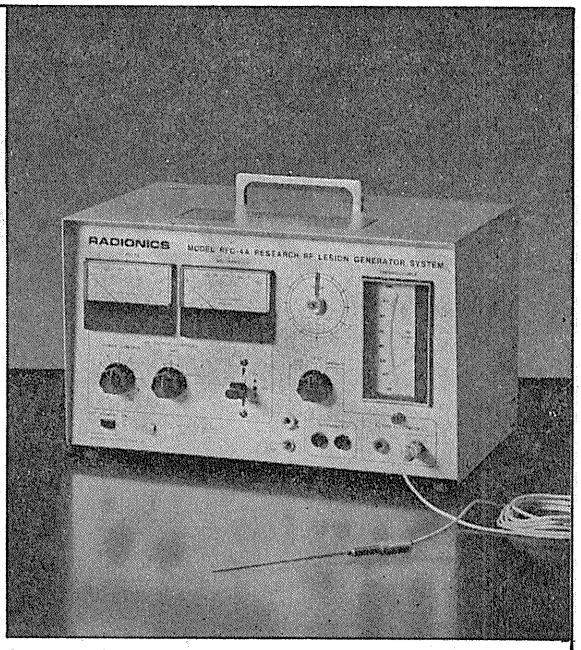
待望の〝0.25mm〟

動物用

リージョン・ジェネレータ
MODEL RFG-4A

直径0.25mmのTC電極により、今迄行ないにくかった極めて微少の損傷作成が可能になりました。

- ■Lesion Generatorによる損傷は、小動物の脳組織の損傷に適しており、また手技が極めて簡単です。
- ■いかなる損傷条件(損傷温度、損傷時間)でも生体組織に出血をひきおこすことはありません。
- ■熱センサーによって損傷組織の温度を正確にコントロールすることができ再現性、均一性に優れた損傷巣を作製することができます。
- ■50°C以上の損傷条件では、損傷温度が高ければ高いほど、また損傷時間が長ければ長いほど大きな損傷巣を作製することができます。
- ■外部の刺激装置と本体を接続することにより、同一電極から電気刺激を与えることもできます。



輸入発売元

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実験動物脳内酵素瞬時不活性化装置

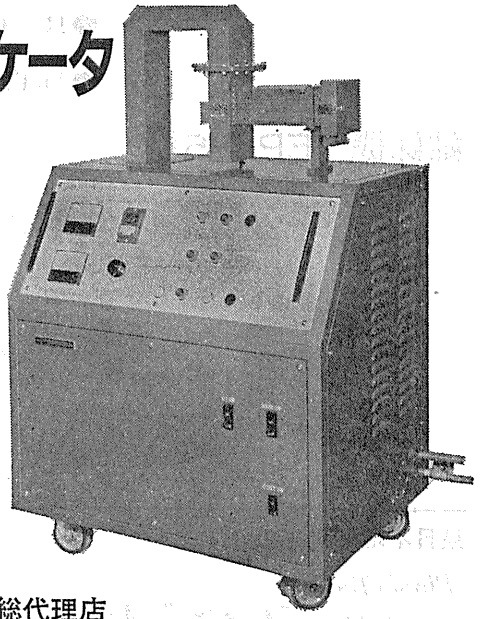
東芝マイクロウェーブアプリケータ
MODEL TMW-6402A

実験動物の脳内物質の測定に先立ち、測定物質に関連する諸酵素を不活性化する方法として凍結法があります。しかしながら凍結法では生体内酵素を不活性化させるまでにかなりの時間を必要とし、この間に測定物質が変化するおそれがあります。

この解決方法としてマイクロウェーブの瞬時照射により諸酵素を不活性化する方法が広く用いられるようになりました。照射後は凍結法で行なわれる低温処理の必要もなく、室温にて処理ができ、安定した測定値が得られます。特に部位別の測定を行なう場合には大変有用です。

●アセチルコリン ●サイクリックAMP ●サイクリックGMP ●GABA ●DOPA ●5-HTP ●セロトニン ●カテコールアミンとその代謝産物 ●エンドルフィン ●プロスタグランディン

などの正確な測定の前処理装置として、薬理学・生化学・生理学・内科学など広い分野に御活用いただけます。



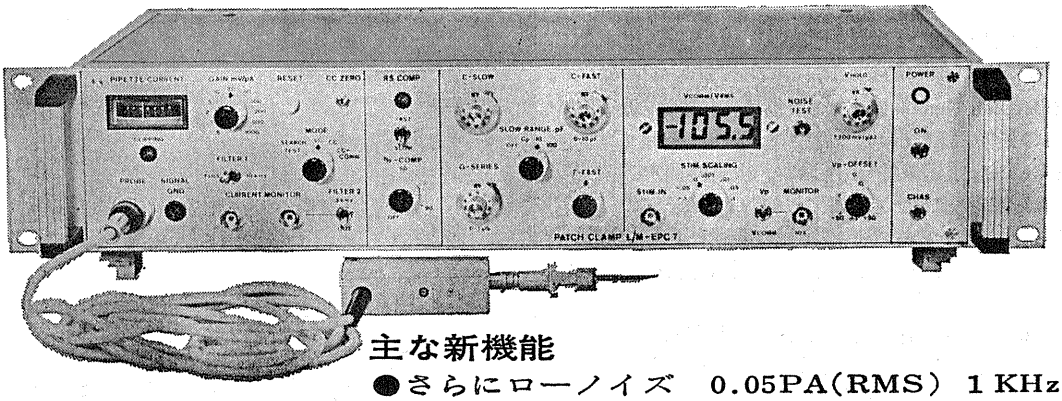
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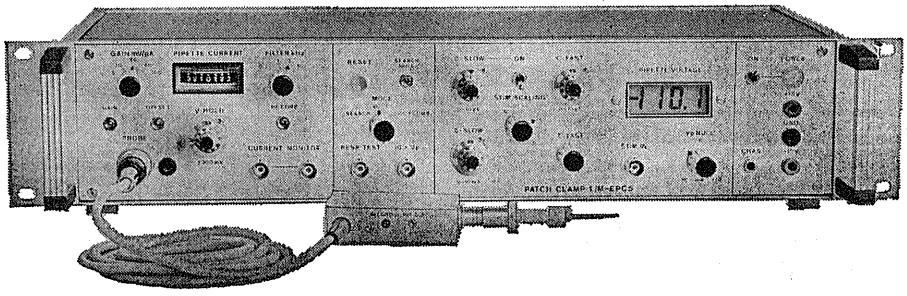
パッチクランプシステム EPC-7



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  0.30PA(RMS) 10KHz
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- 独自のTRANSIENT CANCEL機能

姉妹機 EPC-5型



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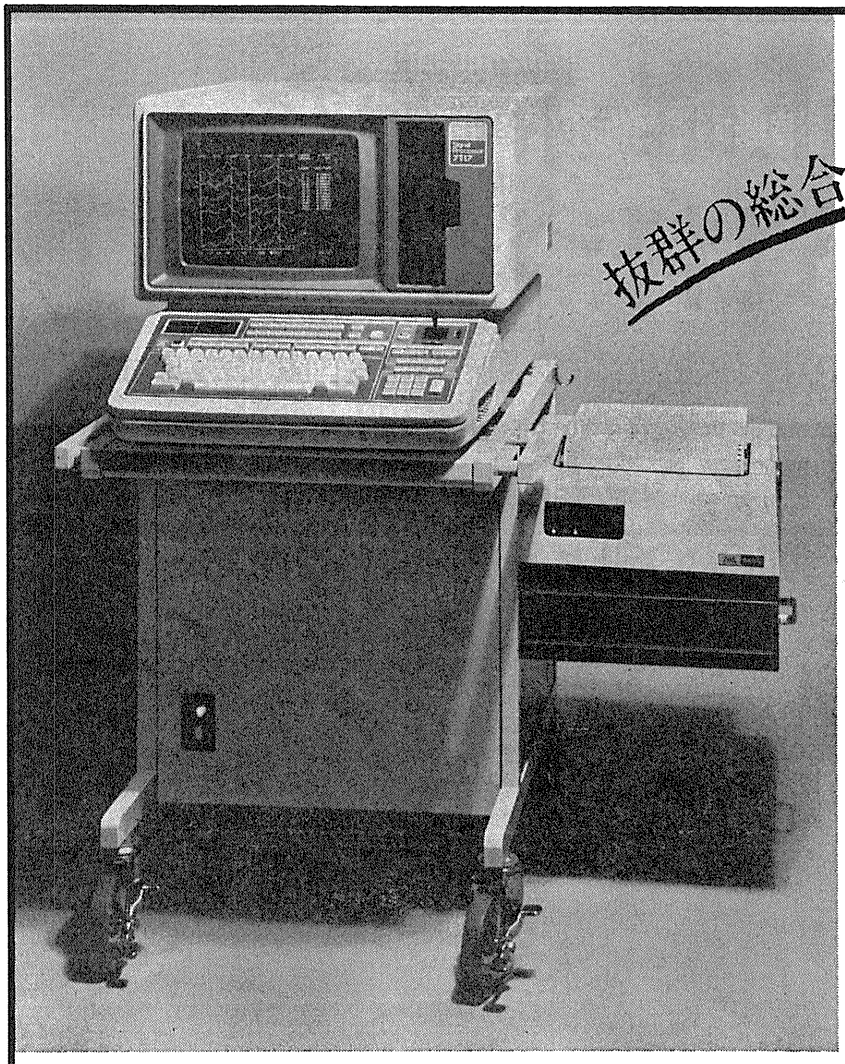
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（特許出願中）



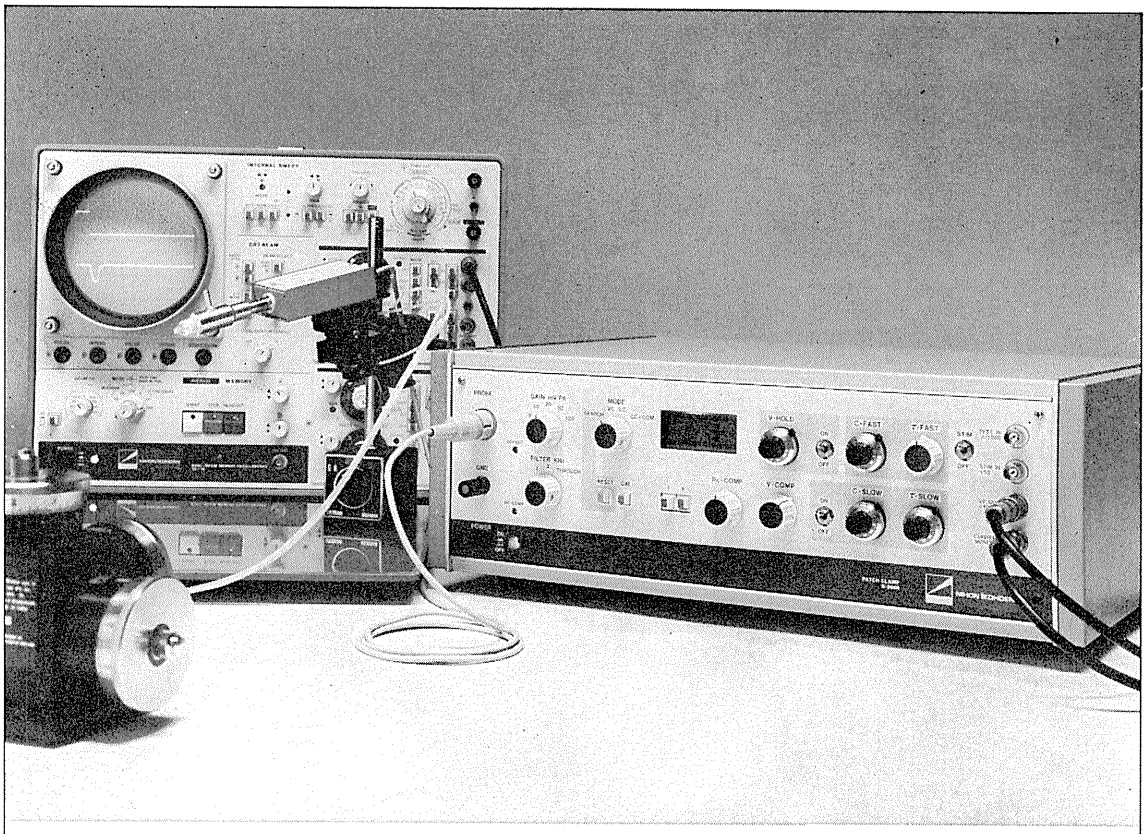
組織・細胞化学用の切片として、凍結または未凍結切片が用いられますが、凍結・融解の過程は細胞の微細形態を破壊するため、できれば未凍結切片を使用すべきであることはよく知られています。しかし、従来の未凍結切片作製用ミクロトームには、組織の破壊が大きく、切片の厚さが一定しない機種や、切片作製に極端に時間がかかり大きな切片や薄い切片が切りにくい機種が多く、また輸入品で高価である等種々難点がありました。弊社ではこれらの欠点をすべて克服した、画期的な未凍結切片作製用ミクロトームとして「マイクロスライサー」を開発しました。

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- 免疫化学
- 生理学
- 神経化学
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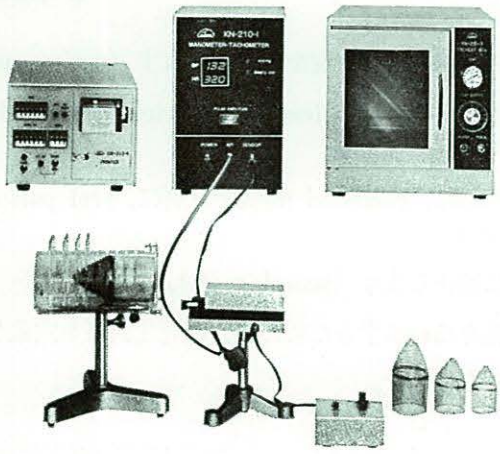
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