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第59回日本生理学会大会　昭和57年３月30日～４月１日　於 慶大日吉校舎

第59回日本生理学会大会を終えて

第59回日本生理学会大会
当番幹事　塚田裕三・村上元彦

本大会は昭和57年3月30日（火），31日（水），4月1日（木）の3日間，横浜市港北区所在の慶応義塾大学日吉校舎において開催されました．また本大会に先立ち，3月29日（月）に常任幹事会およびその他の諸委員会が，新宿区信濃町の医学部内で行われました．

われわれの教室で大会開催をお引き受けするのは，昭和17年に東京で第11回日本医学会総会が開かれた折，故加藤元一教授が当番幹事とならられて以来40年ぶりのことであります．このたびの大会では，何か有意義な企画を樹てようということで色々検討しました結果，次の2点を柱にいたしました．1つは演題数を，無制限にしたことであります．従来多くの大会では1つの研究単位から2題以内ということでありましたが，幸いにも日吉校舎は会場数を充分にとることができるので，この制限を思いきって取りはずし，会員の皆様の日頃の御研鑽の成果を自由に発表して頂き，発表形式も口演を原則とし，御希望ならばポスター発表も可能ということにしました．

もう1つの柱は解説講演です．日本の大学の教育体制は，欧米諸国のそれと異り，大部分の教員の方々が，専門外領域についても学生講義を担当せざるをえない現状であり，この体制は当分の間改善できる見込みはありません．このようなとき一番困ることは，生理学においても専門分野の拡大と細分化の趨勢に伴い，非専門領域では，何をどのように教えたらよいのか，また何がどこまでわかっていて何がわかっていないのか，現在何が中心課題であるかなどを要約して把握することが，なかなか難しいことであります．そこでわれわれは他の2～3の方々の御協力をえて，10のテーマを選び，それらについて生理学会会員が相互的に平易な解説講演を行い，その講演を聞いた人が専門外領域について，講義の指針なりとも把握できるならば，大変有意義であろうと考えた次第です．

以上の方針にしたがって大会を開催しましたところ，参加者総数1,569名，発表演題数702(一般口演630，解説講演20，ポスター52）会場数12という規模になりました．因みに，各研究室分野の演題数（ポスターを含み，解説講演を含まず）を見てみますと，研究方法4，分子生理19，細胞生理46，能動輸送20，興奮性膜38，シナプス・終板26，脊髄・末梢神経14，脳幹・間脳44，小脳6，終脳18，筋運動とその制御10，脳波・誘発電位9，行動・表現26，神経化学17，視覚48，聴覚13，その他の感覚31，骨格筋24，平滑筋14，心筋34，自律神経系32，循環33，血液・腎・体液調節30，呼吸22，消化・吸収5，内分泌・生殖24，運動・体力・疲労17，環境・エネルギー代謝27，体温調節・発汗23，その他8となりました．これらの数字はその分野の研究者の人口分布を直接表わすものではありませんが，今後の大会運営に何らかの参考になると思います．

大会運営につきましては，皆様に御満足頂けますよう意を尽した積りでありますが，当校舎は，100人前後の中講堂がないため，あるいは広すぎたり，あるいは狭すぎたりした会場があり，御不便をおかけしたことをお許し頂きたいと思います．また会場数が増え，プログラムが錯綜したため，お聞きになりたい口演が時間的に重なるという面があったと思われますが，これまた物理的にやむをえぬことでありまして御容赦下さい．

演題数無制限，解説講演とも初めての企画で，当番幹事としては甚だ心配でありましたが，プログラム進行もまずまず大過なく経過し，解説講演会場も殆んど堂にあふれる聴衆を得ることができ，大会を終って安堵している次第です．新企画の評価は後日定まると，思いますが，幾人かの方々からお誉めの御言葉を頂戴し，恐縮いたしております．

懇親会にも多数の方々が御参加下さいまして，大変楽しい会になりました．最後に皆々様の御協力に深甚の謝意を表しまして，この報告を終えたいと思いますが，とくに解説講演をオーガナイズして下さった方々，講演者，座長の方々に厚く御礼申しあげます．また本講演者として日本学術振興会の招聘により海外から参加されましたP. O. Bishop教授，浅沼広教授に謝意を表します．

THE 59th ANNUAL MEETING
OF
THE PHYSIOLOGICAL SOCIETY OF JAPAN

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1

ANALYSIS OF CHEMICAL KINETICS USING AN AUTOMATIC v - S PLOTTER.

HASHIMOTO,M.,HIGASHI,T.,ISOMOTO,A.,IWAKURA,K. AND TYUMA,I. Dept. of Physico-chemical Physiol., Sch. Med., Osaka Univ., Osaka.

The monitered time course of chemical reaction is generally expressed by S - t plot, where S is a signal that proportionally represents the progress of the reaction, and t is time. In this study, we constructed an apparatus with micro-computer that automatically converted S - t plot into v - S plot, where v = dS/dt, and clarified some advantages of this plot as follows by using it in the study of the autocatalytic reaction ; $HbO_2 + NO_2^- + X \longrightarrow metHb + NO_3^- + X$:

(1) The relationship between v and S corresponds to the kinetic equation, so we can easily speculate the kinetics of the reaction.

(2) The v - S relationship is free from the initial conditions of the reaction, so the strict determination of the initial conditions is needless.

The results of our experiments using this method indicated that the reacting species represented by X is H_2O_2 and it initially combines to HbO_2 with the binding constant about 10^6 M^{-1} (pH 7.0, 25°C).

2

DETECTION OF ANTIBODIES SPECIFIC FOR A PEPTIDE-HAPTEN, BRADYKININ, AS WELL AS PROTEINS,WITH AN SOLID-PHASE, ENZYME-IMMUNOASSAY USING POLYSTYRENE MICROTITRATION PLATE AND HORSERADISH PEROXIDASE-PROTEIN A CONJUGATE.

YOSHIDA. T. Department of Physiology, School of Medicine, Mie University, Tsu, Mie.

An indirect, solid-phase, enzyme-immunoassay using polystyrene microtitration plate and horseradish peroxidase-protein A conjugate was applied to the detection of the rabbit specific antibodies for a peptide-hapten, bradykinin(BK). Rabbit antisera were raised against two kinds of BK-immunogens,BK conjugated to bovine serum albumin by means of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide(BSA-WSC-BK) and BK linked to ovalbumin through toluen 2,4-diisocyanate(OA-TC-BK). By the present method, they showed positive binding to the respective carrier-proteins in the dilution up to 1: 100,000, however, they failed to show consistent positive binding to BK itself under the same condition as protein antigens. This difficulty was overcome by the use of a BK-protein conjugate in which the carrier was noncrossreactive to that utilized in the immunogen in the first step of coating the plastic surface with antigens. Thus, both rabbit antiBSA-WSC-BK and antiOA-TC-BK above showed positive bindings to BK-rabbit serum albumin conjugate, which were dose-dependently competed by BK in the range of 0,1 to 100 ug BK/well.

3

FLUORESCENCE SPECTRA OF TISSUES AND ORGANS IN SITU

KAKIUCHI, Y., KIKUCHI, Y. AND KOYAMA, T. Div. Physiol., Res. Inst. Appl. Electr., Hokkaido Univ., Sapporo

A new spectrofluorometer was devised as an approach to detect abnormal degeneration or denaturalization of tissue-organs in situ. The special feature of the instrument is the reflection type of its fluorometric system which allows spectral analysis of such opaque substances as living tissue-organs either in vitro or in situ. A pulsed nitrogen laser (wavelength: 337 nm, pulse width: 1 nsec, and repetition: 30 Hz) is used for excitation. A train of laser pulses is focussed to 0.5 mm circle on the surface of sample tissues, and resultant fluorescence is then introduced into a grating monochromator through a dichromatic mirror. The spectral ranges of wavelength is between 400 and 800 nm which covers most of fluorescence of such metabolic coenzymes as NADH, flavin or cytochrome C. Isolated organs (liver, kidney, heart, lung and pancreas) and cancers of rodents were examined under various conditions. Majority of spectra presented their peaks at the ranges of 460 - 470 nm, however some showed peculiar spectra to each organs with remarkable variations especially at ranges of 420, 520, 640 and 700 nm, which are probably of pathophysiological importance. The instrument was also applicable to map the hematoporphyrin distribution in organs as a fluorescent tracer for blood perfusion.

4

A PRECISION INSTANTANEOUS-FREQUENCY METER FOR THE MULTI-TRACE CRO

OBARA, S. AND NAGAI, T. Dept. of Physiol., Sch. Med., Teikyo Univ., Tokyo

The instantaneous-frequency display of the neuronal activities has been developed in the early sixties. These devices must produce voltage outputs proportional to the reciprocal of the inter-spike intervals by an on-line computation of the hyperbolic function of $V = a/t$. The present circuit combines readily available IC's to give an improved performance of linear F-V conversion over a dynamic range of 4 - 600 Hz with error maxima of c. 1 %. The output is square pulses of 1.5 ms in duration through the use of a flexible S-H circuit which can respond to inputs up to c. 2 KHz. Compared with the Z-modulation method of the earlier models, this display mode is better suited for the chopped-beam multi-trace CRO, and also permits a continuous monitoring of the base line for easier calibration. The required hyperbolic function is approximated by a series of exponentials which increase in time constant by a factor of m. Numerical analysis of a normalized error equation for the approximation indicates possible error maxima of 3.4, 2.4 and 1.1 % for m of 2, 1.8 and 1.5, respectively. This prediction is fully confirmed by the actual performance shown above where m of 1.5 is adopted. A simple and systematic method will be presented for designing a circuit to one's own specifications, and also for compensating for variations in component values.

5

ON THE RNA CODON

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In order to clear the significance of RNA codon of amino acid, first we paid attention to the special case of UUU→Phe under the consideration of the matrix $S=\begin{pmatrix} U & U \\ U & * \end{pmatrix}$. S is the matrix of the linear transformation of coordinate axis, that is X'=SX. The molecular weight of U is 112. The characteristic equation, the characteristic value λ and the characteristic vector e{l,m} are estimated respectively, when the value of * is given according to the formula of $Se=\lambda e$. If the value of * is 1526, the matrix is expressed as following, $S=\begin{pmatrix} 112 & 112 \\ 112 & 1526 \end{pmatrix}$. From this formula, we get $\lambda=103.1837$ as the suitable proper value. Further, we get the values of the components of the characteristic vector as follows: $\cos\theta=$ l=112G, $\sin\theta=$m= -8.8163G. Hence,G is the constant. The rotational angle of the coordinate axis is estimated as -4.4999°. This value is strictly coincidence with $[\alpha]_D^{25}$ (5N HCl)=-4.5°of Phe.

6

STUDIES ON ORIENTED MULTIBILAYER LIPID MEMBRANES BY SATURATION TRANSFER ESR

WATARI, H. AND *SHIMOYAMA, Y. Dept. of Molecular Physiology, Institute Physiol. Sciences, Okazaki, *Dept. of Physics, Hokkaido Educational Univ. Hakodate Branch.

The saturation transfer ESR spectroscopy was applied to the oriented multibilayer membranes of both egg yolk lecithin(EYL) and dipalmitoyllecithin(DPL) in order to explore the molecular order and motion of the lipid membrane organisation. The variation of microwave power changes spectra drastically, especially in the vicinity of the turning point at the central field. The spectra varies with an angle between the membrane surface and the applied magnetic field. The motional parameters were charactrerized by means of the linear relation of rotational correlation time versus peak height ratios. In the case of DPL oriented multibilayers, the correlation time at temperatures below the gel-liquid crystalline phase transition point (ca. 40°C) were found giving the time range of 10^{-3} - 10^{-6} sec. The lipid motion in the EYL multibilayer membranes at the temperatures between -40°C to 0°C was determined by the correlation time, yielding the same range as the DPL membranes. The atempts of the rigorous theoretical treatments have been made to determin order and motion in such the highly anisotropic membrane systems.

7

EFFECT OF CHAIN LENGTH AND INTERACTION BETWEEN SECONDARY STRUCTURE ON CD SPECTRA OF PROTEIN AND PEPTIDE. UYEDA, M., IWAI, T*, FUJIWARA, T*, AND TANIMOTO, O*, Dept. of Physiology, Sch. of Med., Gifu Univ., Gifu and *Dept. of Applied Physics, Fac. of Eng., Osaka City Univ., Osaka

We calculated the CD spectra of a unit α-helical and β-formed segment in polypeptide and protein by difference spectra. The chain length dependence of CD spectra are well establised experimentally and theoretically (End effect). The calculated rotatory strength per residue was 58 % for 7 residue helix, 77 % for 21, 95 % for 31 of 41 residue for each N-π^*, π-π^* transition. But the effect was shown to diminish if helical segment are sandwiched between random or β segment. Our calculation also showed spectral change caused by interaction between ordered structure segments in model polypeptide. CD spectra of two helical segment of S.S.I. (Streptmyces Subtilisin Inhibitor) are same characteristics of helical segment sandwiched in β segment and thus suggest interaction between β and helical segment. CD spectra of C and E helix of Myoglobin in company with minor change in B and D helix suggest helix-helix interaction. Both end effect and inter-segment interaction effect may raise some difficulty on linear multiple regression analysis of CD spectra of protein for secondary structure estimation and probably the failure of the method on S.S.I. are caused by helix-β interaction.

8

LOCAL STRUCTURES OF PROTEINS.

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A newly devised method of searching for the local structures in proteins is reported. The basic idea of this method rests on the success in defining the degree of homology between two different segments of proteins. The degree of homology which is defined as a sum of the second power of the elements of the difference map of the segments enable one to draw a map of conformational homology between two proteins. Two segments which resemble or do not resemble each other are represented on the map as a region of high or low degree of homology, respectively. Well known secondary structures of proteins, α-helix, β-sheet or turn, which are commonly observed in proteins usually make a region of high degree of homology, and distinguished from others clearly. Selecting a proper length of the segments, the map of conformational homology makes it possible to search for not only a small local structure but also a large one like a Ca-binding site in Ca-binding protein. Searching for a class of local structures which has not been discovered yet might be possible with a systematic application of this map.

9

MOLECULAR AGING OF BOVINE PLASMA ALBUMIN (BPA).

INOUYE, H., ERA, S., NAGAOKA, S. AND SOGAMI, M. Dept. of Physiology, Sch. of Med., Gifu Univ., Gifu

Bovine mercaptalbumin (N), when incubated at low ionic strength and alkaline ph, isomerized to a new component (A), having a more positive electrophoretic mobility than N near pH 5. A moving-boundary electrophoresis was employed to separate A from N. By this method, it was found that both Na-caprylate and N-acetyl-L-tryptophan supress an aging reaction (N-A isomerization) and that the former is more effective to protect from this reaction than the later. These results were comfirmed by a differential scanning calorimetry (DSC). The endothermic transition temperatures of BPA in the presence of ligands are as followes : 60°C (BPA), 68°C (BPA + N-acetyl-L-tryptophan, 1 : 40), 79°C (BPA + Na-caprylate, 1 : 40) and 80°C (BPA + N-acetyl-L-tryptophan + Na-caprylate, 1 : 40 : 40).

Only barely detectable amount of A-form was found in crystallized BPA (Armour) but ~10 % in fatty acid-free BPA (Sigma, Pentex). Furthermore, fatty acid-free BPA (Sigma, Pentex), when stored at pH 3.7 at 3°C for several days, produced a new component (A*) up to 20~30 %, having the same mobility of A-form. A* might result from the presence of protease contaminants.

10

HIGH RESOLUTION ^{1}H NMR STUDIES ON THE N-F TRANSITION OF BOVINE PLASMA ALBUMIN IN 0.10 M NaCl.

SOGAMI, M., NAGAOKA, S., INOUYE, H., ERA, S., WATARI*, H., SHIGA*, K. AND AKASAKA**, K. Dept. of Physiology, Sch. of Med., Gifu Univ., Gifu, *Natl. Inst. for Physiol. Sciences, Okazaki and **Dept. of Chem., Faculty of Science, Kyoto Univ., Kyoto

Measurements of the spin-lattice relaxation time (T_1) and the intramolecular cross-relaxation time (T_{IS}) with a presaturation pulse applied at 7.18 ppm upfield from HDO at a constant power (0.20 W) were made on the N- and F-forms of bovine plasma albumin (BPA) in 0.10 M NaCl in D_2O at 25°, using a Brucker WM-360.

		$-CH_3$	LYS(C_ϵ)	AROM	HIS
N-form	T_1 (sec)	0.88	0.86	1.06	1.25
(pD 5.29)	T_{IS}(sec)	0.40	0.41	0.38	0.41
F-form	T_1 (sec)	0.79	0.83	0.98	1.14
(pD 4.03)	T_{IS}(sec)	0.62	0.76	0.51	0.82

T_{IS} values of the F-form were approximately 1.5~2 times of those of the N-form. These results might indicate that the N-form is more rigid structure than that of the F-form. 10 % decrease in α-helix content and almost no increase in the size of BPA molecule were observed in the N-F transition. However, ^{1}H NMR spectra of an aromatic region showed significant changes as in the case of denaturation of proteins, that is, the disorder of side chains.

11

CD-RESOLVED SECONDARY STRUCTURES OF BOVINE PLASMA ALBUMIN. ERA, S., INOUYE, H., NAGAOKA, S., SOGAMI, M. AND ASHIDA*, H. Dept. of Physiology, Sch. of Med., Gifu Univ., Gifu and *Dept. of Physiology, Defence Med. Col., Tokorozawa

Fractions of α-helix (f_α), β-form (f_β) and disordered form (f_r) of bovine plasma albumin (BPA) was estimated using Chen's method with two constraints : $\Sigma f_i = 1$ and $0 \leqq f_i \leqq 1$. Computed CD spectra ($[\theta]_\alpha$, $[\theta]_\beta$ and $[\theta]_r$ at any wavelength) were determined from CD spectra of 5 proteins of known structure using program for multicomponent spectrum analysis (Anal. Chem. 37, 1000 (1965)). Using $[\alpha]_{313}$, the low-pH and the neutral transitions of BPA were seen, one corresponding to the N-F transition and the acid expansion and the other to the N-B transition (N-F : pH 4.4-3.8, ACID EXPANSION : pH 3.6-2.8, N-B : pH 7.0-9.0 in 0.10 M KCl). Values of f_α, f_β and f_r of the N-form in 0.10 M KCl at pH 5.65 were 0.73, 0.15 and 0.12, respectively. In the low-pH transition, changes in secondary structures in 0.10 M KCl were as follows : (N→F), $\Delta f_\alpha = -0.12$, $\Delta f_\beta = 0.07$, $\Delta f_r = 0.05$; (F→E), $\Delta f_\alpha = -0.10$, $\Delta f_\beta = 0.01$, $\Delta f_r = 0.09$. Major increase in the β-form was correlated with the N-F transition. On the other hand, slight changes in secondary structures were seen in the N-B transition.

12

EFFECT OF ERYTHROCYTE SHAPE ON OXYGEN TRANSPORT. KON, K., MAEDA, N. AND SHIGA, T. 2nd. Dept. of Physiol., Sch. of Med., Ehime Univ., Ehime

The effect of shape change on the deoxygenation rate of erythrocytes was studied. Normal discocytes were changed to echinocytes by anionic drugs (trichlorobenzene sulfonate, dehydroepiandrosterone sulfate, SITS, DIDS) and to spherostomatocytes by cationic drug (isoxsuprine). The deoxygenation rate was measured both by the stopped flow apparatus and the continuous flow system.
(1) The deoxygenation rates of discocytes and spherostomatocytes were same. (2) As the degree of echinocytosis increased, the deoxygenation of erythrocytes was retarded, though the membrane fluidity, measured by the spin label method, did not change. The retarded deoxygenation was probably due to the increase of stagnant layer around erythrocytes and the increase of diffusion distance of oxygen inside erythrocytes. (3) The deoxygenation rate of echinocytes, measured with the continuous flow system, depended on flow rate. At lower rate (~50 cm/sec), the deoxygenation of erythrocytes was clearly retarded, while at higher rate (~150 cm/sec), the deoxygenation rate increased to that of discocytes. The deformation of echinocytes by high shear stress would induce the increase of deoxygenation. These findings imply that the deformation of erythrocytes is important not only for blood flow but also for oxygen transport.

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STRUCTURAL AND FUNCTIONAL CHANGES OF HUMAN ADULT AND FETAL HEMOGLOBINS BY ORGANIC PHOSPHATE. TOMITA, S., ENOKI, Y., KOHZUKI, H. and SAKATA, S. Dept. of Physiol., Nara Med. Univ., Kashihara, Nara

In the absence of organic phosphate, especially 2,3-diphosphoglycerate (DPG), the oxygen affinity of fetal hemoglobin(Hb F) is lower than that of adult hemoglobin(Hb A) over the entire pH range. Adding DPG gets the oxygen affinity of Hb F higher than that of Hb A at the physiological pH but, at the lower pH(below 7) the oxygen affinity remains lower than that of Hb A even in the presence of DPG. This low susceptivility for DPG and low oxygen affinity of Hb F are intrinsic characteristics of fetal hemoglobin, which appear to arise from much stable quaternary structure than those of Hb A. This stable structure reduces also the rate of the dissociation of tetramer into its subunits strongly in Hb F than in Hb A.

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EFFECT OF OXYGEN MOLECULES ON THE HEMATO PORPHYRIN SPECTRA ✦AIZAWA,K.,✦SAKAI,S.,✦O'HATA, S.,●KATO,H., and●HAYATA,Y. ✦Dept. Physiol. and●Dept. Surg. Tokyo Med. College Tokyo 160

The fluorescence spectra measurements of Hemato porphyrin derivative (H pD) were obtained with a high spead fluoro-micro-spectrometer equipped with a SIT tube. The emission spectra at 405 nm excitation consists of two peaks at 618 nm and 680 nm in phosphate buffer saline solution (PBS). The HpD emission spectra incorporated into PC-10 culture cell can easily be seen the form red-shifted about 10 nm from the emission spectra of HpD in PBS solution. Next, We have directly measured the effects of oxygen molecules in PBS solution on the HpD fluorescence life time by the technique of synchroscan streak camera (Hamamatsu TV Model C1587X), using a synchronous passive hybrid mode locked CW Ar dye laser at 570 nm (average power of 50 mW, duration 0.8 psec, frequency 12.2 nsec). The fluorescence decays of HpD in aero-,oxygen-bubbled, and oxygen out gassed PBS solution were measured, those life time τ_m were determined to be 239, 176, and 333 psec. This indicates that oxygen molecules quench some excited singlet state HpD by the enhanced $S_1 \rightarrow T_1$ intersystem crossing, due to diffusion controlled collision with that or by the photo-oxidation of the HpD due to sarrounding singlet oxygen molecules produced.

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EXISTENCE OF THE HYDROPHOBIC POCKET IN COMMON IN THE ACTIVE SITES OF SOME SERINE PROTEASES. HIJIKATA, A. AND OKAMOTO, S. Dept. of Physiol., 1st Div., Kobe Univ. Sch. Med., Kobe

4-methyl-L-[N^2-(3-methyl-1, 2, 3, 4-tetrahydro-8-quinolinesulfonyl)-L-arginyl]-2-piperidinecarboxylic acid, one of the representatives of extremely potent and highly selective thrombin-inhibitors characterized by their tri-pod structure (arginine residue (P_1), aromatic group (P_2), hydrophobic group (P_1')) had 4 stereoisomers in 4-methyl-2-piperidinecarboxylic acid portion (P_1') and the stereochemical study was made to search for the stereogeometry of some serine proteases. Thrombin, acetylated thrombin and trypsin showed the same inhibition pattern: ratio of Ki of (2R, 4S) (2S, 4R), (2S, 4S) to that of (2R, 4R) was about 10, 100 and over 1,000 in all three enzymes. Inhibition of plasmin and F.Xa was very weak, nevertheless (2R, 4R) was the strongest. These results indicated that thrombin, acetylated thrombin, trypsin, and possibly plasmin and F.Xa, had a common stereogeometry in their S_1' sites: hydrophobic pocket causing attractive force for 4-CH_3 in R-configuration and causing repulsive force against 2-COOH in S-configuration. The possibility was discussed that the hydrophobic pocket was constituted with Phe(Leu)-Cys-Cys-His in histidine loop in these enzymes.

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THE STRUCTURES OF THE CHARGE TRANSFER COMPLEXES IN THE FLAVOENZYME D-AMINO ACID OXIDASE. Y, NISHINA, K, SHIGA, H, WATARI, H, TOJO*, T. YAMANO*, AND R. MIURA**, National Institute for Physiological Sciences, Okazaki, *Dept. of Biochem.,Osaka Univ. Medical School Osaka, **Dept of Biochem., Res. Inst., National Cardiovascular Center, Suita, Osaka.

The resonance Raman (RR) spectra of the complexes of D-amino acid oxidase (DAO) with benzoate derivatives and the catalytic intermediates of DAO were obtained. In the case of DAO-o-NH_2-benzoate complex, the Raman lines at 1583 and 569 cm^{-1} derived from oxidized flavin and the ligand, respectively, were enhanced. This suggests that the o-NH_2-benzoate molecule lies along the N(5)-C(4a) bond and parallel to the flavin face.

In a catalytic intermediate, a purple complex of DAO with D-proline or D-alanine as a substrate, the resonance enhanced Raman lines around 1605 and 1360 cm^{-1} in either of the complexes were obtained. These lines are probably derived from vibrational modes of reduced flavin molecule. Since the highest energy band at 1692 cm^{-1} in the RR spectrum with D-alanine was shifted to 1675 cm^{-1} upon ^{15}N-substitution of alanine and ammonium, this line in the spectrum with D-alanine or the line at 1658 cm^{-1} with D-proline is assigned to C=N atretching mode of an imino acid corresponding to each amino acid. These results confirm the concept that the purple intermediates of DAO consist of reduced flavin and an imino acid.

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OXYGEN EQUILIBRIUM CHARACTERISTICS OF CHLOROCRUORIN OF *POTAMILLA LEPTOCHAETA*

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Accurate oxygen equilibrium curves of chlorocruorin (Chl) of *Potamilla leptochaeta* were determined under a variety of conditions as to pH, salt, and temperature and the equilibrium characteristics were compared with those of Chls of other species and human adult hemoglobin (Hb A). The overall oxygen affinity of the *Potamilla* Chl was much lower than that of Hb A over the entire pH range examined, pH 6.2 to 9.2. The Bohr coefficient was -0.98 for the Chl compared to -0.53 for Hb A. Cooperativity expressed by the Hill coefficient (n_{max}) for the Chl showed a marked variation upon pH changes with a maximum value 5.82 at pH 8. The shape of the oxygen equilibrium curve for the Chl showed distinct temperature dependences. Heat of oxygenation for the Chl was smaller than that for Hb A. Divalent cations such as Mg^{2+} and Ca^{2+}, when added to the Chl, were found to increase cooperativity ($n_{max} > 5$) and oxygen affinity to physiological range of oxygen pressure. Na^{+} and K^{+} also exerted similar but much smaller effects on cooperativity and oxygen affinity of the Chl. The oxygen binding properties of the *Potamilla* Chl are quite different from that of Hb A and also presents striking constrast to the *Spirographis* Chl. Unlike mammalian hemoglobins, the oxygen transport function of the *Potamilla* Chl seems to be regulated by divalent cations rather than by anions.

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VALIDITY OF CONSTANT-FIELD PRINCIPLE TO EXPLAIN THE PERMEATION MECHANISM OF CALCIUM CHANNEL.

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We examined Ca currents in isolated nerve cell bodies of Helix aspersa, using the suction pipette method for voltage clamp and internal perfusion. Ca current approaches E_{Ca}, but cannot be detected in the outward direction. The current-voltage relationships fit well the curve predicted from constant-field equation with constant permeability. The voltage shifts of the activation parameters by Ca ions can be described by the Guoy-Chapman theory for the diffuse double layer with binding. This theory gives calculated values for a negative surface charge density of 1 e/80 A^2; maximum surface potential of -116 mV, and Ca binding constant of 0.1 M^{-1}. Surface concentration of Ca ions calculated from the theory increases and saturates as the external concentration increases. The relation is identical with that of Ca current magnitude and the external Ca ion concentration. These results suggest that the Ca currents obey the constant field principle presented by Hodgkin and Katz (1949), except their third assumption.

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ENTHALPY CHANGES OF THE BINDING OF CALCIUM TO CARDIAC TROPONIN C AND CALMODULIN.

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We previously reported the study of conformation changes on Ca binding of skeletal troponin C (s-TNC) by microcalorimetric titrations. Conformation changes were discussed in terms of the entropy changes arising from the hydrophobic effect (ΔS_{hyd}) and intramolecular vibrational modes (ΔS_{vib}). In the present report, conformation changes in cardiac troponin C (c-TNC) and calmodulin (CaM) associated with Ca binding were studied and compared with those of s-TNC. In the case of c-TNC only two sites of higher affinity showed enthalpy changes ($\sim$-13 kJ/mol). These two sites of c-TNC gave moderately large positive ΔS_{hyd} and negative ΔS_{vib}, indicating that the Ca binding reduces the interaction of hydrophobic residues with surrounding water molecules and induces a more rigid structure.

The enthalpy titration of CaM showed that CaM has at least two classes of Ca binding sites, two high affinity and two low affinity sites. Enthalpy changes were small and were positive in the absence of Mg. Conformation changes of CaM differ from those of s-TNC and c-TNC in that both ΔS_{hyd} and ΔS_{vib} are small on Ca binding.

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QUANTUM CHEMICAL STUDY ON GATE MECHANISM OF ION CHANNEL.
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The gate mechanism and the selectivity of ions of the channel were studied using a simplified valinomycin model by means of the molecular orbital calculations (the CNDO/2 method). The channel with a diameter of 8 Å provided a low potential barrier for penetration of cation, which corresponds to the open gate. With the diameter of 4 Å, the potential barrier for Na^+ ion was 500 kcal/mole, corresponding to the closed gate. The gates consist of the inward oriented oxygens in the valinomycin type channel. A cation with ionic cloud cannot penetrate the channel. The Na^+-H_2O complex could not penetrate the gate with the diameter of 6 Å, which is open for Na^+ ion itself. The selectivity of cations is based in part on the electromotive force in electric field. In a dielectric field which consists of 4 water molecules between a monovalent cation and anion, Na^+ ion showed the potential differences of 5.6 eV and Li^+ ion, 2.5 eV.

The selectivity of ion channel is a function of the electromotive force and the potential barrier for the ion which is transported through the gate. The interaction of ions with dielectric field and the channel molecule should be studied in the light of quantum chemistry.

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TRANSPORT EQUATION OF GLUCOSE ACROSS RED BLOOD CELL AND BRUSHBORDER VESICLE MEMBRANES.
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The human red blood cells exhibit a biphasic volume change during D-glucose uptake in the presence of monovalent cations at 37 °C. Time course of D-glucose uptake by the brushborder membrane vesicles shows a typical overshoot curve. The reconstituted liposomes which contain the glucose carrier protein extracted from the brushborder membrane shows a biphasic time course of D-glucose uptake. These overshoot time course of volume change and of D-glucose uptake were simulated by means of the membrane equation system for the carrier mediated transport of non-electrolyte. The Katschalsky-Kedem-Curran equation was extended to the carrier transport. The calculation revealed that the D-glucose uptake is accompanied by the volume change, *ie.* the cooperative flow of volume flux and solute flux exhibits the overshoot uptake. The concentration of carrier inside the membrane causes high concentration of solute inside the membrane which is essential for the overshoot uptake. The equation system has limit cycle solution corresponding to the membrane oscillator. The mechanism of active transport was suggested as the dimerization of carrier protein in this system.

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QUANTUM CHEMICAL STUDY OF THE SOLVENT EFFECTS ON RADICALS FROM CATECHOL AND ITS CONGENERS.
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The proton hyperfine coupling constants of radicals from catechol and its congeners were calculated by means of the INDO with molecular geometry adjusting method. The radicals in alkaline and acidic solution, and in various metal ion solutions were calculated. The observed ESR splitting constants were exactly reproduced. The ratio of coupling constants, A_3/A_4, and total energies of the radicals correlate smoothly with length of the C-O bond and the heat of hydration of metal ions. The results correspond to the stability of the radicals increasing in the series, Rb^+, K^+, Na^+, Li^+ < Ba^{++} < Sr^{++} < Ca^{++} < Cd^{++} < Mg^{++} < Zn^{++}. The solvent effects reflect on the hyperfine coupling constants and the molecular geometry, especially the C-O bond length. The Hückel MO method has provided the different assignment of the hyperfine coupling constants of the catechol radicals from that of the INDO method. The difference of the assignment was discussed on the molecular orbital theory. Results from the Hückel MO, that with ω-technique, the iterative unrestricted Hückel MO and the PPP method are compared with that from the INDO method.

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ROLE OF THE HEME SIDE CHAINS IN THE OXYGENATION OF MYOGLOBIN AND HEMOGLOBIN.

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To clarify the functional role of 2- and 4-side chains of heme in the oxygenation of myoglobin (Mb) and hemoglobin (Hb) we synthesized 15 hemins carrying unnatural side chains at positions 2 and 4, reconstituted Mb (sperm whale) and Hb (human adult) with them, and investigated their optical, ionization, and oxygen binding properties. The order of the P_{50} values for the reconstituted Mbs and Hbs does not accord with the order of either bulkiness or the electron-withdrawing power of the substituent. The 2- and 4-side chains are functionally nonequivalent and the modification of the 4-side chain exerts greater influence on the oxygen affinity than that of the 2-side chain. The 2, 4-substitution by isopropyl and propenyl groups increases the P_{50} of Mb whereas the substitution decreases the P_{50} of Hb. All the reconstituted Hbs used showed higher oxygen affinity and smaller cooperativity in oxygen binding than those of native Hb. The magnitude of the Bohr effect and the response to organic phosphates such as 2,3-DPG and IHP were reduced in the reconstituted Hbs. These results indicate that either bulkiness or electron-withdrawing power alone cannot give straightforward explanations for the effect of heme side chain substitutions on oxygen affinity of Mb and Hb.

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PERIODIC INCREASE OF INTRACELLULAR Ca IONS IN GOLDEN HAMSTER EGG DURING FERTILIZATION.
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The golden hamster egg during fertilization shows recurring hyperpolarizing-responses (HRs) superimposed on a gradual shift of the resting potential. The series of the HRs indicate a periodic increase in the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Mechanisms involved in the increase of $[Ca^{2+}]_i$ were analysed by changing $[Ca^{2+}]_o$ or adding other divalent cations. By raising $[Ca^{2+}]_o$ it was found that the Ca^{2+} permeability is increased after fusion of sperm to the egg membrane, and the increased Ca influx produces a hyperpolarizing shift of the resting potential by activating K^+ conductance and produces a favourable condition for generating the HRs. No HRs could occur in Ca^{2+}-free medium. The frequency of HRs was decreased by lowering $[Ca^{2+}]_o$ or by adding Mn^{2+}, Co^{2+} in the presence of Ca^{2+}, while the amplitude of each HR was little affected. In these conditions, the shift of the resting potential was smaller than in normal medium, because of less Ca^{2+} influx. However, it is unlikely that HRs are due to a periodic increase in the Ca^{2+} influx because 1) each HR was not preceded by a depolarizing phase, 2) neither the rate of fall nor the reversal potential of the HR was unaltered by 15-fold change in $[Ca^{2+}]_o$. It is suggested that the increase in $[Ca^{2+}]_i$ for the HR seems to be related to intracellular Ca^{2+} stores or Ca^{2+}-buffering systems.

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ANALYSIS OF THE INCREASE IN INTRACELLULAR Ca^{2+} BY INJECTION OF Ca^{2+} INTO THE HAMSTER EGG.
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Ca ions were injected iontophoretically into the unfertilized hamster egg to observe whether the increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) is enhanced periodically, as suggested by hyperpolarizing responses (HRs) in fertilizing eggs. On injection of Ca^{2+} with a single 2 sec pulse, a hyperpolarization based on K conductance was increased with increasing injection current, and a much larger hyperpolarization (full response) was generated in an all-or-none fashion with an averaged critical injection current of 1.1 nA. Once a full response occurred, the next response by the same pulse was reduced to 20-30%, indicating that there is a refractory period for producing the full response. The refractory period was between 50 and 120 sec. The full response is considered to be due to an enhancement of the increase in $[Ca^{2+}]_i$, since a large and prolonged full response was induced, when Ca buffering system was suppressed by lowering temperature or by replacing external Na^+ with Tris or Li^+. In fertilizing eggs, sperm-induced rhythmic HRs were interrupted by Ca injection: the HR next to the Ca-induced HR occurred with a certain delay, and succeeding HRs appeared again at the original interval. These findings indicate that, although the series of HRs are maintained by continuous Ca influx during fertilization, the rhythm itself is determined by the intracellular condition for Ca ions.

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INFLUENCE OF Ca ION ON THE CELL SWELLING IN THE GUINEA-PIG TAENIA COLI.
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The changes in the tissue weight produced by excess external K were studied in the guinea-pig tenia coli. The tissue weight increased by 10-15% in 2 hrs when NaCl in Krebs solution was completely replaced with KCl. The swelling was significantly increased by removing Ca. Verapamil potentiated the swelling in KCl solution containing Ca(2.4mM), mimicking the effect of Ca removal, but had little effect in Krebs solution containing ouabain(10^{-4}M),in which Ca removal gradually increased the tissue weight. The tissue weight recovered when NaCl was readmitted, replacing KCl isosmotically. This effect was dependent on Na cocentrations and partially inhibited by ouabain(10^{-4}M). Readmission of Ca(2.4mM) did not cause a substantial weight recovery. Most of the results could be explained by the idea that the cell volume is determined by ionic distribution governed by the double-Donnan equiliblium, and that Ca suppresses the membrane permeability to ions. But the effectiveness of Ca-blockers in increasing the tissue weight may suggest some Ca-dependent mechanisms in regulation of the water movement, besides in control of the permeability.

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INTERACTION BETWEEN CALMODULIN AND ITS BINDING PROTEIN. ITANO,T., ITANO,R., TOKUDA, M.,MATSUI,H.,DOI,A. AND HATASE,O., (Dept. of Physiol. Kagawa Med. Sch. Kagawa 761-07)

Calmodulin(CAM) is a ubiquitous and multifunctional Ca^{2+}-binding protein that is believed to be a major translator of the intracellular Ca^{2+} message. It regulates a large number of intracellular enzymes activities, such as, cyclic nucleotide phosphodiesterase, adenylate cyclase, protein kinases, Ca^{2+} ATPase of plasma membrane and NAD kinase. In addition to the effects of CAM on these enzymes, it has been shown to bind strongly to the other proteins which are called CAM-binding proteins. A number of these proteins have been detected, but not characterised. In view of the considerable interest in CAM-binding proteins, we present the evidence concerning an ionic interaction between highly basic protein and CAM. Knowledge of the nature of these interaction is vital in helping to assess the physiological roles of various CAM-binding proteins.

Low concentrations (<10 μg/ml) of basic polypeptides inhibit the CAM-stimulated cyclic nucleotide phosphodiesterase. Inhibitory compounds include synthetic polypeptide [polylysine (D and L) and polyarginine] and basic proteins (protamine, histones and myelin basic protein). In mixtures of CAM and basic proteins, complexes were formed whether Ca^{2+} was present or not. The wide variety of inhibitory basic polypeptides and proteins indicates these interactions are not so specific.

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FURTHER EVIDENCE FOR THE INVOLVEMENT OF Na/Ca EXCHANGE MECHANISM IN CATECHOLAMINE SECRETION BY Na DEPRIVATION, SORIMACHI,M. AND NISHIMURA,S. Dept. of Physiol., Kagoshima Univ.Med.Sch., Kagoshima

The secretory mechanism of CA by Na removal was investigated using Cat adrenals perfused with Ca-deficient media. The response to Na removal was markedly diminished in the presence of Mg, possibly because of reduction in internal Na. In fact, the reduced response was restored after ouabain treatment. In the absence of Mg, however, ouabain did not potentiate the response. Significant effect of ouabain was observed when the stimulation media contained more than 50 mM NaCl. These results suggest that the rate of Ca entry is saturated at the level of internal Na, which reached in the absence of Mg. Previous exposure to Na free(sucrose) media containing EGTA markedly reduced the response to the first stimulation with Ca addition and abolished that to the second one. Similarly, the second addition of Ca during exposure to a medium containing 20 or 50 mM NaCl induced little if any secretion. Restoration of the response was observed either when the concentration of external Na was reduced at the time of Ca addition or when ouabain was added before the second stimulation. These results are in favor of the view that Ca entry due to Na/Ca exchange is determined by Na gradient across the plasma membrane of the chromaffin cells.

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EVIDENCES FOR INCREASE IN K PERMEABILITY OF PANCREATIC ACINAR CELL DURING THE ACTION OF SECRETAGOGUES. ITAGAKI, S., KATOH, K., SUZUKI, Y. and *NISHIYAMA, A. Dept. of Physiol., Yamagata Univ. Sch. of Med., Yamagata, 990-23. *Dept. of Physiol., Tohoku Univ. Sch. of Med., Sendai, 980.

In order to study change in K permeability during the action of secretagogues at the basolateral membrane of pancreatic acinar cell, measurements of ACh reversal potential (*E*ACh) in different K solution and net K flux were carried out in mouse and rat pancreas in vitro. In mouse pancreatic segments superfused with NaCl-free solution, *E*ACh was -19 mV at (K)o 4.7 mM. *E*ACh became more positive in K-rich solution. In the superfused rat pancreatic segments, both ACh and caerulein caused a transient K release. The maximum K release by ACh (5.5×10^{-6}M) was 0.77 μmol./g.w.w./min. and that by caerulein (3.7×10^{-9}M) was 0.72 μmol./g.w.w./min. Both ACh and caerulein also caused a transient increase in K concentration of the effluent from the perfused rat pancreas. The K release by ACh was blocked by atropine and that by caerulein was blocked by db-cGMP. From these results it is concluded that both ACh and caerulein increase the K permeability at the basolateral membrane of pancreatic acinar cell. Unexpectedly, secretin also induced a K release in superfused or perfused rat pancreas. However, neither atropine nor db-cGMP blocked the K release induced by secretin. Secretin may probably increase the K permeability of pancreatic ductule cell.

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STIMULATIVE EFFECT OF SERUM ON THE GROWTH OF HeLa S3 CELLS IN THE MEDIUM OF LOW K^+ AND HIGH Rb^+ CONCENTRATIONS

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Growth of HeLa cells cultured with a chemically defined medium was proved to be slightly stimulated in the presence of 5% dialyzed calf serum. The growth-promoting action of serum was conspicuous when cell growth was suppressed by the replacement of external K^+ with Rb^+. Intracellular K^+ was also substituted by Rb^+ independent of the presence of serum, and was lowered to a content below 0.25 nmol/mg protein in 24 h. Protein synthesis and degeneration was partially inhibited under low K^+ conditions. An addition of serum increased passive Na^+ and K^+ fluxes and stimulated DNA synthesis. But it did not have significant effects on RNA synthesis, protein synthesis, or adhesion of cells to the culture dish. The stimulative fraction of serum was heat-labile and adsorbed to Affi-Gel Blue (20 mM Na-phosphate buffer, pH7.1). From these results we conclude that serum-induced stimulation of cell growth is not a result of direct effect on synthesis of bulk protein, but a reflection of action of serum on other mechanisms.

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CHANGES OF GLUTAMINE METABOLISM IN RAT BRAIN SLICES BY HIGH POTASSIUM MEDIUM.

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The effects of high potassium medium (HKM) on the glutamine (Gln) metabolism, with special reference to glutamine synthetase (GS), were investigated using rat brain cortex slices. When the brain cortex slices were incubated in HKM, Gln content and ^{14}C incorporation into Gln from ^{14}C-glucose increased. The Gln content and ^{15}N incorporation into Gln from $^{15}NH_4Cl$ increased remarkably with the addition of 1mM $^{15}NH_4Cl$ to HKM. Acceralation of Gln metabolism in the slices was owing to an increase of GS activity, which was caused by HKM, but did not by the addition of veratoridine. The K^+-evoked activation of GS, which was not observed in rat liver slices, immature rat brain slices and cultured clonal glial cells (C-6), occurred in adult rat brain slices. The increase of GS activity in adult rat brain slices did not occur with the addition of dBtcAMP or dBtcGMP to the normal medium and was not inhibited by the addition of cycloheximide to HKM ro the omission of Ca^{2+} ions from HKM. The above results suggest that the increase in GS activity by HKM observed in mature brain tissue is not caused by the induction of GS protein synthesis, but may be due to conversion from the non-active form of GS to the active one.

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MEMBRANE POTENTIAL AND ELEMENTAL CONCENTRATION OF THE POSTERIOR SILK GLAND CELLS.

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The intracellular and intraluminal recording of membrane potentials have been made to determine the effects of secretagogues in vitro from the posterior silk gland cells of Bombyx mori. The lumen of the gland was perfused with grace salt solution and the microelectrodes inserted into the cell with reference to the bath solution and/or lumen. The electron probe X-ray microanalysis was also performed to determine the changes in elemental concentrations. Exposure to cytochalasin B (5 μg/ml), a secretion stimulating agent of fibroin, resulted in depolarization of the apical membrane potential and decreased in the apical membrane resistance. In response to juvenile hormone, Methoprene, the basal membrane potential showed depolarization and $[K]_{in}$ of the cytoplasm of the silk gland cells was decreased. The basal plasma membrane produced a typical small hyperpolarization and the apical plasma membrane potential caused a depolarization, with 5-HT (5×10^{-5} M) stimulation. The X-ray spectra obtained from the cytoplasm and the lumen in the stimulating state with 5-HT showed the increase of K concentration and the decrease of Cl concentration. From these observations, the potential responses may reflect changes in the internal K and/or Cl concentrations.

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INFLUENCES OF EXTRACELLULAR IONIC ENVIRONMENTS ON GRP-INDUCED SECRETORY RESPONSES IN THE ISOLATED PERFUSED RAT PANCREAS.

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Gastrin releasing peptide (GRP), 27 amino acid residues, was isolated from hog gastric non-antrol tissue. GRP induced strong enzyme secretion in the isolated perfused rat pancreas. The rate of pancreatic juice flow and protein output increased with increase in GRP concentration, and ED_{50} estimated was 10^{-10}M, which was equal to that of CCK-33 and two fold less than that of bombesin. The secretory responses induced by 3.6 X 10^{-11}M GRP was reduced by one half when the isolated pancreas was perfused with a Ca-deficient solution, and recoverd imidiately after reintroduction of the standard solution contained 2.5 mM Ca^{++}. Lowering extracellular K^+ from 5.6 mM to 1 mM resulted in complete inhibition of the response induced by 3.6 X 10^{-11} GRP and only partial recovery was observed after reintroduction of the standard solution. Lowering $[Na^+]_o$ from 157 mM to 26 mM, however, produced only a partial inhibition of GRP-induced responses. This different from that of CCK and ACh. These results showed that lowering extracellular Ca^{++}, Na^+ or K^+ decreased the secretory responses to 3.6 X 10^{-11} GRP. It is thus possible that GRP may play a physiological role in regulating exocrine pancreas in mammals.

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ELEMENTAL DISTRIBUTION OF AMPHIBIAN RENAL TUBULAR CELLS DETERMINED BY ELECTRON PROBE X-RAY MICROANALYSIS.

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We carried out the electron probe X-ray microanalysis of freshly frozen ultrathin sections of bullfrog kidney to determine the elemental distribution in the proximal tubular cells in correlation with the data obtained in ion-selective microelectrode studies. The scanning transmission electron micrographs of frozen ultrathin sections demonstrated that besides nuclei and mitochondria, many lysosome like granules were present in the cytoplasm of proximal tubular cells. The energy dispersive X-ray spectra from the frozen ultrathin sections of proximal tubular cells showed that high K and P peaks and small Na and Cl peaks were present in the cytoplasm, and relatively high Na, S, Cl, K and Ca peaks and a high P peak were present in the lysome. These quantitative X-ray microprobe data were well concomitant with the data that bipolar frequency for measurement of intracellular Na and Cl ionic activities were present in the proximal tubular cells, which were obtained by ion-selective microelectrode technique. Preliminary electron probe X-ray microanalysis of proximal tubular cells treated with ouabain, amiloride and monensin was also carried out.

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DIFFERENTIATION OF MOUSE BONE MARROW PREADIPOCYTES IN VITRO

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By means of in vitro colony assay and cytochemical staining, we investigated the capacity of mouse bone marrow fibroblastic cells to differentiate into adipocytes and their alkaline phosphatase (ALP) activity. Glucocorticoids enhanced the colony formation and the adipose conversion of the fibroblastic cells, but did not show detectable effect on the ALP activity. In the presence of 10^{-8} M dexamethasone, 55-65% of the colonies became adipocyte-positive. The fibroblastic cells became heterogenous in size and morphology after they grew in vitro. All the types of the cells had ALP activity, and more than 95% of the colonies contained ALP-positive cells. Most of immature adipocytes showed intense ALP activity, while fully matured adipocytes were negative. These results indicate that a vast majority of mouse bone marrow fibroblastic cells formed colonies under our culture conditions are preadipocytes. We suggest that the preadipocytes are originated from adventitial reticular cells in bone marrow stroma, since the reticular cells possess high ALP activity and have been suggested to differentiate into adipocytes.

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FUNCTIONAL CHANGES OF HUMAN AGED ERYTHROCYTES AND THE MEMBRANE STRUCTURE. MAEDA, N., KON, K. AND SHIGA, T. Dept. of Physiol., Sch. of Med., Ehime Univ., Ehime

For studying the causes of the increased suspension viscosity and the decreased oxygen transport function of human aged erythrocytes, the structural changes of membrane proteins and the anion transport function were examined.

(1) Human aged erythrocytes were separated more efficiently by the self-generated density gradient centrifugation of Percoll. The older cells consumed glucose and produced lactate in slower rates than the younger cells. (2) Significant differences in the membrane proteins were not observed between the younger and older cells, as far as examined by SDS-polyacrylamide gel electrophoresis. However, spectrin in the older cells was not completely extracted by 1 mM EDTA, pH 8, while those in the younger cells completely. (3) In relation to the anion transport function of band 3 protein, the self-exchange rate of sulfate between cytoplasm and extracellular fluid was much faster in the older cells than in the younger cells. Organic anion, pyridoxal 5'-phosphate, was incorporated more into the older cells than into the younger cells.

Conclusively, the aging of erythrocytes induces any alteration in the structure or organization of membrane proteins, and the anion transport function and the suspension viscosity of erythrocytes must be modified.

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POTENTIATING EFFECT OF METABOLIC INHIBITORS ON ADRENOMEDULLARY SECRETION INDUCED BY Ca REINTRODUCTION. YAMAGAMI,K. AND SORIMACHI,M., Dept. of Physiol., Kagoshima Univ. Sch. Med., Kagoshima

The adrenal glands of rabbits were perfused with Ca deficient, Mg(1 mM)-containing media and secretion of adrenaline was stimulated with the addition of 2 mM Ca. Treatment of the gland with a medium containing KCN(0.2 mM) markedly potentiated the response to Ca. Inhibition of glycolysis either by monoiodoacetate(IAA) or by NaF plus glucose deprivation was also effective in potentiating the response. Thus, the partial blockade of energy production enhanced the secretion by Ca introduction. On the other hand, the potentiated response in the presence of IAA was markedly diminished after exposure to KCN. This suggests that energy is required for secretion and that either glycolysis or oxidative phosphorylation can fulfill this requirement. These results imply the nivolvement of a component, which is sensitive to decreased energy production, in the potentiation of the response. Na-K pump could be a component, since it was reported that ouabain similarly potentiated the response to Ca. If this is feasible, then a decrease of energy production would increase the concentration of internal Na by reducing Na pumping activity so that Na-dependent Ca influx mechanism is activated. Indeed, the potentiating effect of IAA was found to depend on external Na.

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EFFECTS OF VOLATILE FATTY ACIDS ON THE SECRETORY RESPONSES OF EXOCRINE AND ENDOCRINE PANCREASES IN SHEEP. HARADA, E., NAKAGAWA, K. AND IKEI, N. DEPT. PHYSIOL. FAC. VET.MED. HOKKAIDO UNIV., SAPPORO 060, KATO, S. DEPT. PHYSIOL. FAC. VET. MED. COLL. DAIRYING, EBETSU, 069-01

The secretory responses of the endocrine and exocrine pancreases to volatile fatty acids have been studied in anesthetized sheep and in isolated lobules. Butyrate and propionate (625 μmoles/Kg) stimulated insulin and glucagon releases in the anesthetized sheep, whereas acetate has little effect. Pancreatic juice flow, amylase and protein outputs rapidly increased after intravenous administration of all 3 fatty acids (625 μmoles/Kg). These peak values were about 3 times higher than those obtained in response to pancreozymin (2U/Kg). A detectable response was obtained with 7.9 μmoles/Kg butyrate, 125 μmoles/Kg propionate and 312.5 μmoles/Kg acetate. The secretory response to butyrate (625 μmoles/Kg) was not affected by pretreatment with atropine and hexamethonium. In the isolated lobule preparation, amylase release increased in response to butyrate in a concentration dependent manner, reaching maximal levels at 1 mM and declining at 100 mM. It is concluded that volatile fatty acids act directly on pancreatic acinar cells to stimulate secretion. The physiological implications of these findings and considered.

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SERUM-FREE GROWTH OF CELL LINES FROM THE MOUSE MAMMARY GLAND AND A MAMMARY TUMOR.
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We have previously reported the establishment of three mammary-derived cell lines, mammary fibroblasts (MF), preadipocytes (MTF) and mammary tumor cells (MTD). To define the factors necessary for growth of these cells, serum-free culture conditions were developed. Basal culture medium consisted of a mixture of Dulbecco's modified Eagle's medium and Ham's F12 (1:1) containing bovine serum albumin and transferrin. Fibronectin-coated dishes were used to facilitate the attachment of these cells. Basal medium plus insulin greatly stimulated the growth of MTD cells. After growth under serum-free conditions, these cells still retained the ability to produce mammary tumor virus in response to dexamethasone. In contrast, MF cells required the presence of conditioned medium (CM) from either normal or neoplastic mammary epithelial cells or MTD cells in addition to the basal medium plus insulin. Likewise, the growth of MTF cells was stimulated by CM from mammary tumor cells and MTD cells. Thus, the growth of the cells of possible stromal origin was stimulated by CM from epithelial cells. Since MF cells produce a growth factor for mammary epithelial cells and MTF cells support the long-term growth of mammary epithelial cells, the present results suggest that epithelial cells and stromal cells of the mammary gland affect the growth mutually.

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INDUCTION OF CELL DIVISION IN PRIMARY CULTURES OF ADULT RAT HEPATOCYTES.
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Conditions required for the induction of mitosis in primary cultures of adult rat hepatocytes were examined. When hepatocytes were cultured in a serum-free, hormone -supplemented medium containing 1 mM of Ca^{2+}, DNA synthesis was induced in about 50% of hepatocytes. However, hepatocytes became aggregated and detached from culture flasks after 3 days. On the other hand, when Ca^{2+} concentration was reduced to below 0.4 mM, not only survival time of hepatocytes was prolonged but mitosis was observed in cells morphologically identified as hepatocytes. In the time lapse cinemicrography, three different types of division were observed. First, one mononucleated hepatocyte divided into two mononucleated daughter hepatocytes. Second, one binucleated hepatocyte divided into two mononucleated daughter hepatocytes. Third, soon after one mononucleated hepatocyte divided, two daughter hepatocytes fused, forming one binucleated hepatocyte. With insulin and glucagon, 50% increase in cell number was observed. In contrast, insulin in combination with EGF showed 100% increase. These results suggest that low concentration of Ca^{2+}, insulin and glucagon or EGF as well as FD, non-essential amino acids, a high concentration of pyruvate and Trasylol® as we have previously reported are essential for the induction of hepatocyte proliferation in primary cultures in a serum-free system.

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COMPARISON OF STRUCTURE AND PROPERTY L929 CELL AND ITS DERIVED CELL(Ln).
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A new cell line Ln cells were spontaneously transformed from L929 cells(Lo) during Long-term culture. Ln cells can grow in serum- and protein-free medium. In the present study, we focused on the differences in the structure of the cell surface. In order to observe the cell surface of each phase of cell cycle, we carried out synchronization of Lo and Ln cells by excess thymidine method. At the same time, the changes in cell number and cell volume in the time course after release from excess thymidine were measured by a Coulter counter. Cell number increased twice after 6-10 h. Cell volume slightly increased in early phase and commenced to decrease at 6 h later. The period of G_2 phase was culculated about 5 h. Two types of cells in both of Lo and Ln cells were observed by scanning electron microscopy, i.e., microvilli-rich and blebs-rich cells. However, the blebs-rich cells were found much around the mitotic phase of Ln cells. We did not see any significant differences in the surface ultrastructure in the other phases of cell cycle. The electrophoretic patterns of membrane proteins of Lo and Ln cells were different from each other in SDS-polyacrylamide-gel electrophoresis.

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MACROMOLECULAR SYNTHESIS DURING THE CELL CYCLE IN RELATION TO CELLULAR ATP LEVEL IN L CELLS.(3) YONEZU,T., HOSOKAWA,K., OSAKA,N., YAMAGUCHI,H., MIYAMOTO,H., AND OKA,Y. Dept. of Physiol., Sch. of Med., Tokushima Univ., Kuramoto-cho, Tokushima

The effect of cellular ATP level on macromolecular synthesis durig the cell cycle was studied in the population of L cells synchronized by selective detachment of mitotic cells. These cells were treated with inhibitors of cellular ATP generation such as 2-deoxy-D-glucose(2-DG), carbonylcyanide chlorophenylhydrazone(CCCP) and iodoacetate (IAA). When two of these inhibitors were combined, for example, 2-DG plus IAA, 2-DG plus CCCP or IAA plus CCCP; cellular ATP level decreased rapidly and fell to 32-60% of the control level in late S, and 5-30% in G_2. We found that the decrease in cellular ATP level was related to inhibition of ^{3}H-uridine incorporation in late S and G_2. However, the inhibition was not so severe only upon treatment with 2-DG plus IAA in spite of a strong decrease in the ATP level in G_2. Also, incorporation of ^{3}H-amino acid mixture was proved to be inhibited in late S and G_2 when the ATP level was decreased, but the incorporation was not significantly affected in early and middle S.

These results revealed that dependency of macromolecular synthesis on cellular ATP level was distinctive at different stages in the cell cycle.

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PAROTID ACINAR CELLS: IONIC DEPENDENCE OF ISOPRENALINE-EVOKED MEMBRANE POTENTIAL CHANGES. IWATSUKI, N. AND * NISHIYAMA, A.
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The effect of microionophoretic application of isoprenaline on membrane potential and resistance of mouse parotid acinar cells was investigated using two intracellular microelectrodes technique. Simple depolarizations were found at relatively high resting potentials, while biphasic potential changes to isoprenaline (hyperpolarization followed by depolarization) were observed at low resting potentials. Both depolarizing and hyperpolarizing responses to isoprenaline were accompanied by a reduction of membrane resistance. The isoprenaline equilibrium potential of the initial phase was about -53 mV and -24 mV of the delayed phase. The initial isoprenaline-evoked potential change was sensitive to alterations in extracellular Na, K and Cl concentrations, whereas the delayed depolarizing response to isoprenaline was markedly reduced by replacing extracellular Na by Tris or extracellular Cl by SO_4. These results indicate that isoprenaline opens up the independent conductance pathways permeable to Na and K.

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CATECHOLAMINE RELEASE AND CHANGES OF CYTOCHROMES IN THE ADRENAL GLANDS. SAITO, T., ASADA, N., SAITO, A. AND KANNO, T. Dept. of Physiol., Fac. Vet. Med., Hokkaido Univ., Kita-ku, Sapporo 060

Isolated adrenal glands of dogs were perfused via the adrenolumbar vein with an oxygenated Locke's solution in the reverse direction at 35 °C or room temperature. Oxidation-reduction of cytochromes, $a(a_3)$, b, $c+c_1$, was recorded with the scanning spectrophotometer. The release of catecholamine (CA; major component was adrenaline) from the glands was increased in response to acetylcholine (ACh, 50 μM), and the release coincided with cytochrome reduction at 35 °C. At room temperature, however, only slight reduction of cytochromes was observed although a similar ACh-induced secretory response was maintained. CA output was remained even in an anoxic environment. The present study suggests that reduction of cytochromes may coincide with ATP production, which, in turn, supplies a part of metabolic energy for the ACh-induced secretory response.

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CORRELATION OF FIELD POTENTIALS WITH TISSUE NADH FLUORESCENCE AND OXIDATIVE CYTOCHROMES DETERMINED BY FLUORO- AND SPECTROPHOTOMETRY. TAKANORI FUJII, Dept. of Physiol., Kyoto Pref. Univ. Med., Kyoto 602 Japan

Field potentials evoked by stimulation of the lateral olfactory tract in guinea pig olfactory cortical slices, were studied under normoxia and hypoxia in relation to tissue pyridine nucleotide (NADH) fluorescence and the oxidative reflection spectrum of cytochromes (cyt.) determined by fluoro- and spectrophotometry.
1) The tissue NADH fluorescence increased slowly after the onset of hypoxia and the discontinuation of perfusion, and attained a plateau (5.8% increase over the control) 6 min after the onset of hypoxia. It was restored to the control level 14 min after the resumption of the oxygen supply and perfusion. 2) The oxidative spectra of cyt. a, c and b began to decrease with the onset of hypoxia and reached almost steady states (ca. one third of the control) 3 min later. With the resumption of oxygen supply after 3 min hypoxia, the spectra recovered to 90% within about 1 min. 3) The rate of the decrease in amplitude of the potentials was slower than that of the oxidative spectra, and the amplitude decreased to almost half of the control level 3 min after the inception of hypoxia. It recovered to 90% of the control 5 to 7 min after the restoration of oxygen.
When the hypoxic period lasted less than 8 min, both cyt. spectra and potential amplitudes recovered to 90% of the control.

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TRANSMITTER RESPONSIVENESS IN NEURONAL CELL CLONES DEPENDENT ON CULTURE PERIODS
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A neuroblastoma × glioma hybrid cell line NG115-301 responded with K^+-dependent membrane hyperpolarizations to both serotonin and dopamine applied iontophoretically. The responses were concluded to be mediated by discrete receptors from the following findings. 1) All cells responded to serotonin, while a considerable proportion did not to dopamine. 2) No cross-desensitization was observed. 3) α- And β-adrenergic antagonists preferentially suppressed the dopamine response.
The dopamine response of NG115-301 cells appeared noticeably only after >1 week cultivation with a medium containing 1mM dibutyryl cyclic AMP and increased thereafter, while the serotonin response was present before and through the exposure to Bt_2cAMP. The dopamine response decreased when Bt_2cAMP was withdrawn from the culture medium.
A similar Bt_2cAMP/culture period-dependent expression of specific receptor type was found in acetylcholine-elicited responses of a neuroblastoma cell line N1E-115.

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NATURE OF MUSCARINIC RESPONSES IN THE CULTURED SYMPATHETIC GANGLION CELLS OF THE RABBIT. HARUO KOBAYASHI & SUMIKO MOCHIDA. Department of Physiology, Tokyo Medical College, Shinjuku-ku, Tokyo 160.

Superior cervical ganglia of adult male rabbits were dissociated by collagenase treatment. Nerve cells, isolated and cultured for 3-8 days, were penetrated by glass microelectrodes and the membrane changes associated with the responses to extrinsically applied cholinergic substances were analyzed. Cells responded to acetylcholine with depolarization consisting of two components: earlier one with increase in membrane conductance and later occurring, longer persisting, one with small decrease in conductance. Methacholine and muscarine elicited only the latter type response. By the analysis of voltage-current relationship, the muscarinic decrease in conductance was found to occur only in the range of membrane potentials (Vm) more positive than about -65 mV, probably due to the suppression of 'M-current'. Additional, voltage-independent, type of muscarinic depolarization was also detected at all levels of Vm tested, with no apparent change in conductance. A depolarization similar to this second type was often produced by dibutyryl cyclic GMP at doses lower than 100 μM. Further, the 'hump' on the falling phase of action potential, with the after-spike-hyperpolarization as well, was depressed by muscarinic agents. The cultured sympathetic neurons thus retain the basic feature of muscarinic responsiveness found in neurons in the intact ganglia.

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EFFECT OF PARASYMPATHECTOMY ON ELECTRICAL CELL COMMUNICATION BETWEEN ACINAR CELLS IN RAT SUBMANDIBULAR GLAND. KANNO, Y., SASAKI, Y., ENOMOTO, T. AND SHIBA, Y. Dept. of Physiol., Hiroshima Univ. Sch. of Dent., Hiroshima

Parasympathectomy is well known to cause the degeneration of rat submandibular acinar cells. Submandibular acinar cells have well-developed cell coupling. We have investigated the effect of parasympathectomy on the maintenance of cell coupling between the acinar cells. Parasympathectomy was carried out by cutting the right chorda tympani nerve and lingual nerve in male Wistar rats. The wet weight of the denervated gland was decreased gradually two or three days later. Cell coupling was rapidly decreased after denervation; one day after denervation, coupling ratio between the most acinar cells declined from 0.55 to 0.31. Three or four weeks later, most acinar cells degenerated, but some acinar cells remained intact morphologically and kept high coupling ratio.
These results might suggest that the parasympathetic nerve innervation was required for the maintenance of cell coupling between the most acinar cells of rat submandibular gland.

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IONIC DEPENDENCE OF REVERSAL POTENTIAL OF ACH-EVOKED DEPOLARIZATION IN HAMSTER PANCREATIC ACINI. SUZUKI, Y. AND NISHIYAMA, A. Dept. of Physiol., Yamagata Univ. Sch. of Med., Yamagata 990-23. *Dept. of Physiol., Tohoku Univ. Sch. of Med., Sendai 980.

ACh evoked membrane depolarization in hamster pancreatic acinar cells and its reversal potential (EACh) was -11 mV in control solution. EACh became more negative in Na-deficient solution, while it became more positive in K-rich or Cl deficient solution. Applying the present results of EACh and the previously reported values of intracellular ionic activity of mouse pancreatic acini to the Goldman equation (Petersen, Maruyama, Graf, Laugier, Nishiyama and Pearson, 1981), the relative ionic permeability at the EACh (PK/PNa/PCl = 1/0.91/0.43) was calculated. In contrast to the previous study on mouse pancrease (PK/PNa/PCl = 1/1.2/5), PCl/PK or PNa was characteristically low in hamster. Intracellular injection of Ca evoked membrane depolarization which was very similar to the action of ACh. In Ca-dificient solution EACh became more positive while Ca-rich solution it became more negative. It is suggested that the increase in intracellular Ca concentration induced by ACh-receptor interaction increases PNa and PK while the extracellular Ca concentration controls PNa/PK during the action of ACh.

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RELATION BETWEEN REDUCTION OF CYTOCHROMES AND SECRETORY RESPONSE INDUCED BY LOW OR HIGH DOSE OF ACETYLCHOLINE IN THE ISOLATED PERFUSED RAT PANCREAS.
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Redox states of cytochromes, a(a_3), b, and c + c_1, was continuously recorded with a scanning organ spectrophotometer (Tateisi Institute of Life Sciences). Continuous stimulation with acetylcholine (ACh) induced immediate reduction of the cytochromes followed by increase in secretory response. A linear relation was obtained between the degree of cytochrome reduction and the amount of pancreatic protein output induced by ACh at concentration of 10^{-7}M or lower. On the contrary, a reverse relation was obtained between the degree of cytochrome reduction and the amount of pancreatic protein output induced by 10^{-6}M ACh. These results show that the cellular mechanism induced by ACh at low (physiological) concentration may differ from that induced by ACh at high (pharmacological or pathological) concentration. The former mechanism is associated with increase in ATP production, whereas the latter may involve excess $[Ca^{2+}]_i$ and hypoxia.

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PANCREATIC EXOCRINE SECRETION AND REDOX STATE OF THE CYTOCHROMES IN THE ISOLATED PERFUSED RAT PANCREAS AND THE PANCREATIC LOBULES OF RAT.

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Secretory responses of the exocrine pancreas and redox state of the cytochromes, a(a_3), b, and c+c_1, were compared between in the isolated perfused rat pancreas and in the pancreatic lobules of rat. The concentration of carbachol (CCh) required for half-maximal amylase secretion (ED_{50}) in the isolated perfused pancreas were about seven times lowere than that in the pancreatic lobules. In the isolated perfused pancreas, marked reduction of the cytochromes was recorded during continuous stimulation with 3×10^{-7} M CCh, whereas reduction of the cytochromes induced by 3×10^{-6} M CCh was scarcely detected in the pancreatic lobules. These result show that the isolated perfused pancreas is most sensitive for the CCh stimulation, that the intracellular respiratory system is well reserved. The pancreatic lobules are less sensitive to CCh, and the intracellular respiratory system may be shifted to hypoxic state event at resting state.

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RATE-LIMITING STEP OF CYTOCHROME P-450-DEPENDENT DUG OXIDATION IN PERFUSED LIVERS

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Infusion of drugs (hexobarbital and aminopyrine) into isolated rat livers resulted in spectral changes of cytochrome P-450(P-450), which could be readily detected by reflectance spectrophotometry. The difference spectra thus induced in livers from fed, phenobarbital(PB)-treated rats could be ascribed to a mixture of the ferric P-450-substrate complex and reduced (oxygenated) P-450. In livers, the steady-state level of reduced (oxygenated) P-450 was dependent on the concentration and the kind of drug substrate added, and this level correlated well with mixed-function oxidase activity (estimated from the substrate-induced increase in oxygen uptake). In the absence of exogenous substrates, the steady-state level of reduced (oxygenated) P-450 was very low. It was suggested from these observations that in livers from fed,PB-treated rats the mixed-function oxidase activity is controlled by the substrate binding to P-450.
(This study was collaborated with Dr.T.Iyanagi, The Univ. of Tsukuba)

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METABOLIC STEADY STATES OF MITOCHONDRIA IN SITU

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We have already reported that glucagon or norepinephrine not only stimulates oxygen uptake but also produces cytochrome reduction in perfused rat liver (1979,1981). To analyze the elevated-respiration coupled cytochrome reduction, we estimated metabolic steady states of mitochondria in resting perfused rat liver utilizing Amytal, FCCP, Antimycin A, and 95%N_2+5%CO_2 perfusion. The percentage reduction levels of the mitochondrial electron carriers in the resting state were 8% for cytochrome a(a_3), 11% for cytochrome c+c_1, 18% for cytochrome b, 26% for flavoprotein, and 55% for NAD, indicating that they range between State 3 and State 4 except for cytochrome a(a_3).

It was concluded that the cytochrome reduction in connection with stimulated respiration caused by the glucose releasing hormones in liver cannot be interpreted in terms of metabolic states obtained in isolated mitochondria by Chance and Williams (1955).

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DIELECTRIC ANALYSIS OF ISOLATED RAT LIVER MITOCHONDRIA

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Dielectric measurements of mitochondrial suspensions were carried out over a frequency range of 10kHz to 500MHz by the use of a computer-controlled Impedance Analyzer System. The dielectric behavior of intact mitochondria suspended in 130mM-KCl was successfully approximated by a superposition of two Cole-Cole type dispersions, of which the characteristic frequencies (and β-factors) were 2MHz(β = 0.9) and 20MHz(β = 0.8), respectively. In contrast, so-called swollen mitochondria that were deprived of their outer membranes by osmotic shock showed a familiar Cole-Cole type dispersion having one characteristic frequency of 1-2MHz(β = 0.8) when suspended in the same medium. Such an observation suggests that the presence and intactness of the outer membrane in addition to the inner membranes is responsible for the dielectric behavior of the intact mitochondria. On the basis of a "double-shell" model(Irimajiri et al.,1978), therefore, we analyzed the observed dispersion curves to evaluate electrical parameters for submitochondrial phases. The results obtained are as follows: (1) Membrane capacities were 1.4-1.9μF/cm^2 for the outer membrane and 0.4-0.9μF/cm^2 for the inner one. (2) Conductivity for the mitochondrial outer space, a space lying between the outer and the inner membranes, was close to that of the suspending medium. (3) Conductivity for the matrix was lower than for the external medium with a ratio of 0.2-0.3 irrespective of the medium KCl concentrations examined.

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THE PURIFICATION AND CHARACTERIZATION OF CALMODULIN FROM RAT LIVER MITOCHONDRIA

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We communicated the purification and characterization of calmodulin, a acidic, heat-stable and multifunctional Ca^{2+}-binding protein, from rat liver mitochondria by 2-chloro-10(3-aminopropyl)-phenothiazine-Sepharose 4B affinity chromatography. The results were as follows:

1) Rat liver mitochondria contained about 7% of total cellular calmodulin. About 80% of the mitochondrial calmodulin was proved to be cytosol origin. It was easily detached by washing with buffer containing EGTA. The other 20% was intramitochondrial one; half of it was in the matrix space, and half in the membrane.
2) It co-migrated with bovine brain calmodulin in non-denaturing and SDS-containing polyacrylamide gel electrophoresis and isoelectric focusing. Its apparent molecular weight was 18,000 dalton and the isoelectric point was 3.9.
3) It activated Ca^{2+}-calmodulin-dependent phosphodiesterase of bovine brain in the presence of Ca^{2+}, and did not in the absence of Ca^{2+}. The activation was inhibited by some neuroleptic drugs. (Biochem. Biophys. Res. Commun. in print)

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EFFECT OF CHOLECYSTOKININ OCTAPEPTIDE ON THE REDOX STATES OF CYTOCHROMES OF PERFUSED BULLFROG BRAIN.

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Redox state of cytochromes, a(a_3), b, and c+c_1, in the perfused bullfrog brain were recorded with a scanning organ spectrophotometer (Tateisi Institute of Life Sciences, Kyoto, Japan). The tip of the light guide of the spectrophotometer was placed on the surface of cerebral hemispheres. Continuous perfusion with a solution containing CCK-8 (0.01-30 nM) induced significant reduction of cytochromes without any additional procedure in some preparations. In other many preparation, the CCK-8 induced cytochrome reduction was observed after the procedure of reversibl osmotic opening of the blood-brain barrier by preceding perfusion with 300 mM arabinose. In any cases in which the cytochrome reduction was observed, the peak level reached to about 25 % of the maximum reduction level. The CCK-8 induced cytochrome reduction does not appear to be hypoxia, for oxygen consumption and ATP content of the perfused brain were both increased during perfusion with the solution containing CCK-8.

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Effects of extracellular Ca^{2+} on the redox state of mitochondrial respiratory chain in perfused liver.

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Though the role of Ca^{2+} as a regulatory factor in the liver has been investigated in great detail, the relation of Ca^{2+} and mitochondrial redox state in vivo is not completely understood.

Ca^{2+} added to the liver perfused with Ca^{2+} -free Krebs- Henseleit solution caused the remarkable but transient reduction of cytochromes and NAD, and decreased the respiration following short live activation. Digitonin with normal Krebs-Henseleit (2.5mM Ca^{2+}) caused the same changes described above on mitochondrial respiratory chain, whereas the addition of digitonin with Ca^{2+} -free perfusate caused no change. Those Ca^{2+} effects were inhibited by the addition of an inhibitor of mitochondrial Ca^{2+} transport, ruthenium red. These data suggest the direct effect of Ca^{2+} to the mitochondrial redox state rather than through the cytosolic metabolic changes, and different control mechanism by Ca^{2+} on the mitochondrial repiratory chain in vivo is suggested.

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HEPATOCYTE ATTACHMENT FACTOR IN FIBRINOGEN DIGESTS.

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We have reported in this meeting that DNA synthesis and mitosis were induced in primary cultures of adult rat hepatocytes under serum-free conditions. To cultivate hepatocytes in a serum-free medium, attachment factors need to be supplemented during initial plating. In our plating medium, fibrinogen (Cohn Fr. I) digests degraded by plasmin have been supplemented. To isolate attachment factors, fibrinogen digests were applied on a Sepharose CL-6B column. Attachment activity was found in the fractions between V_o and the main protein peak which contained fragments D and E. The active fraction, whether reduced or unreduced, gave double bands corresponding to molecular weight 200,000 daltons on an SDS-polyacrylamide gel electrophoresis. When fibronectin was removed from Cohn Fr. I by affinity chromatography on a gelatin-Sepharose 4B column, attachment activity in fibrinogen digests was lost. These results suggest that attachment factors in fibrinogen digests are 200,000-dalton fragments of fibronectin. Hepatocytes anchoraged by fibronectin synthesized DNA and underwent mitosis to the same degree as those anchoraged by fibrinogen digests.

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LIVER INJURY DIRECTLY INDUCED BY MAST CELL DISRUPTION

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Mast cells are known to be present within the connective tissue of the liver, and it is suggested that basoactive amines from these cells might be involved in the local regulation of blood flow through the sinusoids. Though increase in IgE and blood histamine level or decrease in the number of liver mast cells are reported in acute hepatitis, the involvement of mast cells in the pathogenesis of this disease have not yet been elucidated. An experimental model for the direct evaluation of the relationship of mast cells to the injury or increase in the membrane permeability of liver cells was devised. An antiserum highly specific to rat mast cells was prepared from rabbits immunized with purified mast cells (over 97 %) with a modified coil planet centrifuge. This antiserum could liberate histamine completely at a 1:30 dilution and the cytotoxicity was not decreased after absorption with non-adherent cells of various lymphoid or hematopoietic organs origin. Moreover, only mast cells were stained with FITC in indirect immunofluorescence technique, performed with frozen sections of rat liver tissues. The IgG fraction of this antiserum was administrated to a perfused rat liver. The activity of hepatic enzymes in the perfusate remarkably increased just at the time when histamine was released from mast cells. This finding suggests the liver injury secondary caused by mast cell disruption.

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PHASE ANGLE OF THE LIVING BODY APPLIED BY FINITE ALTERNATING POTENTIAL,
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Applying the finite alternating potential $E_m \sin\omega t$ to the series curcuit of the resistance r and capacitance c, the formulae V and τ are following,

$$V = \frac{RE_m}{R+r}\cos\phi \ , \qquad \tan\omega\tau = \frac{1}{\omega(R+r)c}$$

V is the flowing potential, τ is the phase angle deviation time at the rest condition, and R is the input resistance of the potential meter.

And in this case $\phi > 0$.

When the curcuit is composed by r and c parallel,

$$V = \frac{RE_m\sqrt{1+\omega r^2c^2}}{\sqrt{(R+r)^2+(\omega Rrc)^2}} \ , \qquad \tan\phi = \frac{-\omega r^2 c}{R+r+\omega^2 Rr^2c^2}$$

By the following fomula led from the upper formulae, the values r, c are gotten.

$$\omega k_2(\omega Rk_2\tan\phi - 1)r^2 + (2\omega^2 Rk_1k_2\tan\phi - \omega k_1)r + R(\omega^2 k_1^2 + 1)\tan\phi = 0$$

$$k_1 = \frac{V^2+E_m^2}{V^2-E_m^2}\cdot\frac{\tan\phi}{\omega} \qquad k_2 = \frac{V^2}{V^2-E_m^2}\cdot\frac{\tan\phi}{\omega R} \qquad c = \frac{k_1}{r} + k_2$$

In this case $\phi < 0$.

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INTRACELLULAR TRANSPORT AND RECYCLING OF ASIALOGLYCOPROTEIN RECEPTOR IN RAT HEPATOCYTES.
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The intracellular transport of a rat hepatic binding protein specific for asialoglycoproteins was studied in vivo. After pulse-labeling for 10 min with ^{3}H-leucine and chasing, a maximal labeling of the binding protein was in the rough microsomes at 15 min, in the smooth microsomes and the heavy Golgi subfraction (GF_3) at 25 min and in the intermediate plus light Golgi subfractions (GF_{1+2}) at 30 min. By 60 min, the labeling in the GF_{1+2} had decreased and leveled off. In the plasma membrane fraction, the radioactive binding protein first appeared at 20 min, rapidly increased and reached a constant level at 40-60 min. These results indicate that the binding protein is transported rapidly from the endoplasmic reticulum to the plasma membrane via the Golgi apparatus and then recycled back to the Golgi apparatus and equilibrated between the two structures.

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DIRECT FERRITIN IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF AN ASIALOGLYCOPROTEIN RECEPTOR ON RAT HEPATOCYTE CELL SURFACE.
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Direct ferritin immunoelectron microscopy was applied to visualize the distribution of the asialoglycoprotein receptor on hepatocyte cell surface. The specific antibody conjugates were incubated with the hepatocytes, which were isolated from rat liver homogenate after fixation by glutaraldehyde perfusion. These cells preserved well the original polygonal shape and polarity, and it was easy to identify the sinusoidal, lateral and bile canalicular faces. The surface density of the ferritin particles bound to the sinusoidal face was about 4 times higher than that bound to the lateral face, while the laleling of the bile canalicular face was almost at the control level. Using the surface area of hepatocyte measured by morphometrical analyses, it was estimated that ∿90% of bound ferritin particles were at the sinusoidal face, less that 10% at lateral face and ∿1% at the bile canalicular face. The average number of the receptor on all the cell surface was estimated as 1.1×10^6/cell.

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THE EFFECT OF PROTEIN SYNTHESIS INHIBITOR ON SUPPRESSION OF FL CELL COUPLING BY TUMOR PROMOTER.
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Tumor promoter, 12-O-tetradecanoyl phorbol-13-acetate (TPA) reversibly suppresses the formation of electrical cell coupling between FL cells. TPA-mediated suppression of cell coupling might result from TPA-induced modification of cell surface (insertion of new protein or destruction of gap junction components). We have investigated above possibility with cycloheximide (CY) and actinomycin D (Act.D). The following results have been obtained. 1) electrical cell coupling appeared in most paired cells 3-4 hr after culture in 10%cs MEM medium with and without CY (1.0 μg/ml) or Act.D (1.0 μg/ml). TPA (100 ng/ml) suppressed the formation of cell coupling in the presence of CY or Act.D. 2) When TPA was removed after 5 or 24 hr treatment, cell coupling recovered to control level 4-5 hr later even in the presence of CY or Act.D. These results suggest that TPA-mediated uncoupling does not result from new protein synthesis by TPA, nor the destruction of the apparatus for cell coupling formation, but the suppression of the function of gap junction.

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ROLES OF ENERGY METABOLISM AND MEMBRANE IN THE CIRCADIAN CLOCK SYSTEM
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Two clonings of ciliate protozoan, Paramecium bursaria were used to study roles of energy metabolism in the circadian clock system; one cloning inhabited by symbiotic green algae, Chlorella and the other Chlorella-free. Circadian changes in their activities were compared with each other. The activity was recorded with the number of cells per an hour which traversed a mark swimming randomly in a culture dish (traverse frequency= TF). The TF of both clonings were high during the day and low at night. Free-running rhythm of TF in the Chlorella-containing specimens lasted longer and clearer than that in the Chlorella-free specimens when they were placed in continuous darkness (DD). The free-running period of TF of Chlorella-containing specimens was influenced by the light intensity of previous light-dark (LD) cycles before they were placed in DD. The period of specimens previously exposed to LD cycles, the intensity of which was higher than about 1500 lux, was cretically prolonged. The intensity of 1500 lux was cretical for Chlorella to survive in its host. The free-running period of Chlorella-free specimens was little affected by the light intensity of their previous LD cycles before they were exposed to DD. Roles of energy metabolism and membrane in the circadian clock system were diacussed.

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MEASUREMENT OF WATER CONTENT IN LYMPHOCYTES BY GAS-LIQUID CHROMATOGRAPHY: EFFECT OF PHA, CON A AND OLEIC ACID ON THE WATER CONTENT
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To study intra-cellular water change accompanied with biological behaviour of lymphocytes, a new, rapid and precise method for measuring water content of lymphocytes by gas-liquid chromatography correcting the trapped water with sucrose or ^{3}H-sucrose was developed. Effect of PHA(20 ug/ml), Con A(8 ug/ml) and oleic acid(5 ug/ml) on water content of lymphocytes before and after incubation at 37 C for 1 h were investigated by this method. Then, potassium content of the cell samples was determined by atomic absorption spectrophotometry. Percent water contents of thymus and lymph node lymphocytes were 75.90 ± 1.33 and 77.22 ± 0.82 respectively. PHA reduced markedly the both value of them, but Con A only the value of thymus cells. The dehydrative activity of PHA and Con A to the lymphocytes was inhibited with simultanous addition of oleic acid. The phenomena were not seen on incubated thymus cells and opposite tendency was observed. On the other hand, changes in intra-cellular potassium content were associated with changes in water content of the lymphocytes without incubation.

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SEPARATION OF THYMUS HUMORAL FACTOR (THF) SENSITIVE LYMPHOID CELLS FROM HUMAN TONSIL

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Three subsets of small lymphocytes [LSL (lighter), ISL (intermediate) and HSL (heavier)] were separated from the excised human tonsil by combining multi-layer centrifugation with adhesion column method. These subsets were incubated with calf THF. Then, their THF sensitivity, which reflected an induction of precursor T cells (PTC) to T cells (thymus-dependent lymphocytes) was checked by an acquisition of Con A-responding capacity and capability to form rosette with sheep red blood cells. An acquirement of the lectin-responding capacity was examined by an enhancement of DNA synthetic activity of Con A-stimulated subsets with the factor. The lymphocytes, to which more than three sheep erythrocytes bound, were counted as the positive rosette-forming cells. In the systems the optimum concentration and incubtaion time of THF were 5 ug/ml and 24 hours, respectively. These experiments indicated that THF sensitive cells (PTC) existed undoubtedly in human tonsil and that the cells were concentrated into the LSL subset. It was assumed from the results that the tonsil was not only peripheral lymphoid tissue, but also central lymphoid one.

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ADP-INDUCED RESPIRATORY INHIBITION OF MITOCHONDRIA.

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Addition of ADP in the presence of mersalyl (6-12 nmoles/mg protein of mitochondria) caused an immediate deceleration of respiration instead of acceleration, and the ADP-induced inhibited state of respiration was termed as "state 4'". The inhibited state looks like to a condition termed "state 6" observed on addition of Ca^{++} or Mn^{+} to mitochondria buffered with Tris-Cl in the absence of phosphate (Chance, 1963), but the mechanism of these two states seems to differ. This work is the first step toward the elucidation of the mechanism of st. 4'. Mersalyl didn't affect the respiration of st. 4 antecedent to ADP addition. Mersalyl abolished not only the acceleration of respiration on addition of ADP but also decelerated the respiration. The inhibited state was disinhibited with thiol reagents (dithiothreitol, cysteine, Penicillamine and Thiola). DNP also released the inhibited respiration. Preincubation of mitochondria with mersalyl in the presence of phosphate or substrate fortified or lessened the mersalyl action. Preincubation with minute anount of ADP (14 nmoles/mg protein) augumented the inhibition (ADP-fortified mersalyl effect). As a mechanism of the respiratory inhibition, a constriction of substrate availability by the configuration change (shrinkage) of mitichondria by ADP and the concomitant unmasking or sensitization of substrate anion translocator to the action of mersalyl was suspected.

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REGULATION OF ESTEROPROTEASE SYNTHESIS IN THE MOUSE SUBMANDIBULAR GLAND.

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The submandibular glands of both male and female mice have androgen receptors and exhibit distinct morphological and biochemical sex differences. Whereas most esteroproteases in these glands (male or female) are enhanced by androgen, we found that the synthesis of one of them (pI=4.6) was inhibited by androgen in males only; castration of male mice caused de novo synthesis of this enzyme. By an immunocytochemical technique, this esteroprotease was shown to be localized in the androgen-dependent, granular convoluted tubules. The purified enzyme had a molecular weight of 27,600, consisting of two subunits of 10,000 and 18,000 daltons. Being similar to rat tonin, the enzyme had an optimum pH of 9.0 and was strongly inhibited by Hg^{++}, Cu^{++}, and leupeptin. However, the enzyme is somewhat different from tonin, since this new esteroprotease cleaves Arg^{2}-Val^{3}, Pro^{7}-Phe^{8}, and His^{9}-Leu^{10} bonds but not the Phe^{8}-His^{9} bond of angiotensin I.

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ON THE EXISTENCE OF CELL GROWTH REGULATORY FACTORS AND TRYPSIN INHIBITOR-LIKE PROTEINS IN A SUPERNATANT OF HATOMUGI

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Inhibitory activity for L_{929} cell proliferations was found in a supernatant(Sup) of Hatomugi (Coix Ma-yuen Roman), and it was sensitive to heat treatment (100°C, 1 min). The Sup was partially purified by Sephadex G-100 chromato.; the 3rd and the 4th of fractions eluted showed the inhibitory activity, suggesting the Sup contained multiple inhibitory factors. The hydrodygation of casein by trypsin was modulated by Bran (B) and Embryo (E) of Hatomugi.
The results showed that (B) system had stronger inhibitory activity than (E) one. Trypsin inhibitor-like activity of the main peak of (B) fractionated by Sephadex G-100 chromoto. was shown at the same fraction of the Sup in the same chromatography.

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EFFECT OF INTRACELLULAR MONOVALENT CATIONS UPON ACTIVE Rb^+ TRANSPORT IN HeLa CELLS.

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A kinetic study of membrane cation transport was made by use of rewarmed cells. Intracellular Na^+ contents were previously changed by chilling the cells with cold media of different external Na^+/K^+ ratios. However, sum of internal Na^+ and K^+ contents proved to be kept at an almost constant value (about 1.4 μmoles/mg protein), provided that sum of external Na^+ and K^+ concentrations were held at 140 mM. Double reciprocal plot of the square root of active Rb^+ uptake against external Rb^+ concentration showed a linear relation between them. When external Na^+ concentration increased the apparent half saturation concentration of external Rb^+ was linearly raised, but the apparent maximal Rb^+ uptake was unchanged. This suggests that Na^+ competes Rb^+ at the Rb^+ binding sites of the cation pump. Also, the linear curves indicated by the similar double reciprocal plot at varied internal Na^+ contents were shown to be parallel. We obtained an equation in relation to the active Rb^+ uptake, which fairly described these results.

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ASSAYS OF PHOSPHORUS COMPOUNDS IN PERFUSED SALIVARY GLAND BY 31 P-NMR.

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^{31}P-NMR (nuclear magnetic resonance) was used to continuous measurement of phosphorus in perfused dog submandibular gland. The gland was perfused at constant flow rate (2 ml.min^{-1}.g^{-1} tissue weight) by Krebs-Henseleit Ringer solution saturated by 95%O_2 & 5% CO_2, at 25°C and set in a large bore NMR tube (30 mm in diameter). 31 P-NMR spectra was sequentially collected with 256 or 500 pulses and required 6 or 11 minutes. At resting, ATP and creatine phosphate levels were 0.43 ± 0.04 mM (mean ± S.E. in 9 glands) and 0.62 ± 0.04 mM. By the clamp of perfusate line, tissue content of ATP and creatine phosphate decreased, that of ADP increased and tissue pH decreased. The reperfusion recovers tissue content of the phosphorus compounds and tissue pH. The Acetylcholine administration induced a salivary secretion and decreased in ATP and creatine phosphate level, increased in ADP and tissue pH decreased. Based on above data, energy metabolism of salivary gland was discussed.

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EXAMINATION OF PROCEDURES FOR ISOLATION OF CARRIER BRUSH BORDER MEMBRANE

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In order to obtain Ca^{++}-free and detergent-free protein extract from the brush border membrane (BBM), ficoll-density gradient centrifugation was carried out for the homogenate of rat renal cortex or rabbit ileum to isolate the BBM and the basolateral membranes (BLM). The BBM fraction was found to be trapped mainly on 11 - 13 % ficoll and the BLM on 13 - 15 % ficoll. The purity was assessed by measuring Na -dependent D-glucose uptake and alkaline phosphatase activity for the BBM and Na^+-K^+ ATPase activity for the BLM. In order to determine a small amount of detergent contaminated protein extracts, a very sensitive method for measuring the contaminated detergent was devised. The solutions containing detergent were sonicated for 10 sec and the heights of froth produced and fluid column were measured. At least, 10^{-10} % order of Triton X-100 could be detected by this new device. After testing by this method, batch-wise treatment with detergent-adsorbent was found to be more efficient in removing detergent from protein extracts than conventional column method. Schlieren pattern of BBM fraction was changed after treatment with detergent, addition of detergent (Triton X-100) caused separation of two groups of particles having different values of density, indicating the isolation of smaller particles, probably vesicles. The smaller particles were determined to be about 1 S.

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CONSTRUCTIOU OF A TWO-DIMENSIONAL LIPID BILAYER MEMBRANE FROM PREFORMED LIPID MONOLAYERS

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In order for the study of Na^+-linked cotransport systems reconstituted in a planar artificial membrane, we examined the Montal's method for construction of a planar membrane from lipid monolayers spreading on water surface. The following apparatus was used ; two troughs made of Tefoln were separated by thin Teflon film which was ca. 10 um in thickness and had a small hole, ca. 140 um in diameter, at the center region. The volume of each trough was 1.1 ml. The troughs and the film were washed repeatedly in chloroform-methanol and in hexane to clean before use and the film was coated with silicone grease before clamping between the troughs in order to prevent electric leakage. Phosphatidylcholine (PC) was first spread on the surface of water placed in both troughs below the level of the hole. Optimal concentration of PC was evaluated by change in surface tension and 0.3 - 0.6 mg PC/ml hexane were found to be approproate. After formation of the monolayers, the solvent, hexane, was allowed to evaporate for 5 min (25 ^{o}C). Thereafter, the monolayers of both sides were brought into contact by raising the water level. The bilayer membrane thus formed at the hole had a capacity of 1.1 uF/cm^2, an electric conductance of 10^{-6} - 10^{-8} S/cm^2. Form the estimated value of membrane capacity, the thickness was determined to be 20 Å.

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MODELING OF MEMBRANE TRANSPORT SYSTEM BY NETWOTK THERMODYNAMICS

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Using the bond graph notation of network thermodynamics, we try to model the dynamic system of membrane transport which allows both volume flow and ionic flows, and in which various power dissipative processes and stored processes may occure. The membrane subsystem is represented as a resistive multiport network which represents both power coupling and dissipation. The solution compartment subsystem is represented as a capacitive multiport network which represents both power coupling and stored processes. These subsystems are combined as whole membrane transport system, in which the power balance can be maintained in both dissipative and stored processes. The Donnan condition, the active transport and the epithelial transport systems are also discussed.

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INTRACELLULAR MEASUREMENT OF Na ION ACTIVITY USING NEUTRAL CARRIER Na ION-SELECTIVE MICROELECTRODES.

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To measure intracellular Na ion activities (a^i_{Na}) and membrane potentials (PD) simultaneously, double-barreled Na ion-selective microelectrodes were constructed with a neutral carrier (ETH 227). At Na^+ activities $\geq$ 10 mM, changes in electromotive force (EMF) of the electrode were linear with a slope constant of about -56 mV. Detection limits of these electrodes were 3 $\sim$ 5 mM, and selectivities of Na^+ over K^+ were 40 $\sim$ 50. The electric resistance of ionic barrel was an order of $10^{11}\Omega$ and the time required for 95% of full response in EMF was 5 $\sim$ 10 sec.

Micropuncture studies carried out on the renal proximal tubules and the sartorius muscles of bullfrogs revealed the following results: In the proximal tubule, the average a^i_{Na} was 14.4 ± 7.0 (SD) mM and PD -54.4 ± 10.2 mV (n=22), whereas in the sartorius muscle a^i_{Na} was 12.6 ± 4.1 mM and PD -72.1 ± 5.7 mV (n=7). It was experimentally demonstrated that an addition of Ouabain (10^{-4} M) into the perfusates of muscle or proximal tubule produced a progressive depolarization of membrane PD with increasing a^i_{Na}. Amiloride (10^{-4} M), added to the luminal side of the proximal tubule, caused a hyperpolarization and decrease of a^i_{Na}. Thus, this electrode can be a powerful tool for biological researches of intracellular Na.

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THE EFFECT OF INSULIN ON THE Na-K ATPase ACTIVITY OF MEMBRANE VESICLE PREPARED FROM FROG MUSCLE. KITASATO,H., MURAYAMA,K., MARUNAKA,Y. AND NISHIO,K. Dept. of Phsiol., Shiga Univ. of Med. Sci. Ohtsu, Shiga 520-21

Previous experiments revealed that insulin stimulated Na efflux from frog muscle. To elucidate whether the stimulating effect of insulin on Na efflux is mediated by the alteration of intracellular events for which insulin is responsible or is a result of the direct action of insulin on the Na-K transport system, the effect of insulin on the Na-dependent ATPase activity and Na transport were examined under a cell-free condition. Crude microsomal preparation was first passed through a Sepharose 2B gel filtration column. The microsomes in a fraction of high ATPase activity were loaded with Ca. After having been partially disrupted by the French press procedure of 500Kg/cm^2, the Ca loaded microsomes were further separated in a sucrose density gradient centrifugation. The ATPase activity was confined in a region of 24% sucrose. The digitoxin-sensitive ATPase activity was about one third of the nonspecific ATPase activity. The specific activity of the membrane vesicles prepared from insulin-pretreated muscle was lower than that of the membrane vesciles prepared from the control muscle. The addition of insulin into the assay solution caused no observable change in the ATPase activity. The ^{22}Na uptake by vesicles measured by the Millipore filtration method seems to increase by an addition of insulin.

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EFFECTS OF RESTING MEMBRANE POTENTIAL ON 3-O-METHYL-D-GLUCOSE UPTAKE IN SKELETAL MUSCLES MARUNAKA, Y., KITASATO, H., MURAYAMA, K. AND NISHIO, K. Dep. of Physiol., Shiga Univ. of Med. Sci., Ohtsu

We studied the mechanism of glucose transport in skeletal muscles, using 3-O-Methyl-D-Glucose(3-O-MG),non-metabolized sugar, and paired sartorii of *Rana catesbeiana*. 3-O-MG uptake increased in proportion to $[Na]_o$, while muscles with high $[Na]_i$(high Na muscles) took up more 3-O-MG and more Na than muscles with low $[Na]_i$ (low Na muscles). The resting membrane potential of high Na muscles was more negative than that of low Na muscles. This hyperpolarized membrane potential of high Na muscles might cause large driving force for Na-influx. The increase of Na uptake might stimulate 3-O-MG uptake in high Na muscles. From these results, we suspect that 3-O-MG was taken up with both Na-influx dependent- and independent-mechanisms. Further, we examined effects of insulin, phloridzin and $[3\text{-O-MG}]_o$ on 3-O-MG uptake. Insulin stimulated Na-dependent 3-O-MG uptake but had no effect on Na-independent component. Insulin hyperpolarized the muscular membrane and increased the amount of Na uptake. The increase of Na uptake might be due to the increase of driving force for Na-influx caused by hyperpolarization. This increase of Na uptake might stimulate 3-O-MG uptake. Phloridzin blocked only Na-dependent component. Around 50mM of $[3\text{-O-MG}]_o$, the rate of Na-dependent 3-O-MG uptake was saturated but that of Na-independent 3-O-MG uptake still kept increasing linearly.

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CELLULAR MECHANISM OF ACTIVE CHLORIDE TRANSPORT BY THE RENAL TUBULE. HOSHI, T., YOSHITOMI, K., MIURA, M. and ITOI, K. Dept. of Physiol., Faculty of Med., Univ. of Tokyo, Bunkyoku, Tokyo

The early segment of the distal tubule of Triturus kidney was found to be a unique portion where the transtubular PD was lumen-positive and ion transport was sensitive to furosemide. To clarify the mechanisms of ion transport, particularly Cl^- transport, in this segment, genesis of the lumen-positive voltage and conditions affecting ion transport were studied. The transtubular PD under control conditions was 13.7 ± 1.0 mV (lumen-positive) and this positive potential was abolished by replacement of Cl^- with NO_3^-, while choline chloride substitution for NaCl had little effect on it. The rate of fluid absorption as observed by Gertz method was highest (t1/2 = 1.15 min) among nephron segments tested. The gradient-limiting nature of NaCl transport was seen, the equilibrated luminal NaCl concentration being about 35 mM. The ion transport and fluid absorption were completely inhibited by furosemide (10^{-5}M) but not by amiloride (10^{-4}M). An increase in luminal NaCl concentration caused a transient increase in the luminal positivity and this PD increase was sensitive to furosemide. Furosemide also caused a significant hyperpolarization of the basolateral membrane. These data show that Cl^- entry mechanism at the luminal membrane is electrogenic and sensitive to furosemide, but this process is not contributing to the formation of the lumen-positive voltage.

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ELECTRICAL PROPERTIES OF THE NEWT COLLECTING DUCTS-A COMPARATIVE STUDY OF I_{oc} AND I_{sc}.
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Little is known about ion transport and electrical properties of the collecting duct of amphibian kidney. The present study aimed at characterization of Na^+ ion transport in the collecting duct of newt kidney. The transepithelial PD[ψ_{ms}] averaged -87.8 ± 3.8 mV lumen-negative, in the presence of 105 mM NaCl Ringer's solution in luminal and peritubular sides. ψ_{ms} was dependent on the luminal Na^+ concentration. Both the plots of $1/\psi_{ms}$ v.s. $1/[Na^+]_\ell$ and $[Na^+]_\ell/\psi_{ms}$ v.s. $[Na^+]_\ell$ are linear over a wide range of Na^+ concentration. The maximum value of ψ_{ms} and the half saturation Na^+ concentration are -87.5 mV and 3.9 mM, respectively.

Amiloride at 10^{-4} M inhibited ψ_{ms} by 96.2 %, and half-maximum inhibition was seen at 2×10^{-6} M.

The specific transepithelial resistance (R_t) was measured according to the principle of cable analysis. Estimated value of R_ℓ was 17.5 $K\Omega\cdot cm^2$. From the values of ψ_{ms} and R_t, the open-circuit current (I_{oc}), was calculated to be 5.4 $\mu A\cdot cm^{-2}$. The results indicate that the amphibian collecting duct is a typical tight epithelium transporting Na^+ through amiloride-sensitive Na^+ channels. Short-circuit current [I_{sc}] was measured by clamping ψ_{ms} at zero by the use of an axial Pt wire inserted into the lumen. The I_{sc} averaged 8.3 $\mu A\cdot cm^{-2}$. I_{oc} and I_{sc} agree remarkably with eath other.

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PURIFICATION OF Na^+/GLUCOSE COTRANSPORT CARRIER OF INTESTINAL BRUSH BORDER MEMBRANES
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In order to characterize the molecular properities of Na^+/glucose cotransport carrier, solubilization and purification of the carrier proteins were attempted. The brush border membrane vesicles were isolated by the methods of Schmitz et al., then the vesicles were subjected to negative purification. After treatments with deoxycholate(0.06%) and papain (0.5mU/mg protein), phlorizin binding capacity and Na^+dependent overshoot glucose uptake per mg protein increased 2-fold. Thereafter the vesicles were treated with Triton X-100 (2-4%) to solubilize proteins, then the solubilized proteins were separated with a Bio-Gel A-5m column and subsequently by chromatofocusing(pH 7-4). Transport activity of separated proteins were tested by incorporating them into soy bean phospholipid liposomes and observing Na^+dependent overshoot uptake of glucose. Transport activity was seen only in proteins of around 340K dalton and elutable in pH range of 5.5-5.0 . The reconstituted liposomes with proteins exhibited 20-fold higher transport activity than proteoliposomes with non-purified proteins. The initial rate of glucose uptake by the reconstituted liposomes exhibited S-shaped relation to glucose concentration, the Hill's number being about 2. The 340K dalton protein was splitted by boiling with SDS into two subunits of 200K and 160K dalton.

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TRANSCELLULAR MOVEMENT OF CHLORIDE ION IN THE RENAL TUBULE.
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Using double-barreled ion-selective microelectrodes, intracellular ion activities of Na $(Na)_i$ and Cl $(Cl)_i$ were measured directly in bullfrog nephrons, and behavior of Cl movement across the membrane was analyzed. The results obtained are summarized as follows: 1) The mean values of (Cl) in the lumen, cell and peritubular interstitium were 71.1, 9.9 and 72.5 mEq/L, whereas the membrane potential difference (PD) across the peritubular and transepithelial borders were -68.4 and -8 mV, respectively, in the early proximal tubule. 2) Replacement of Cl with SO_4 did not cause changes in PD, while $(Cl)_i$ was decreased gradually, indicating that the $(Cl)_i$ movement is relatively independent of PD. 3) Luminal applications of furosemide or amiloride produced a significant fall in $(Na)_i$. The fact that the $(Cl)_i$ was reduced concomitantly with Na in the former, but remained unchanged in the latter suggests that luminal entry of NaCl as a neutral salt is sensitive to furosemide, but not to amiloride. 4) Change of external pH and administration of ouabain produced changes in the membrane PD without changing $(Cl)_i$ within the range of membrane PD from 10 mV hyperpolarization to 15 mV deploarization. But beyond this range $(Cl)_i$ started changing in the direction that expected for balancing the anion gap. All these finding would agree well with the concept that the movement of Cl is passive in nature.

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EFFECTS OF INTRACELLULAR Ca ON THE ION TRANSPORT ACROSS THE PROXIMAL TUBULE OF BULLFROG KIDNEYS. KOTERA,K., KAJINO,K., YANTORNO,K.P., MORIMOTO,Y., SATAKE,N. and FUJIMOTO,M. Dept. of Physiol., Osaka Medical College, Takatsuki, Osaka.

To determine ion activities of Ca and Na in the proximal tubule cells, micropuncture experiments were perfromed on perfused bullfrog kidneys using double-barreled Ca- and Na-selective microelectrodes with neutral ion ligands, ETH 1001 and ETH 227, respectively. The detection limit of Ca ion activity was 63 nM. The mean intracellular Ca, $(Ca)_i$, was 10.7 ± 6.6 μM. Negative correlations were observed between the peritubular membrane potential (E_M, -45 ∿ -70 mV) and $(Ca)_i$ (3 ∿ 25 μM), and E_M and $(Na)_i$ (5 ∿ 31 mM). The procedures for increasing $(Ca)_i$ (e.g., an addition of Ca^{++} (3 ∿ 5 mM) or Ca-ionophore, A23187, to the peritubular perfusate and a partial substitution of peritubular NaCl with mannitol or choline chloride) caused depolarizations of E_M (by 6 ∿ 11 mV) and moderate rises of $(Na)_i$ (by 2 ∿ 5 mM). It is suggested that the increased $(Ca)_i$ does not necessarily decrease the luminal membrane permeability to Na, but rather increases, thus producing a depolarization of E_M and a rise of $(Na)_i$, which may in turn decrease the luminal entry of Na and stimulate a Na-K pump on the peritubular membrane. Therefore, an electrochemical feedback mechanism might operate to keep pace with the rate of luminal entry and the peritubular extrusion of Na across the proximal tubule without mediation of intracellular free Ca.

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POTENTIATING EFFECTS OF β-ADRENERGIC STIMULATION OF K RELEASE INDUCED BY ACH OR PHENYLEPHRINE IN RAT SUBMAXILARY GLAND. KANEKO, K., KATOH, K., SUZUKI, Y. T. AND *NISHIYAMA, A. Dept. of Physiol., Yamagata Univ. Sch. of Med., Yamagata. *Dept. of Physiol., Tohoku Univ. Sch. of Med., Sendai.

Measurements of net K-flux from the superfused segments of rat submaxilary gland were carried out in order to investigate effects of β-adrenergic stimulation on the K release induced by cholinergic or α-adrenergic against. Isopreterenol (2.5×10^{-5}M) induced a K uptake which was blocked by ouabain. The K release induced by ACh (5.5×10^{-7}M) or Phe (5×10^{-6}M) was significantly potentiated in the presence of isoproterenol. The potentiating effect of isoproterenol was mimicked by dibutyryl cyclic AMP (3 mM). The potentiating effect of isopreterenol on the ACh-induced K release was abolished in the presence of ouabain (10^{-3}M). The potentiation of Phe-induced K release by isoproterenol was also reduced in the presence of ouabain but not abolished. The K release induced by noradrenaline (3×10^{-7}M) in the presence of ouabain was reduced by 17 % by addition of propranolol (5×10^{-6}M). These results indicate that the most part of potentiating effects of β-adrenoreceptor stimulation concerns with increase in intracellular K concentration through activation of Na-K pump, which enhances the chemical driving force for the passive K transport. However, some part of the potentiation is not explained by the K accumulation into the cell.

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SODIUM CHANNEL AT THE APICAL MEMBRANE OF NECTURUS URINARY BLADDER. SUZUKI,Y.T. Dept. of Physiol., Yamagata University School of Medicine, Yamagata

Current-voltage relations of the amiloride-blockable Na channel at the mucosal membrane of Necturus urinary bladder were determined using a computer driven clamp of transepithelial PD and intracellular microelectrodes. The relations were well described by the Goldman-Hodgkin-Katz flux equation over a wide range of membrane potentials. However, when the activity of Na in the mucosal solution was increased, the Na current showed saturation. Furthermore, K in the mucosal solution inhibited the Na current in a manner that its magnitude was greater when the intracellular electrical potential became more nagative. These characteristics were incompatible with "independence principle" which must be obeyed in constant-field theory. Another model based on Eyring rate theory was considered. The flux equation of one-well two-barriers model can explain both saturation and the potential-dependent K inhibition of the Na current quantitatively.

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ELECTROLYTE TRANSPORT AND MEMBRANE POTENTIAL OF RAT BLADDER EPITHELIAL CELLS. HOTTA, K. AND YAMAMOTO, Y. Dept. of Physiol., Nagoya City Univ. Med. Sch., Mizuho-ku, Nagoya 467

To examine the properties of ion transport throuth mammalian urinary bladder, effect of various ions and drugs which modify the transport of Na^+, on membrane potential of epithelial cells was investigated using microelectrode. Physiological and morphological changes upon treatment of the animals with BBN(tumor inducer) were also examined. In normal Krebs solution at 37°C, epithelial cells exhibited membrane potential of about -22mV and -24mV to the solutions of mucosal and serosal sides, respectively. Substitution of mucosal Krebs to Na^+-free Krebs or amiloride increased the potential difference whereas ouabain and high K^+-solution(50mM) induced membrane potential change from the serosal side. Administration of BBN to the animals for 4 weeks induced significant increase of membrane potential of epithelial cells. These results were analyzed in terms of Na^+ channel and Na-pump activity located on the mucosal and serosal surfaces of the cell membrane. Co-operation of Prof. N. Ito, Drs. Imaida and Oshima of Pathol. Dept. are greatly acnowledged.

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KINETIC ANALYSES OF ACTIVE Na TRANSPORT THROUGH FROG SKIN IN DIFFERENT SETUPS. TAKADA, M. and HAYASHI, H. Dept. of Physiol., Saitama Med. Sch., Moroyama, Iruma-gun, Saitama 350-04

Kinetic analyses of active Na transport in SO_4-Ringer through frog skin were made by using three different experimental setups; (I) cannula whose inside facing to the dermis, (II) cannula whose inside facing to the epidermis, and (III) Ussing-type chamber with gaskets. Various concentrations of [Na], 2 mM Cd, and 0.1 μM amiloride (Am) were applied to the epidermal side of the skin. Short circuit current (SCC) was measured by adjusting the potential differences (PD) of the frog skin in Na-free condition. In type I setup, Eadie plot of normalized SCC (SCC %) vs. (SCC %)/[Na] was fitted by a single straight line. Cd activated the SCC uncompetitively and Am inhibited it competitively. However, in type II or III setups, Eadie plot of (SCC %) vs. (SCC %)/[Na] was fitted by two lines (higher [Na] range over 60 mM and lower [Na] range under 30 mM [Na]). Cd activated the SCC noncompetitively. Am inhibited the SCC competitively in high [Na] range but the type of inhibition was mixed in lower [Na] range.

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MICROCALORIMETRIC STUDIES ON Na^+, K^+-ATPase.
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Calorimetric experiments were carried out in LKB batch-microcalorimeter using calorimetric titration method which developed originally by T. Kodama and R.C. Woledge (J. Biol. Chem. 251 1976). We improved this automated device of titration and LKB microcalorimeter. Microcalorimetric measurement were carried out at high sensitive and stable condition, namely the value of heat effect could be recorded at full scale deflection of 5 μV, equivalent to 86 μW, within maximum baseline drift 0.1 μV/24 hours. We have already shown strong exothermic heat production specific to the overall reaction of Na^+, K^+-ATPase measuring by LKB flow-microcalorimeter (J. Physiol. Soc. Japan 43 1981). Here we reexamined overall reaction of this enzyme with batch-microcalorimeter which has two reaction vessels connected in a twin principle arrangement, because the heat effect in biological system was associated with different reaction in it. We established to get heat change specific to Na^+, K^+-ATPase. A calorimetric titration method was used to study Mg^{2+} binding to Na^+, K^+-ATPase. Calorimetric study revealed exothermic enthalpy changes associated with binding of Mg^{2+}. The dissociation constant of low affinity substrate site for the binding of Mg^{2+} derived from calorimetric measurements is in good agreement with the value obtained from kinetic studies.

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FLUORESCENT PROBE (diS-C_3-(5)) ANALYSIS OF SODIUM COTRANSPORT IN ISOLATED CHICK ENTEROCYTES KINOSHITA, Y., OGAWA, M., ASAMI, K. and IRIMAJIRI, A. Dept. of Physiol. Kochi Med. Sch., Nankoku, Kochi

In an attempt to correlate the so-called sodium cotransport mechanism with the membrane potential (MP) changes of transporting cells, fluorescence changes (ΔF) were monitored for enterocytes suspended in an isotonic buffer containing diS-C_3-(5). Addition of D-glucose or D-galactose, in the presence of Na^+, elicited a change in ΔF (Ex = 620 nm, Em = 670 nm) which is known to reflect depolarization in MP. Such effects were also observed with L-alanine, L-glycine and L-leucine, but not with L-glucose, D-fructose and L-glutamic acid. Phloridzin inhibited D-glucose-evoked changes in ΔF, in a dose-dependent manner, with a full inhibition attained at 0.5 mM. Phloridzin showed no inhibitory effect on L-alanine-evoked changes in ΔF. No considerable effect was found with amiloride in the concentration range tested. Such sugar-evoked changes in ΔF followed a saturation kinetics, giving rise to a linear relationship between $1/\Delta F$ and 1/[sugar] or $1/[Na^+]$.

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THE INTERACTION OF TRANSPORT BETWEEN PEPTIDES AND AMINO ACIDS Himukai, M., Kato, T. and Hoshi, T. Dept. of Physiol., Fac. of Med., Univ. of Tokyo

Inhibition of peptide transport by amino acids was studied in isolated guinea pig ileum. It is now established that many di- and tripeptides are transported across the mucosal membrane by mediated process(es) distinct from amino acid transport systems. For example, intact peptide absorption of glycyl-L-leucine was not competed by glycine, isoleucine and leucine (Leu) in guinea pig small intestine. However, a weak but significant interaction of transport between dipeptides and amino acids has been occasionally reported by several investigators, although the mechanism of inhibition remains unknown. Present kinetical study demonstrated that the inhibition of glycyl-glycine (Gly-Gly) transport by Leu was "pseudo-competitive" type (Robinson & Alvarado) , i.e. competitive type of inhibition on a Lineweaver-Burk plot but non-linear (hyperbolic) relationship between the reciplocal of Gly-Gly influx and the concentration of Leu on a Dixon plot. This suggests that Gly-Gly carrier has another binding site to which Leu can bind, that Leu binding to this site is inhibitory to Gly-Gly transport, and that Leu is partly transported by Gly-Gly carrier system. So, it can be said that Gly-Gly carrier has a feature of polyfunctional carrier.

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NA-CHANNEL ACTIVATION BY ACETYLATION WITH N-ACETYLIMIDAZOLE IN GLUTAMATE H TYPE NEURONES OF THE SNAIL.

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In snail suboesophageal ganglia, Na^+ permeability increase responses induced by ACh or Glu in the Glu-D type neurones reduced their amplitude by N-actylimdazole (AI), irreversibly.

In Glu-H type neurones, Glu-induced responses augmented three to five times by application of AI with Glu, simultaneously, This augmentation is due to Na^+ channel activation, and the AI effect is reversible. The activation of Na channel by AI is lost with TNBS for modification of amino groups. These results suggest that the Na channel in the Glu-H type neuronesis inactivated by tyrosyl residue is binding with the channel gate site. These AI effects on the Glu-induced responses both in Glu-D and Glu-H type neurones indicate that the tyrosyl residues have some important roles to control or inhibit the ion-transport mechanisms in the receptor-ion channel complex.

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SEPARATION OF Cl ION CHANNEL AND GABA ACTION IN THE FROG DORSAL ROOT GANGLION CELLS.

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The experiments were performed on isolated neurons in the lumber dorsal root ganglion of the bullfrog (Rana catesbeana). The Cl-channel in the neurons was separated from other Na-, K- and Ca-channels by the suction pipette technique which allows current clamp and intracellular perfusion. Na, K and Ca ions in the external and internal medium were replaced with equimolar Tris, Cs and Co ions, respectively. The reduction of the Cl ion concentration in the external or internal solutions were made by replacing Cl ion with isethionate ion externally or aspartate ion internally. The Cl ion concentration of the internal solution was increased by replacing aspartate ion with an equimolar Cl ion, an increase over 130 mM Cl was made by adding extra CsCl to the medium. The experiments were run at temperatures between 18 to 20° C. GABA was applied by perfusion.

The GABA equilibrium potential (E_{GABA}) was equal to that for Cl (E_{Cl}) and behaved as a Cl ion electrode following changes of external and internal Cl ion concentrations. The GABA response was blocked competitively by bicuculline but non-competitively by picrotoxin.

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EFFECT OF SEA ANEMONE TOXIN ON THE SODIUM INACTIVATION IN CRAYFISH AXONS.

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Sea anemone toxin (PaTX) from Parasicyonis actinostoloides slowed the sodium inactivation without changing kinetics of the sodium activation or that of the potassium conductance in crayfish giant axons under voltage clamp conditions. An analysis on the sodium current profiles during the toxin treatment suggested all-or-none modification of individual sodium channels. In consequence, toxin-induced steady-state sodium current density was regarded as a good indicator of the toxin binding to its receptor. PaTX binding thus evaluated showed a large suppression under depolarization. This suppression seems to occur in a rapid response to the membrane potential rather than slowly changing channel properties such as refered to as "ultra-slow inactivation" under sustained depolarization. In accordance with these electrophysiological results, the potential-dependent toxin binding was exhibited directly in biochemical techniques using [^{3}H]propionyl-PaTX with crayfish leg nerves.

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ROLE OF Ca^{2+} ON K^+ CURRENT OF THE ISOLATED SNAIL NEURON. AKAIKE, N., *OYAMA, Y. AND *NISHI, K. Dept. of Physiol., Fac. of Med., Kyushu Univ., Fukuoka and *Dept. of Pharm., Kumamoto Univ. Med. Sch., Kumamoto

The effect of Ca^{2+} on the delayed K^+ current (I_{KD}) and the fast transient K^+ current (I_A) in the identified neurons of Helix aspersa were investigated by using suction pipette technique which allows voltage clamp and internal perfusion. I_{KD} was suppressed with replacing Ca^{2+} with Co^{2+} externally and the reduction of I_{KD} occurred more quickly when the external solution was made hypertonic by adding sucrose or caffeine was directly added to the bathing medium. Furthermore, the reduced I_{KD} was almost abolished by successive perfusion with EGTA or F^- intracellularly, suggesting that I_{KD} is a quite same kind of current to the K^+ current activated by transmembrane Ca^{2+} current (I_{Ca}). On the other hand, I_A was also reduced by Co^{2+} applied externally but not affected by the internal perfusion of EGTA or F^-. The different sensitivities upon the internal EGTA or F^- between two currents, suggest that the interaction between the site and ions in the membrane K^+ channels differs between them.

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THE CALCIUM ACTIVATED POTASSIUM CONDUCTANCE IN RABBIT NODOSE GANGLION CELLS. MORITA, K.*, TOKIMASA, T.*, HIGASHI, H., NORTH, R. A.* AND NISHI, S. Dept. of Physiol., Kurume Univ. Sch. of Med., Kurume and *Dept. of Nutr. & Food Sci., MIT, Cambridge

One class of type C nodose neurons showed a prominent and long-lasting hyperpolarization (AH) following a train of tetanic stimuli. This AH reversed its polarity at about -90 mV, and its value changed by 54 mV when the external K concentration was changed ten-fold. The AH was abolished in a Ca-free or Co-containing (1 mM) solution; suggesting that the AH is due to a Ca activated K conductance. Ionophoretic injection of Ca into a C neuron which had been immersed in a solution contining no Ca, 3 mM Co and 0.5 μM TTX induced a hyperpolarization. Intracellular injection of EGTA, on the other hand, nullified the post-tetanic AH. The evidence implies that the intracellular Ca plays an important role in production of the post-tetanic AH. The AH elicited by 1 to 5 impulses in 2 sec showed an ixponential time course with a τ value of a few sec, while the AH produced by 20 to 60 impulses in 2 sec consisted of double exponential time courses with corresponding τ values of a few sec and some 10 sec. NaCN (1 μM) depressed the slow component without affecting the fast component of AH, while caffeine (1 μM) prolonged both the fast and slow components of AH with no appreciable effect on the peak amplitude.

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THE INACTIVATION MECHANISM OF CALCIUM CHANNEL IN THE SENSORY NERVE TERMINAL OF FROG MUSCLE SPINDLE. ITO,F., KOMATSU,Y., FUJITSUKA,N. and KATSUTA,N. Dept. Physiol., Nagoya Univ. Sch. Med., Nagoya.

Calcium inflow following individual sodium spikes in spontaneous discharges has been found in the sensory nerve terminal of isolated frog muscle spindle. The amplitude and duration of the Ca component were increased in isotonic sodium salt solutions with a group of anions, of which the size in the aqueous solution ranged from 0.74 to 1.32 times that of hydrated sodium ions. Several small spikes occurred superimposed on a plateau of the increased slow component 10 min after treatment of isotonic sodium salt solutions with some of the anions in the group. The effects of those anions were competed with divalent cations, without discrimination between the agonists and the antagonists to calcium ion. It is hypothesized that the anions with the size close to that of hydrated sodium ions may play a role as a carrier for sodium ions to inflow through calcium channel, which may be inactivated by the divalent cations binding to a site inside the membrane after inflow through the channel.

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MITOCHONDRIAL REGULATION OF CALCIUM IONS IN THE SENSORY NERVE TERMINAL OF FROG MUSCLE SPINDLE. FUJITSUKA,N., ITO,F., KOMATSU,Y. and KIM,N.

Calcium spike in the sensory nerve terminal of the frog muscle spindle has been known to play an important role in the mechano-transduction. The hypothesis that the calcium ions entered into the terminal are regulated by mitochondria was emphasized electrophysiologically and ultrastructurally after application of the inhibitors of mitochondrial function. Afferent discharges and spindle potential during stretch of the isolated muscle spindle were abolished by treatment of 0.5 mM ruthenium red, 10 μM N-ethyl maleimide (NEM), or 2 μM carbonyl cyanide M-chlorophenyl hydrazone (CCCH). The muscle spindles incubated with ruthenium red revealed an accumulation of electron dense deposits in mitochondria, which were verified to contain the dye by an energy dispersive X-ray analysis, instead of calcium particles in normal Ringer solution. This implies that the dye may be uptaken instead of calcium ions by the mitochondria.

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DEVELOPMENT OF CALCIUM AND SODIUM CHANNELS DURING THE DIFFERENTIATION OF A MYOGENIC CELL LINE, MC3T3-A1. AMAGAI, Y., KODAMA, H., IIJIMA, M., AND KASAI, S. Dept. of Physiol., Tohoku Dental Univ., Koriyama, Fukushima

The clonal myogenic cell line, MC3T3-A1/M13, has the capacity to differentiate in vitro into myotubes closely resembled striated muscle fiber in vivo. Fully matured myotubes generated fast Na spikes but immature myotubes showed no or abortive action potentials. We found that even the immature myotubes which only showed delayed rectification in normal saline could generate overshooting action potentials when bathed in high-Ca saline (>5mM). Amplitude of the overshoot was dependent on the external Ca concentration. The action potentials were elicited even in Na-free saline but suppressed by the addition of Co. These observations demonstrate that the potentials are Ca action potentials. They were often accompanied by prolonged (1-5sec.) hyperpolarizing afterpotentials which were thought to be mediated by Ca-dependent K conductance system. These results indicate that during the development of excitability in the M13 myotubes, Ca channels appear first and then Na channels developed subsequently to rearrange the cation channels, ultimately to act as majority of the spike generating system, when the Ca current were no more detected.

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OSCILLATION OF CYTOPLASMIC FREE CALCIUM CONCENTRATION MEASURED WITH CALCIUM ION-SELECTIVE MICROELECTRODES IN FIBROBLASTS.
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Fibroblastic L cells show spontaneous oscillation of membrane potential composed of repeated hyperpolarizing responses. Using Ca^{2+}-selective microelectrodes made with Simon's neutral carrier, the intracellular concentration of free calcium ions was measured. The order of cytosolic free Ca^{2+} concentrations was 0.1 μM in the resting state. The spontaneous hyperpolarization coincided with a significant increase in the cytoplasmic free calcium ions over 1 μM. Thus, synchronous oscillations of the free Ca^{2+} level were observed within the cell, during the oscillations of membrane potential. The external application of a high dose of calcium (10 mM) or of a flavonoid quercetin (100 μM), a Ca-ATPase inhibitor, produced a marked increase in the intracellular concentration of free calcium ions (up to several micromolar) in company with a prolonged hyperpolarization. It is concluded that recurrent stimulation of Ca-activated K-channels is responsible for the membrane potential oscillations in the fibroblasts.

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EXTRA- AND INTRA-CELLULAR CALCIUM AND PENTYLENETETRAZOLE INDUCED BURSTING ACTIVITY IN SNAIL NEURONS.

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To determine the role of the extra- and intra-cellular calcium in the manifestation of pentylenetetrazole(PTZ) induced bursting activity(BA) in the neurons of Euhadra peliomphala, the change of the bursting form was examined after the extra- and intracellular calcium concentration was changed. When the extracellular calcium concentration was reduced, the plateau phase of BA was prolonged, the interburst period was shortened, and the inward as well as the outward current were reduced. When the 10^{-8}M Ca-EGTA buffer solution was injected intracellularly in the PTZ-sensitive neuron, BA could be induced as in the case of PTZ application. In the PTZ-non-sensitive neuron, a 10^{-8}M Ca-EGTA buffer intracellular injection did not induce BA but induced only a slight depolariztion.

The above findings together with the former experimental facts suggest that the reduction of intracellular calcium concentration induces a release of the intracellularly stored calcium and induces a series of intracellular successive changes such as gathering of intracellular calcium at the inner surface of the cell membrane, calcium binding state change which were previously indicated, and finally induces pathological channel changes and manifest BA.

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ROLES OF Ca-(Sr-)TRANSPORTS AT THE CELL MEMBRANE IN THE MECHANISM OF SLOW RHYTHMIC HYPERPOLARIZATIONS OF A SYMPATHETIC GANGLION CELL. NOHMI, M., KUMAMOTO, E., MINOTA, S. AND KUBA, K. Dept. Physiol., Saga Med. Sch. Saga 840-01.

Slow rhythmic hyperpolarizations (SHP) generated in a bullfrog sympathetic ganglion cell in the presence of caffeine (1 mM) result from alternate release and uptake of Ca^{2+} at intracellular Ca-stores. The maintenance of the intracellular Ca^{2+} concentration ($[Ca]_i$) at a certain level seems necessary for this oscillation. The removal of all Na^+ from Ringer, which would reduce the Na^+ concentration gradient ($[Na]_o/[Na]_i$) and eventually $[Na]_i$, hyperpolarizes the cell membrane, increases the interval of SHPs and shortens their decay phase. On the other hand, ouabain (0.01 mM) which would decrease $[Na]_o/[Na]_i$ and raise $[Na]_i$ prolonged the decay phase and interval. These results suggest that $[Na]_o/[Na]_i$ determines the interval of SHPs presumably by regulating $[Ca]_i$ during silent intervals between SHPs through a mechanism other than a Na/Ca exchange mechanism, while $[Na]_i$ regulates the decay phase by acting on the rate of either uptake of Ca^{2+} into a Ca-store or active Ca^{2+}-extrusion at the cell membrane. Replacement of total Ca^{2+} with equimolar Sr^{2+} increased the rates of rise and fall of a SHP and decreased the interval. These effects of Sr^{2+} were constant for more than two hours and completely reversible, indicating that Sr^{2+} is a complete substitute for Ca-transports at the cell membrane and the membrane of an intracellular Ca-store.

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ANALYSIS OF CALCIUM CURRENT INACTIVATION OF *Helix* NEURONS. OYAMA, Y., NISHI, K. AND AKAIKE, N.* Dept. of Pharmacol., Kumamoto Univ. Med. Sch., Honjo, Kumamoto, Japan.
*Dept. of Physiol., Fac. of Med., Kyushu Univ., Maidashi, Higashi-ku, Fukuoka, Japan.

It has been shown that inactivation of calcium current occurs at two rates with fast and slow time constants, during depolarizing pulses for 400-600 msec in the intracellular presence of EGTA (Brown et al. 1981). Under the same conditions, we found that for short pulses of 600 msec or less in duration the falling phase of calcium current could be fit by two exponential functions (namely fast and slow phases) but for long pulse of 8000 msec four exponential functions were required. The time constant of the forth component was over 10000 msec. Therefore, for estimating the true time constants for fast (first) and slow (second) phases, the third and forth components should be subtracted from total currents. The time constants of fast and slow phases, thus obtained, before and after subtraction were 61 msec and 23 msec, 1209 msec and 125 msec repectively. The forth component was less sensitive to changes in temperature, pH and several drugs than other components.

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QUALITATIVE CHANGE OF CALCIUM CHANNELS DURING DEVELOPMENT.
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The embryo of an ascidian whose cleavage was arrested with cytochalasin B just after fertilization could differentiate and generate Ca spikes such as seen in an epidermal cell. The Ca channel in this cleavage-arrested embryo was compared with egg Ca channel with respect to ion selectivity and the mechanism of inactivation. The ion selectivity of the egg Ca channel was Ca = Ba < Sr, however that of the cleavage-arrested embryo was Ca < Sr < Ba. The inactivation of the egg Ca channel was voltage-dependent, however that of the cleavage-arrested embryo was dependent on Ca current. The properties above mentioned changed from the egg type to another at about 40 hours after fertilization at 8°C.

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IONIC PERMEABILITY OF MOUSE OOCYTE MEMBRANES BEFORE OVULATION.
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Mouse oocytes were isolated from the ovaries, and excitability of the membranes was investigated by intracellular recording.

The oocytes produced action potentials in Na-free solution, and they were suppressed when Ca^{++} was replaced by Mn^{++}. The spikes were blocked by Co^{++}. Thus, they were identified as Ca spikes.

The overshoot increased with a 28-mV slope for a 10-fold elevation of the external Ca^{++} concentration. The threshold membrane potential for the spike initiation became more positive (stabilizing action) and the maximum rate of rise of the Ca spike increased as the external Ca^{++} concentration increased. Sr^{++} or Ba^{++} substituted for Ca^{++} in producing action potentials. It is concluded that the Ca channels exist in the mouse ovarian oocyte membranes.

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ENHANCEMENT OF THE IONIC CURRENTS THROUGH VOLTAGE-GATED Ca CHANNELS IN THE MOUSE OOCYTE AFTER FERTILIZATION.
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The changes of voltage-gated Ca channels in the mouse oocyte were investigated after fertilization under voltage clamp. Superovulated oocytes were collected from the ampulla of the oviduct and were fertilized in vitro. After electrical recordings, the oocytes were fixed and stained with lacmoid acetate for visualization of chromosomes and/or sperm head. About 60 minutes after insemination, the amplitude of inward current through Ca channels increased, which occurred at anaphase during the second meiotic division. The peak amplitude of the maximum inward current at metaphase was 5.3 $\pm$ 1.0 nA. It was 6.8 $\pm$ 2.1 nA at anaphase and 8.4 $\pm$ 1.3 nA at telophase. The selectivity among alkaline earth cations, V-I curve, and kinetics of the Ca channels were not altered after fertilization. Therefore, it was suggested that the enhancement of Ca channel currents was due to the increase in the number of Ca channels.

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CHARACTERISTICS OF ION-CHANNELS IN MOUSE PANCREATIC B-CELLS.

NISHIO, K.,KITASATO, H., MURAYAMA, K. AND MARUNAKA, Y. Dept. of physiol., Shiga Univ. of Med. Sci., Ohtsu

Membrane potentials in B-cells from mouse pancreatic islets of Langerhans in the presence of 11.1 mM glucose were measured under various conditions using microelectrode. Attention was paid mainly to the effects on spike bursts of drugs or experimental conditions that would be expected to affect characteristics of spike generations. Removal of Na^+ from the perifusing solution depolarized the membrane but spike bursts remained. Complete removal of Ca^{2+} by a Ca-chelator, Nifedipine (2 mg/L) depolarized the membrane and completely inhibited spike discharges. Both Mn^{2+} and Co^{2+} rapidly suppressed spike bursts and shifted the membrane potential toward depolarizing direction by ca. 10 mV. Addition of Ca^{2+} (7.5 mM) to the Mn^{2+} or Co^{2+} containing solution, spike discharges on the rapid depolarization restored but with prolongation of the slow depolarization and the plateu phase. TTX (1 uM) had no effect on both spike discharges and silent phases. The Na-channel activator, veratridine (0.1 mM) caused a depolarization of about 5-7 mV in the presence of either 2.8 mM or 11.1 mM glucose and decreased the duration of the silent phase. The membrane potential to which the peak of spikes reached was unchanged in the presence of veratridine. These findings strongly suggest that mainly Ca-entry is responsible for the spike generation in mouse pancretic B-cell membrane.

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ELECTRICAL MEMBRANE RESPONSES TO ACETYLCHOLINE IN CULTURED EPITHELIAL CELLS (INTESTINE 407).

YADA, T. AND OKADA, Y. Dept. of Physiol., Fac. of Med., Kyoto Univ., Kyoto

Cultured epithelial cells (Intestine 407) exhibit spontaneous oscillations of membrane potential, and respond with hyperpolarizing responses to mechanical or electrical stimuli. In addition, the application of acetylcholine (ACh) or carbachol onto the cell surface elicited a hyperpolarizing response. Such cholinergic responses were blocked by atropine but not by curare, suggesting the involvement of muscarinic receptors. Quinine, an inhibitor of Ca^{2+}-activated K^+-conductance, inhibited both the ACh response and the spontaneous oscillation. The ACh response was inhibited by the deprivation of external Ca^{2+}, while the spontaneous oscillation was still maintained in a Ca^{2+}-free medium. On the other hand, dantrolene suppressed the spontaneous oscillation, but not the ACh response. Based on these results, it is concluded that the stimulation of muscarinic receptors triggers Ca^{2+}-inflow across the cell membrane, which, in turn, stimulates Ca^{2+}-activated K^+-conductance, and that a repetitive Ca^{2+} release from microsomes is implicated in the spontaneous oscillation. Since the other intestinal secretagogues, histamine and vasoactive intestinal polypeptide, also induced the hyperpolarizing responses, it is hypothesized that these electrical activities are closely associated with Ca^{2+}-dependent secreting activities in the cells.

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EFFECTS OF INTRACELLULAR Ca, Mg, AND Mn IONS ON MEMBRANE CURRENTS OF SQUID GIANT AXONS.

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The membrane current was first measured under the internal perfusion of a K-glutamate solution at the concentration ranging 25 to 250 mM as a control. Thereafter, the effects of various concentrations of Ca, Mg, or Mn ions were examined 10 sec, 1 min, and 2 min after the onset of perfusion, and each set of valtage-current relation was obtained within 25 seconds. By the divalent cation perfusion, common remarkable changes appeared on the membrane current. One minute later, the threshold voltage for initiation of inward current lowered by 10 to 15 mV than the control (the order of change; Mg>Mn>Ca). The inward current was suppressed by about 20 to 30 percent (Mn>Ca>Mg), and the outward current was also suppressed by 40 to 50 percent (Mg>Mn>Ca). Thus, the internal divalent cations affect the membrane to open the excitation channel more easily but to suppress the Na and K channels.

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ASYMMETRICAL CAPACITANCE CURRENTS IN THE 'SLOW' Na INACTIVATION PROCESS IN THE CRAYFISH GIANT AXON. MURAYAMA, K., KITASATO, H., NISHIO, K., AND MARUNAKA, Y. Dept. of Physiol., Shiga Univ. Med. Sci., Ohtsu

Nerve membranes are mostly provided with the 'slow' Na inactivation system in addition to the 'fast' Na inactivation expressed by the h parameter in the Hodgkin-Huxley model. The relation between these two processes was studied from the asymmetrical capacitance current (I_s) that is possibly originated from the gating charge displacement in the Na channel. I_s was obtained from voltage clamped crayfish giant axons internally perfused with 188 mM TEA-F solution bathed in Tris-Cl + TTX solution by summing the capacitance currents during depolarizing and exactly matched hyperpolarizing pulses. The gating charge displacement was immobilized by prolonged depolarization in two phases, the fast (τ=1.7 ms) and slow (τ=5 sec) immobilization. The time course of the former corresponded to that of the fast Na inactivation, but the latter was slower than the slow Na inactivation. The slow immobilization exhibited voltage dependency. Internal application of Pronase (0.01-0.03 mg/ml) eliminated ca. 80% of the fast immobilization along with the prolongation of time course of the fast Na inactivation, but the slow immobilization remained unchanged. These results suggest that these two inactivation processes are not sequential and the molecular mechanism of the slow Na inactivation might be completely different from that of the fast Na inactivation.

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MODULATION OF THE SENSITIVITY OF SQUID AXON MEMBRANE TO GRAYANOTOXIN(GTX) AFTER THE TREATMENT WITH VARIOUS ENDOPEPTIDASES. SEYAMA, I. AND YOSHIOKA, Y., Dept. Physiol., Sch. Med., Hiroshima Univ., Hiroshima.

GTX has been shown to exert a depolarizing action on squid axon due to specific increase in membrane permeability to Na ion. Since the gating machinary of Na-channel is composed of macromolecules, one can expect to see the change of sensitivity to GTX after chopping the binding sites by various endopeptidases. Voltage clamp experiments were conducted on internally perfused squid giant axon. Because the site of action of GTX is on the internal surface, both GTX and endopeptidases were applied intracellularly. After treatment of squid axon with endopeptidases(1 mg/ml), such as pronase, BPN', St. fradiae protease, trypsin and St. fradiae trypsin for 1 - 2 hr, both peak and steady state currents were suppressed. Subsequent application of 100 μM GTX(ED_{50}=30 μM) did not depolarize the nerve membrane. In spite of suppression of both peak and steady state currents to the same extent as before, thermolysin, α-chymotrypsin, pepsin and neutral protease failed to eliminate the depolarization caused by GTX. These results indicate that the presence of the basic amino acid, such as Arg and Leu, may be essential for the binding of GTX.

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STRUCTURE-ACTIVITY RELATIONSHIPS OF INHIBITORY DERIVATIVES OF TYROSINE ON AN IDENTIFIABLE MOLLUSCAN GIANT NEURONE. TAKEUCHI, H. and *ARIYOSHI, Y. Dept. of Physiol., Sch. of Med., Gifu Univ. Gifu; and *Central Research Lab., Ajinomoto Co., Inc., Kawasaki

Inhibitory effects of N-β-phenylpropionyl-L-tyrosine (L-phenylalanyl-L-tyrosine without an amino group), N-β-phenylpropionyl-L-tryptophan (L-phenylalanyl-L-tryptophan without an amino group) and their derivatives on an identifiable giant neurone, TAN (tonically autoactive neurone), of an African giant snail (Achatina fulica Férussac) were examined in an attempt to elucidate which structural features are necessary to produce their effects.

The structural features are proposed to be as follows:
The active compound has a phenyl or a cyclohexyl group (hydrophobic binding group) at the top, after a suitable distance a peptide bond (proton donor and proton acceptor), adjacently a carboxyl group (proton acceptor), and a phenolic hydroxyl or an indolyl imino group (proton donor) at the end in the molecule.

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CHANGES IN Na SPIKES IN TISSUE-CULTURED NERVE CELLS AFTER INHIBITION OF PROTEIN SYNTHESIS BY ANTIBIOTICS.
FUKUDA, J., YAMAGUCHI, K. AND MAEDA,K. Dept. of Physiol., Fac. of Med., Univ. of Tokyo, Bunkyo-ku, Tokyo 113.

In order to study roles of protein synthesis on maintenance of membrane excitability, tissue cultured nerve cells from dorsal root ganglia of adult guinea-pigs were exposed to various species of antibiotics which inhibit protein synthesis. Inhibition of RNA synthesis by actinomycin D, a-amanitin and chromomycin A_3 caused a marked suppression of neurite outgrowth. Intracellular recording with a glass microelectrode showed no change in resting membrane potential or in input resistance. A marked reduction of $\dot{V}_{max}$ of pure Na spikes (elicited in a cobalt-containing medium), which is considered as proportional to a transient inward Na current, was observed. Inhibition of protein synthesis in ribosomes by puromycin and cyclohexamide or inhibition of protein glycosilation in Golgi stack by tunicamycin caused a marked suppression of neurite growth and reduction in the Na currents. While no change was detected in the resting membrane properties. These findings suggest that the protein synthesizing processes inhibited by the antibiotics contribute an essential role on appearance of Na channel molecules in the plasma membrane.

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A FACTOR FROM NERVOUS TISSUE PROMOTES THE DEVELOPMENT OF TETRODOTOXIN-SENSITIVE SODIUM CHANNELS ON CHICK SKELETAL MUSCLE CELLS IN CULTURE (II).
KANO, M., YAMAZAKI, S., SATOH, T. and YAMAMOTO, M. Dept. of Physiol., Kitasato Univ. Sch. Med., Sagamihara

The development and maintenance of the tetrodotoxin(TTX)-sensitive sodium channels in the vertebrate skeletal muscle cells are under the trophic control of innervation. We previously reported that proteins present in embryonic chick brain exrract can duplicate at least a part of the trophic effect of innervation on the development of the TTX-sensitive sodium channels in cultured chick skeletal muscle cells in the absence of innervation. In order to identify the biologically active protein, the extract of embryonic chick brain were fractionated biochemically and added to the cultures of embryonic chick skeletal muscle cells. The trophic effect of the protein fractions on the development of the TTX-sensitive sodium channels was examined by measuring the maximum rate of rise of TTX-sensitive spike potential of myotubes in 2-week-old cultures. The active protein was isolated by consecutive ammonium sulfate precipitation. Gel-filtration on Sephacryl S-300 of ammomium sulfate precipitation revealed two distinct peaks of trophic activity. The active protein fractions from gel filtration were further fractionated by ion-exchange chromatography on DEAE-cellulose. The active protein bound at neutral pH and was eluted with a 0.25 or 0.3 M Nacl solution.

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ACTION POTENTIAL GENERATION IN IMMATURE CULTURED NEURONS FROM CHICK CEREBRAL CORTEX.
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Spike generating mechanisms were investigated in cultured cortical neurons from chick embryos. Action potentials were intracellularly recorded from immature neurons cultured for 6-11 days. The maximum rates of rise of action potentials were increased by the addition of 10mM Ca^{2+} and 1-2mM TEA into Tyrode's solution, and such action potentials were not completely blocked by 10^{-5} g/ml tetrodotoxin(TTX). Action potentials with high maximum rates of rise were often elicited in the presence of TTX, TEA and 10mM Ca^{2+} and these TTX-resistant action potentials were blocked by Co^{2+}(10mM). The maximum rates of rise of action potentials were also increased by 10mM Mn^{2+}; though less effectively than 10mM Ca^{2+}. Even in a high concentration of Mn^{2+}(20mM) showing a membrane stabilizing effect, action potentials with high maximum rates of rise were frequently observed. The increase of the maximum rate of rise by Mn^{2+} was partially blocked by TTX(10^{-5} g/ml). On the other hand, the action potentials recorded from mature neurons(25-40 days) were suppressed by 10mM Mn^{2+}. These results suggest, in addition to the confirmed partial contribution of Ca ions to action potential generation, that Mn ions can pass through Ca channels in immature cultured neurons.

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RECEPTOR-MEDIATED HYPERPOLARIZING RESPONSES TO NUCLEOTIDES IN CULTURED FIBROBLASTS.
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The external application of ATP (> 0.2 mM) induced a transient hyperpolarizing response, while the intracellular ATP injection (by electrophoresis or pulses of pressure) did not affect the membrane potential profiles in fibroblastic L cells. A rapid hyperpolarization was also induced by extracellular ADP, GTP, ITP, UPT, CTP or TTP, but not by AMP, cyclic AMP, adenosine and PNP. A high dose of AMP rather suppressed the ATP-induced response. The membrane became desensitized by the prolonged application of ATP. Since non-hydrolysable ATP analogues (β,γ-methylene-ATP, AMP-PNP) also induced the membrane responses, it is obvious that ATP acts on the cell surface without its enzymatic hydrolysis. After desensitization of the ATP-induced response, the fibroblast still exhibited a receptor-mediated hyperpolarizing response to β-lipoprotein or complement. Thus, it is concluded that the fibroblasts have independent receptors for nucleotides, in addition to those for lipoproteins and complements. These receptor systems are presumably implicated in the physiological functions of fibroblasts. The intracellular microinjection of ATP using pressure was made in the collaboration with Dr K. Machida, Fac. of Sci., Kyoto Univ.

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HYPERPOLARIZING RESPONSE IN SQUID GIANT AXONS INDUCED BY STRETCHING OF THE MEMBRANE
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When the squid axon membrane was stretched by injecting a certain amount of intracellular solution, the membrane hyperpolarized by a few mV. This hyperpolarizing response induced mechanically is very unique among all mechano-electrical tranducer cells. The amplitude of hyperpolarization increased with the amount of solution injected. This hyperpolarizing response was associated with an increase in membrane conductance. Application of inward current through the membrane reversed the sign of the response. The reversal potential thus obtained was at a level 5 - 15 mV hyperpolarized from the resting level, and was greatly affected by extracellular potassium concentration. Tetraethylammonium, applied intracellularly, reduced the amplitude of the hyperpolarizing response. However, 4-aminopyridine, tetrodotoxin, and procaine did not suppress the response at all. The response became larger when the temperature was reduced or when a prolonged plateau was produced. The hyperpolarizing response could be attributed to activation of a type of the K-channel on the squid axon membrane.

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CHANGE IN OPTICAL ACTIVITY OF NERVE FIBERS WITH EXCITATION
WATANABE, A. Dept. of Cell Physiol., Nat. Inst. for Physiol. Sci., Okazaki 444

Squid axons were excised, cleaned and stained with fluorescein isothiocyanate, and mounted on a chamber which was furnished with two windows sealed with cover slips for allowing light beam to go through. The light source was a 250W tungsten incandescent lamp. Using interference filters, a quasi-monochromatic light at a wavelength between 470 and 520nm was obtained. The light was linearly polarized with a polarizer, went through the axon and an analyzer, and was accepted by a photodiode which transformed the light signal into the electric signal. The signal was amplified by an AC amplifier and was fed to a signal averager. In each trial 4096 stimuli were applied to the axon and the optical response was accumulated. In trial A the azimuth of the polarizer transmission axis was in parallel with the longitudinel direction of the axis and the azimuth of the analyzer transmission axis was 45° from the longitudinal direction of the axon. In trial B the azimuths of the analyzer and the polarizer were exchanged. The difference of the records in the trials A and B was interpreted as the indication of the optical activity. It was found that the change took place at the the rising phase of the action potential. At 510nm the change was in the direction of increased dextrorotation.

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THE ANESTHETIC ENHANCEMENT MATERIALS AND THEIR EFFECT ON THE RHEOLOGICAL ASPECT OF NERVE MEMBRANE ICHIKAWA, O., MIYOSHI, M. AND HIJI, Y. Dept. of Physiology, Tottori Univ. School of Medicine, Yonago 683

The enhancing effect of local anesthetics induced by organic acid salts was studied both electrophysiologically and rheologically using the giant axon of crayfish. The blockade of the nerve conduction by local anesthetics such as procaine and lidocaine was remarkably enhanced when they were used together with sodium salt of organic acid which had few effect on the action potential for itself. In the presence of either 0.2% sodium acetate, sodium benzoate or sodium salicylate, for example, the minimum concentrations needed for blocking the action potential was reduced to less than half of those in the absence of them. It was proposed that the following molecular structure of organic acid was essential for enhancing anesthesia. The molecule should have a lipophilic moiety such as aliphatic hydrocarbon chain or aromatic ring and simultaneously a carboxyl group attached to it as a hydrophilic residue. The analysis of the stress relaxation curves recorded by means of direct stretch of the giant axon revealed that the organic acid salt softened the axonal membrane. It was suggested that this makes the anesthetic even at a low concentration to reach the effective site of membrane easier.

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EFFECT OF MYRMICACIN ON THE SQUID GIANT AXON.
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Myrmicacin (3-hydroxydecanoic acid) which is isolated from the secretion of South American Leaf-cutting ants (*Atta sexdens*) inhibits the germination of collected seeds and spores in the ant's nest during strage. The effect of myrmicacin on the nerve membrane was studied by using a voltage clamp in squid giant axons. Suppression of the Na-current was observed when the agent (1 to 2 mM) was applied externally and internally. At the same time, a slight depolarization of the resting potential was accompanied, but K-current remained unchanged. These effects were completely reversed after washing with normal sea water. This suppression effect of Na-channel was also observed by appling thier analogous compounds, pelargonic acid, capric acid, 2-decenoic acid, etc. From these results it is possible to consider that myrmicacin and analogous compounds inhibit the function of Na-channel.

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FUNCTIONAL FRACTIONATION WITHIN A NON-SPIKING INTERNEURON OF CRAYFISH IN THE CONTROL OF MOTONEURON ACTIVITY. TAKAHATA, M. AND HISADA, M. Zool. Instit., Fac. of Science, Hokkaido Univ., 060 Sapporo.

The membrane potential of crayfish motoneurons is controlled effectively by anaxonic, non-spiking interneurons. These interneurons have both input and output synapses on their numerous fine branches. The function of these interneurons depends hence on the electrical distance from one part of the neuron to others within the same neuron. We calculated voltage decrements within the interneuron using the steady-state cable equation. The neuron morphology was studied with the cobalt staining method. We found that less than 2% of the applied voltage at one branch reached the other branches at a distance of 800μm. The result indicated that a particular input synapse could only activate neighboring output synapses due to the non-spiking nature of the interneuron. The conclusion was consistent with the result of current injection test that a certain interneuron activated a motoneuron but other interneurons did not, although they showed similar location and branching pattern to the effective one. This was presumably caused by the different site of electrode penetration relative to the output synapse onto that motoneuron in each interneuron.

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CHAOS IN THE ONCHIDIUM PACEMAKER NEURON UNDER SINUSOIDAL STIMULATION

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Onchidium pacemaker neuron beats spontaneously and its interspike interval is about two seconds (Temp. 20 °C). A single-microelectrode voltage clamp technique was used to analyse subthreshold current-voltage (I-V) relationship (the microelectrode was switched at 2.0 kHz for measuring the membrane potential and injecting current). Steady state (I-V) relationship showed non-linearity with a negative resistance region (N.R.R.) between -70 mV and -40 mV of the membrane potential.

This non-linearity leads the time-ordered dissipative structure. The response of the dissipative structure to the external force has been of interest in recent years. The nonperiodic oscillation of the Onchidium pacemaker neuron was observed under a peiodic stimulation with appropriate amplitude and frequency. The stroboscopic mapping of the phase space is an invariant set, i.e. a strange attractor and depends on the phase. The stroboscopic transformation reveals a single valued transition function with period three. This suggests that the nonperiodic oscillation is chaotic, considering the work of Li and Yorke (1975).

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MEMBRANE CURRENTS IN INSECT MUSCLE FIBERS.

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A two electrode voltage-clamp was used to investigate membrane currents in the ventral longitudinal muscle fibers of the larval mealworm, *Tenebrio molitor*. Three components of membrane currents elicited by step depolarization were distinguished, an inward current, a transient outward current and a delayed outward current. Increasing external calcium concentration increased the size of the inward current and shifted the reversal potential to a positive direction. The current was suppressed by cobalt ions. Therefore, the inward current was associated with the inward movement of calcium ions down its electrochemical gradient. A transient outward current followed an inward current at about -20 mV. The outward current was absent in calcium-free or cobalt saline. From the instantaneous current-voltage relation, the reversal potential for the transient outward current was determined as -46 mV, a slightly more negative than that for the delayed outward current. The reversal potential of the delayed current was about -80 mV in cobalt saline containing 3M-K, and was dependent on the external potassium concentration suggesting that the delayed outward current is carried by potassium ions. The graded electrogenecity produced in many arthropod muscle fibers may result from the various degree of activation of the transient outward current.

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HODGKIN'S IONIC THEORY AND MATUMOTO'S STRUCTURE THEORY FOR ELECTRICAL ACTIVITY OF NERVE.

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Matumoto, one of the authors presents a new structure theory for electrical activity of nerve or muscle fiber which is based on the equivalent circuit of nerve fiber in its resting and excited states. The equivalent circuit of a nerve fiber is a double polarized layer. The inner layer is self polarized, but the outer layer(surrounding tissue of the fiber) is polarized by the discharge current of charge of the inner layer. In resting state, the resistance of the inner layer(Rm) has a certain high value which is transiently reduced to $R_\epsilon \fallingdotseq 0$ by the stimulating current. The resting equivalent circuit then changes to one of excitation, so that excitation occurs and recovers to produce an action potential. Rm and the other parts of the equivalent circuit in the resting state are called the "active element" and the "field of excitation", respectively, and together they constitute the "excitable system". The structure theory permits the making of a hardware model(MMES) with electric elements which can be tested under various experimental conditions. Results obtained under known conditions were the same as those for the nerve, and under conditions not yet reported, new facts were obtained which were later proved for nerves. In this report, the origins of certain essential activity is described which might help to determine whether the ionic or structure theory is more nearly correct for the electrical activity of nerve and muscle.

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THE EFFECT OF ALKALINE EARTH METAL IONS ON THE ACTION POTENTIAL OF CULTURED CHICK EMBRYONIC HEART CELL. YAMASHITA, E. AND IGARI, T. Dept. of visual physiol., Fucul. Ed. Tohoku Univ., Sendai

The action potential of cultured chick embryonic heart cell was recorded with the microelectrode method. The increase in external Ca ion shortened the duration of action potential with or without previous application of TEA. Sr and Ba ions with the removal of Ca in the solution both much prolonged the action potential. This prolonged duration was reversed by the addition of 1.8 mM Ca ion in the solution, but the effect of Sr was different from that of Ba in the progressive prolongation of action potential with increased concentration of K ion in the solution. The normal action potential or prolonged one by the treatment with TEA shortened with increased K ion.

TEA, Sr and Ba were all found to produce prolongation of action potential, but their mechanisms seem to be different each other because of the different responses on the concentration of K ion.

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ELECTRICAL PROPERTIES OF Ca-CHANNEL IN THE INTERNALLY PERFUSED FROG PRIMARY AFFERENT NEURON. KURAOKA, S., *OYAMA, Y., * NISHI, K. AND **AKAIKE, N. Dept. of Physiol., Ginkyo College, Kumamoto, *Dept. of Pharm., Kumamoto Univ. Med. Sch., Kumamoto and Dept. of Physiol., Fac. of Med., Kyushu Univ., Fukuoka

The properties of Ca-channel of the isolated primary afferent neurons of the bullfrog (Rana catesbeana) were studied by using suction pipette technique which allows current clamp and internal perfusion. Ca-component of the action potential was separated from both Na^+ and K^+ components by replacing Na^+ in the external solution with $Tris^+$ and by replacing K^+ in the external and internal solutions with Cs^+. Ca-spike was completely abolished either by subsituting Co^{2+} for Ca^{2+} or by adding organic Ca-antagonists such as Diltiazem, D-600 and Verapamil. Foreign divalent cations (Ba, Sr, Mn, Zn and Cd) could evoked spikes. The order of the spike amplitude was in Ba > Sr > Ca > Cd > Zn > Mn. The nature of Ca-channel of the frog primary afferent neurons for divalent cations was essentially similar to that of snail neuron (Helix aspersa).

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INTERNAL PERFUSION OF ISOLATED FROG DORSAL ROOT GANGLION CELLS. KURAOKA, S., *OYAMA,Y., **AKAIKE, N. Dept. of Physiol., Ginkyo College, Kumamoto, *Dept. of Pharm., Kumamoto University Med. Sch., Kumamoto and **Dept. of Physiol., Fac. of Med., Kyushu Univ., Fukuoka.

The experiments were performed on isolated neurons in the lumbar dorsal root ganglion of the bullfrog (Rana catesbeana) by the suction pipette technique which allows current or voltage clamp and internal perfusion. Na-spike was obtained by substituting $Cs^+$and Co^{2+}in the external solution for K^+ and Ca^{2+}, respectively. In this condition, the isolated nerve cell body was internally perfused by the solution containing 130 mM Cs^+, 118 mM $aspartate^-$ and 12 mM Cl^-.

Na-spike was sensitive to the externally applied TTX and blocked dose-dependently by the drug. In the presence of TTX, addition of Na^+ to Ca-free Mg^{2+} solution did not cause any spikes, suggesting that in this preparation Na^+ cannot pass the Ca-channel. Na-spike was also completely abolished by replacing NaCl in the bathing medium with Tris Cl or isotonic sucrose. In addition, the action potentials in this preparation consist of two components of Na- and Ca-spikes, though Na-spike is predominant.

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Ca-K BI-IONIC ACTION POTENTIAL IN SQUID GIANT AXONS

TERAKAWA, S. Dept. of Cell Physiol., Natl. Inst. for Physiol. Sci., Okazaki

Under intracellular perfusion with a solution containing potassium as the sole cation species, squid giant axons were found to be capable of developing all-or-none action potentials when immersed in a medium in which $CaCl_2$ was the sole electrolyte. The action potential appeared only when the concentration of external Ca-ion was reduced to a level below 20 mM. In this range of Ca concentration, the level of reversal potential measured by the voltage-clamp method varied directly with the logarithm of the Ca concentration; the proportionality constant was close to RT/2F. The action potential observed under this Ca-K bi-ionic condition could not be suppressed by addition of tetrodotoxin or saxitoxin to the external medium. The external Ca-ion could be replaced with Co- or Mn-ion without eliminating the action potential. D-600 could not suppress the inward current observed under the voltage-clamp condition, but 4-aminopyridine could suppress it. Therefore, in spite of the absence of extracellular K-ion, this Ca-dependent action potential is to be explained as an activity of the K-channel.

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LESS CONTRIBUTION OF K-PERMEABILITY TO THE RESTING POTENTIAL OF MOUSE NEUROBLASTOMA CELLS IN PHYSIOLOGICAL $[K^+]_o$. M. Miyake, K. Kurihara. Faculty of Pharmaceutical Sciences, Hokkaido Univ. Sapporo 060

The resting potential (RP) of N-18 neuroblastoma cells was depolarized with an increase in $[K^+]_o$ above 15 mM at a rate of 55-58 mV per 10-fold change in $[K^+]_o$ but practically unchanged with varying $[K^+]_o$ below this concentration. The membrane resistance (R_{in}) was constant in the range of lower $[K^+]_o$, but decreased sharply by a factor of 1/4-1/5 with an increasing $[K^+]_o$ above 15 mM. The above observations and ^{86}Rb efflux measurements indicate that the N-18 cells carryed the K-channels which opened at high $[K^+]_o$ but closed at low $[K^+]_o$ including physiological concentrations. These K-channels were suggested to be the $[Ca^{2+}]_i$-dependent ones on the basis of their pharmacological properties. The question arises here what kind of mechanism is responsible for the generation of RP in physiological $[K^+]_o$ where these K-channels were closed. The elimination of Na^+ and Cl^- from the external solution brought about no change in RP in the range of $[K^+]_o$ below 10 mM. On the other hand, polyvalent cations (Ca^{2+}, Mg^{2+} and La^{3+}) and proton depolarized the cells accompanying no decrease in their R_{in}. All of these cations and proton decreased similarly the zeta-potential measured by cell electrophoresis. These findings suggest that a surface potential contribute significantly to the RP observed in N-18 cells.

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DISTRIBUTION OF SYNAPTIC TERMINALS ON GIANT INTERNEURONS(GIN) OF LAMPREY SPINAL CORD

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The ratio of conductance increases by bath applied 1 mM glutamate, aspartate, glycine and GABA were 1, 0.7, 32 and 6.5(n=4) in Ca-free 0.5 ug/ml TTX and 1 mM 4AP perfusate. This may arise from the differences of single-channel conductance, mean open time, number and distribution of excitatory and inhibitory transmitter receptors and so forth. The distribution of transmitter receptors may be reflected by that of presynaptic terminals, according to spherical(S) or flat(F) synaptic vesicles. The vesicle area(VA,$x10^{-9}$ mm^2) and elongation factor(EF=long axis/short axis) of 10 S terminals were 1.56±0.04 and 1.33±0.02(vesicle number, n=1782) and those of 6 F terminals were 1.45±0.03 and1.62±0.02 (n=1921), respectively. The mean of the shortest distance between the synaptic vesicles (MD, $x10^{-6}$mm) in S and F terminals were 5.05±0.70 and 6.10±0.52. This may reflect the loosely packed pleomorphism of vesicles in F terminals. The differences of EF and MD were statistically significant($p<0.01$). S or F terminals associated with flattened subsurface cistern(CS or CF) were encountered(10-20%). The ratio of F terminals to S terminals on soma was 2.16(n=136) and that on dendrites was 0.69(n=159). These distributional differences of terminals may be one reason why inhibitory conductance is fairly larger than excitatory one.

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RELATION BETWEEN PHARMACOLOGICAL ACTIVITY AND MOLECULAR STRUCTURE OF THE PRESYNAPTIC RELEASE MODULATORS

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While studying the pharmacological interaction of 4-aminopyridine (4AP) and streptomycin (SM), we found that several diamines with a hexagonal skeleton modify the amplitude of endplate potentials and the fractional release (*P*) in a dose-dependent manner. This was interpreted to mean that these chemicals modulate the transmitter release from the motor nerve terminal *via* combination with a certain receptive site in the hypothetical Ca-sensitive release component X. To elucidate the characteristics of X, pharmacological activities of *m*-, *o*-, and *p*-phenylenediamines (mPDA, oPDA, and pPDA), 2,4-toluenediamine (24TDA), and 4-aminopyrimidine (4APm) were compared in the present experiments. Among the compounds studied, pPDA showed the strongest presynaptic action comparable to tetraethylammonium, oPDA was next, and mPDA, 24TDA, and 4APm followed. Peculiarity of these chemicals was their slow development of facilitatory presynaptic action. The increase in *P* was complete within 15 min in 4AP, whereas it required more than 30 min to reach a steady state in PDAs and 4APm. SM counteracted the facilitatory presynaptic action of pPDA in a competitive manner, as was the case with 4AP. Contrary to 4AP, no appreciable prolongation of the action potential invading the nerve terminal region was observed with these compounds.

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PHYSIOLOGICAL FUNCTION OF THE IDENTIFIED NEURON R14 WITH HIGH GLYCINE CONCENTRATIONS IN APLYSIA ABDOMINAL GANGLION.

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The purpose of this study was to demonstrate the physiological function of the neurons R3-R14 with high glycine concentrations in Aplysia abdominal ganglion. In previous studies it was shown that Aplysia neurons R3-R14 have high glycine concentrations, a glycine uptake system, a glycine axonal transport system and that glycine is associated with characteristic vesicles in those neurons. Their morphology indicates that R3-R14 innervate circulatory vessel musculature. Simultaneous recording from the soma of R14 and a varicosity in a branch of R14 in the aorta muscle confirmed the innervation of the muscle by R14. Intracellular recordings were obtained from R14 and the muscle fibers while simultaneously recording muscle tension with strain gage. Neither firing of R14 nor exogenous glycine resulted in any electrophysiological effect on the muscle or on junctional potentials produced in the muscle fibers by identified motoneurons. However, both bath applied glycine and firing of R14 enhanced muscle contractions induced by stimulation of the vulvar nerve. Thus glycine appears to modulate Aplysia muscle contraction. Mechanisms of the action of glycine on the anterior aorta muscle were discussed.

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IONTOPHORETIC APPLICATION OF EXCITATORY AMINO ACIDS IN PLOTOSUS ELECTRORECEPTORS

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Certain amino acids are known to excite afferent nerves in the acoustico-lateralis receptors. L-glutamate (Glu) and/or L-aspartate (Asp) were applied iontophoretically by multi-barrel electrodes to in situ ampullae of Plotosus electroreceptor, to induce single-unit afferent discharges. In suppressed ampullae Glu induced a facilitation of a smooth time course, with peak frequency reaching up to 120 - 210 Hz (30 cases). The peak response increased sigmoidally over one log unit in applied current. Hill's plot of the response maxima gave straight lines with slope of 2 - 3 (mean: 2.7). On paired application Glu response appeared to simply summate to result in further facilitation. Applied to the same spot, Asp also produced a facilitation, but at a higher dose. The latency and time course of Asp responses clearly differed from those of Glu response, suggesting different receptor sites for them. Combined application further revealed that Glu response was markedly potentiated by a conditioning Asp pulse even at a dose well below the threshold to Asp. The potentiation long persisted even for 5 sec after a 100 msec Asp pulse. In contrast, a conditioning Glu pulse of similar duration only resulted in a summated response as expected. These data suggest that Glu mimics the action of neurotransmitter, while Asp may exert its action by a different mechanism.

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EXCITATORY ACTIONS OF GLUTAMIC ACID AND HOMOCYSTEIC ACID ON CA3 NEURONS IN THE HIPPOCAMPUS.

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The actions of D- and DL-homocysteate (DH and DLH) on CA3 neurons were studied in thin sections of the guinea pig hippocampus. DH and DLH administered to the stratum radiatum induced large depolarizations in CA3 neurons. The responses to DH and DLH were accompanied by increases in membrane conductance. The amplitudes of the responses increased and decreased, respectively, during tonic hyperpolarizing and depolarizing currents. In neurons injected with Cs^+, these responses were reversed in polarity at membrane potentials of -13 to -19 mV. These results indicate that receptors for homocysteate in the hippocampus have different properties from those found in the spinal cord and are quite similar to glutamate receptors.

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Effects of a spider (Nephila clavata) toxin on the glutaminergic synapses

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Effects of a neurotoxin (JSTX) separated from a spider (Nephila clavata, Joro spider) venom on glutaminergic synapses were studied using crustacean neuromuscular junctions. JSTX selectively and irreversibly blocked the excitatory postsynaptic potential (EPSPs) of lobster neuromuscular junctions without affecting the inhibitory postsynaptic potentials. Threshold dose of JSTX for suppressing EPSPs corresponds to a few percent of one venom gland. Glutamate potential in the postsynaptic membrane was abolished by JSTX but aspartate-induced depolarization was unaffected. Kainic acid potentiated the glutamate potential in lobster muscle membrane but it had no effect after the toxin application. Depolarization produced by quisqualic acid was suppressed by JSTX showing that quisqualate is a strong agonist of glutamate. Effects of JSTX were further studied using brain slice of guinea pig. A relatively large amount of JSTX suppressed EPSPs in the pyramidal cells of CA_3 of the hippocampus and also postsynaptic potentials in the piriform cortex. Whereas the toxin failed to block postsynaptic potentials in the superior colliculus by optic tract stimulation.

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EFFECTS OF AMINO ACIDS ON PYRAMIDAL NEURONS IN RAT PREPYRIFORM CORTEX NEURONS. HORI, N. AND CARPENTER*, D. O. Dept. of Pharmacol., Faculty of Dent., Kyushu Univ., Fukuoka and *NY State Dept. of Health, Albany, NY 12201

It is generally believed that the excitatory neurotransmitters released by the lateral olfactory tract (LOT) on the pyramidal cell (p-cell) of the rat are amino acids, probably glutamate and/or aspartate. Those evidence, however, are not definitive. Electrical patterns produced by amino acids iontophoretically applied to the dendrites of P-cells varied depending on the amino acid applied. Tangental slices were cut by hand from the olfactory cortex upon which intracellular recordings were obtained using microelectrodes. The resting potentials of P-cells were about 70 mV, and action potentials respond to LOT stimulation were about 75 mV. Depolarization superimposed by train pulses were observed by iontophoresis of amino acids; glutamate, aspertate, N-methyl-DL-aspartate (NMDLA) and kainic acid (KA). The depolarization produced by NMDLA and KA was of a long duration. These amino acids caused an increase in conductance. The mechanisms by which NMDLA and KA produced the depolarization is discussed.

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PROPERTY OF ACETYLCHOLINE-RECEPTORS ON CHOLINOCEPTIVE NEURONS IN THE BRAIN STEM CULTURE. SAJI, M. AND MIURA, M. Dept. of Physiol., 1st Div., Gunma Univ. Sch. Med., 3-39-22 Showa-machi, Maebashi 371

Cultures of dissociated cells from the lower brain stem of 14-15 day-old rat embryos were prepared. The property of acetylcholine(ACh)-receptors on cholinoceptive neurons was studied by electrophysiological and pharmacological techniques. Test substances were applied iontophoretically on the surface membrane of the cell body through a multibarrel micropipette. ACh facilitated the unit discharges in 41 out of the total 89 neurons tested, and inhibited those of only one neuron. The ACh-responses were completely blocked by atropine, and also partially by d-tubocurarine. Both nicotine- and muscarine-responses were equally antagonized by the independent application of atropine and hexamethonium. Since these cholinoceptive neurons showed always a mixed nicotinic-muscarinic property, we thought both nicotinic and muscarinic receptor-sites in these cholinoceptive neurons may be functionally linked each other. Furthermore, we tried to express the mixing degree of the two kinds of receptor-properties, and found that they were varied widely from neuron to neuron with nicotinic property dominancy.

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MODULATION OF THE SENSITIVITY OF NICOTINIC ACH-RECEPTORS BY NEUROTRANSMITTERS. KOKETSU, K., AKASU, T. AND MIYAGAWA, M. Dept. of Physiol., Kurume Univ. Sch. Med., Kurume

In general, the efficiency of a monosynaptic transmission is represented by the amplitude of postsynaptic current which is generated at postsynaptic membrane. It has been well known that the amplitude of postsynaptic current induced by a neurotransmitter is modulated by the actions of other neurotransmitters. The present paper reports the experimental evidences supporting our new hypothetical concept that the amplitude of postsynaptic current can be modulated not only by the changes in the amount of active release of a neurotransmitter (presynaptic modulation) but also by the changes in the sensitivity of receptor to this particular neurotransmitter (subsynaptic modulation).

The sensitivity of receptor can be estimated by measuring the postysnaptic current, which is induced by direct application of the neurotransmitter to receptor, by means of the voltage-clamp experiment. The maximum amplitude of ACh-currents induced by iontophoretic applications of ACh to the nicotinic receptor of the frog skeletal muscle end-plate or the bullfrog sympathetic ganglion cell, was decreased by catecholamine, 5-HT, histamine and some polypeptides (P-substance, LHRH), while it was increased by ATP. The mechanisms underlying these subsynaptic modulatory actions have been analysed to some extents.

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NOISE ANALYSIS OF ACh-OPERATED CHANNEL IN A BULLFROG SYMPATHETIC GANGLION CELL.
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Experiments were carried out in the presence of atropine, whose depressant actions on the nicotinic response was almost desensitized under the present experimental conditions. The fast excitatory postsynaptic current (f-EPSC) decayed with a bi-exponential time course, the fast phase comprising about 80% of the total amplitude. The time constant (τ_f) of the fast phase was 3.5 msec, while that of the slow phase (τ_s) was 20 msec. The decay phase of a miniature EPSC showed a similar bi-exponential function. Both τ_f and τ_s increased with membrane hyperpolarization. Measurement of ACh noise also revealed two-kinetic components, the time constants of which corresponded to τ_f and τ_s for f-EPSC. The single channel conductance (γ) obtained from the mean and variance of ACh-current was 8.7 pS, being about one-third of that of end-plate. Using fractions of the mean ACh-current for fast and slow components, which were estimated by linear extrapolation of two exponential functions of the f-EPSC decay to a peak time, the single channel conductances of fast (γ_f) and slow (γ_s) components were calculated from respective maximum spectral densities and half power frequencies. They were almost the same and about 8 pS. These results suggest that ACh-operated channel in the bullfrog sympathetic ganglion cell closes with two rate-limiting steps or that there are two distinct classes of ACh-operated channels.

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AN EPSP-AFTER HYPERPOLARIZATION AS A FUNCTION OF M-CHANNELS.
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The 9th or 10th curarized and atropinized ganglion in the isolated sympathetic chain of the bullfrog was studied both with intracellular (3M KCl) electrodes and with extracellular ones. At a given Vm following impalement, close to -50 mV, each epsp had an after-hyperpolarization (HP) whose amplitude increased with increase in amplitude of epsp. With spontaneous resting Vm's between -45 and -60 mV, 53 cells all exhibited epsp-after HP, while 5 cells at > -70 mV had none. With resting Vm hyperpolarized by applied current, epsp-after HP decreased, finally disappearing at Vm between -60 and -65 mV. An after-HP following depolarizing current pulse with 20 msec in duration showed the same membrane potential dependancy as that of epsp-after HP, indicating that epsp-after HP results from a transient depolarization of cell membrane but not from any transmitter mediation. Epsp-after HP was selectively eliminated by 10 μM muscarine but not by 5 mM tetraethylammonium. It is thus concluded that epsp-after HP results from a temporary opening of "M-channels" (Brown & Adams, 1980). In the intact frog's sympathetic ganglion (10 preparations), the surface-recorded compound epsp was never followed by an after-HP. This indicates that the resting membrane potential of intact cell is assumed to be more negative than at least -60 mV.

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EFFECTS OF DENERVATION AND AXOTOMY ON INCREASED CYCLIC GMP LEVEL INDUCED BY ELECTRIC STIMULATION AND HIGH POTASSIUM-DEPOLARIZATION IN SUPERIOR CERVICAL SYMPATHETIC GANGLION OF THE RAT.

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When electric stimuli were applied to preganglionic nerve of excised superior cervical sympathetic ganglion of the rat during aerobic incubation, strong acceleration of cyclic GMP content was observed in the ganglion. This increase was blocked by the addition of muscarinic ACh antagonist, atropine, or operations of either denervation or axotomy.

Increase of extracellular potassium ion concentration or addition of veratridine in the medium containing calcium ions caused remarkable increase of ganglionic cyclic GMP content. This increase was not affected by the addition of ACh antagonists, but was completely blocked by denervation treatment. The depolarization-induced increase of cyclic GMP level was fairly maintained for 4 days after axotomy operation, then it went down gradually.

These data suggest that there are two sites of cyclic GMP production system in the ganglion; one is in postganglionic, and is coupled with muscarinic ACh receptor in the ganglion cells, the other is in preganglionic nerve terminals, where ACh release mechanism is associated in response to membrane depolarization in the presence of calcium.

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EFFECTS OF K-CHANNEL BLOCKERS ON TRANSMITTER RELEASE IN BULLFROG SYMPATHETIC GANGLIA.

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Effects of tetraethylammonium (TEA), 4-aminopyridine (4-AP) and Cs^+ on transmitter release in bullfrog sympathetic ganglia were studied with an intracellular electrode. TEA (25-500 μM) produced a reversible and dose-dependent increase in the quantal content of fast excitatory postsynaptic potential (f-EPSP) with a decreased quantal size. Both 4-AP (0.6-5 μM) and Cs^+ (5-50 x 10^3 μM) caused a progressive and dose-dependent rise in quantal content which was slowly reversible. In contrast to TEA actions, the quantal size and ACh potentials were unaffected by these two agents or increased slightly at high concentrations of Cs^+. All these K-channel blockers lengthened the synaptic delay, suggesting that the increased quantal content is due to a prolongation of the falling phase of an action potential at the presynatpic terminal through an action on K-channels. The frequency of miniature EPSP was increased in the presence of 4-AP (2.5 μM) and Cs^+ (100 μM), while it tended to be reduced by TEA (25 μM). It was suggested that K-channel blockers affect transmitter release at the preganglionic terminals not only by increasing a Ca^{2+}-current during an action potential, but also by other mechanisms, e.g. alteration in the resting level of the intracellular Ca^{2+} concentration.

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TRANSFORMATION AND SYNAPTOGENSIS OF DISSOCIATED ADRENAL CHROMAFFIN CELLS OF RAT IN VITRO

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Adrenal chromaffin cells, dissociated from 10 day-old rats, were plated in culture dishes containing 2.5S nerve growth factor. Electronmicroscopical examination showed that the chromaffin cells were transformed into neuronal cells and formed synapses with each other. Synaptic interactions were demonstrated electrophysiologically with intracellular recording techniques. Stimulation of single presynaptic cells gave rise to simple or complex excitatory postsynaptic potentials (EPSPs) in postsynaptic cells. The responses depended on the extracellular calcium and magnesium concentration, the evoked EPSPs increased in size and duration with the calcium concentration and decreased with high magnesium. Cobalt and manganese counteracted calcium and not only abolished the response but also suppressed the firing of cells. The reversal potentials of the evoked EPSPs were estimated by changing the membrane potential of postsynaptic cells and were between -10 and -18 mV (six cases), these values corresponded with acetylcholine reversal potentials which were evoked by iontophoretically applied acetylcholine. The nicotinic blocking agents, d-tubocurarine, hexamethonium and tetraethylammonium attenuated or abolished the EPSPs at moderate concentrations. However, α-bungarotoxin was effective only at high concentrations.

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THE EFFECTS OF PENTOBARBITAL-SODIUM AND DIAZEPAM ON THE POSTSYNAPTIC RECEPTOR ACTIVITIES OF VARIOUS TYPES.

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The ganglion cells of Aplysia include postsynaptic receptors for acetylcholine(ACh), dopamine(DA), GABA, glutamate, glycine, noradrenaline, serotonin, and β-alanine. The receptor activities of these types were evaluated by a conductance change recorded from each receptor membrane, and the effects of pentobarbital-sodium(PB) and diazepam(DZ) were compared among different types of receptor activities.

The dilute solutions of both PB and DZ below 10^{-6} M exhibited a slight but significant augmenting effect on certain types of receptor activities. Augmented by DZ were ACh-induced Cl^-- and Na^+-dependent responses, DA-induced K^+-dependent response, and GABA-induced Cl^--dependent response, whereas the augmented by PB was only GABA-induced Cl^--dependent response. The higher concentrations above 10^{-5} M of both PB and DZ depressed dose-dependently almost all types of receptor activities examined, though the rate of depression varied among the types.

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LIDOCAINE ACTION ON THE EPSP OF FROG SPINAL MOTONEURONE

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It has been reported that synaptic transmission is susceptible to local anesthetics at spinal motoneurone. In this study, lidocaine actions on amplitude and time course of the monosynaptic EPSP produced by lateral column stimulation were investigated in the isolated, intra-arterially perfused spinal cord. In the presence of low concentrations of lidocaine not to produce conduction block of the presynaptic fibers, decrease in peak amplitude, shortening in time to peak and slowing in decay time were observed in stimulus-induced and unit EPSPs. Decrease in amplitude of the EPSPs was greater in the former than the latter. Lidocaine also decreased frequency of spontaneous miniature EPSPs and increased occurrences of failure of the responses to the stimulus. Moreover, lidocaine decreased in amplitude of Ca^{2+}dependent afterhyperpolarization following spike potential. These results suggested that lidocaine may have direct action on the postsynaptic membrane and a possible effect on transmitter release from presynaptic terminal.

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EFFECT OF NEUROTRANSMITTERS ON THE VOLTAGE-DEPENDENT CURRENTS IN BULLFROG SYMPATHETIC GANGLION CELLS.

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It is well documented that the transmission of nerve impulses from presynaptic to postsynaptic neurones is modulated both presynaptically and postsynaptically at a synaptic junction. A common mode of the postsynaptic modulation is to control the membrane excitability by producing postsynaptic potentials. Another postsynaptic modulation has been reported to take place by inducing the changes in action potentials. The present experiment was performed to evaluate the hypothetical concept that neurotransmitter directly controls the action potential of the neurones by use of voltage clamp technique.

Voltage-dependent inward current is composed of Na^+ (I_{Na}) and slow Ca^{2+} (I_{si}) current components, while outward K^+ current is composed of the delayed rectifier current(I_{K1}) and slow outward current (I_{K2}) which corresponds to the M current.

Muscarinic action of ACh markedly depressed the I_{K1}, I_{K2} and I_{si}. Adrenaline (Ad) depressed I_{K1} and I_{si}, while it caused no significant changes of I_{K2}. Muscarinic action of ACh and Ad did not suppress the I_{Na} recorded in low Na^+ Ringer solution at 4 °C. Luteinizing hormone releasing hormone (LHRH), substance-P and ATP mainly depressed I_{K2}. The modes of these actions were analysed to some extents.

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ANALYSES OF ADRENALINE ACTIONS ON THE Na^+-K^+ PUMP IN FROG SKELETAL MUSCLES.

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Adrenaline (Ad) markedly increased the ouabain-sensitive component of $^{22}Na^+$-efflux due to the stimulating effects on the Na^+-K^+ pump activity in frog skeletal muscles. These facilitatory effects of Ad were manifested with the following modes of actions: 1) Characteristic dose-response action of Ad; effects of Ad was revealed at 0.1 μM and increased with increasing concentrations, then finally saturated at more than 10 μM. 2) Specificity of Ad action; the other biogenic amines were less effective (noradrenaline, dopamine) or non-effective (histamine, serotonine). 3) Elimination of Ad action by β-blocker; the Na^+-efflux increase caused by 1 μM Ad was blocked by 3 μM propranolol but not by 3 μM phenoxybenzamine. 4) Desensitization of Ad action; when Ad was applied repeatedly, facilitatory effects were reduced in secondary application as compared with first application. These modes of the facilitatory action of Ad on the ouabain-sensitive Na^+-efflux suggest that β-adrenergic receptive site plays an important role to modulate the Na^+-K^+ pump activity in the frog skeletal muscle membrane. The proteinic complex localized on excitable membranes, namely the Na^+-K^+ ATPase-β-adrenergic receptor complex, may be a physiological functioning unit to operate and also regulate the membrane machinery driving the Na^+-K^+ pump system.

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ROLE OF CHLORIDE IONS IN HIGH POTASSIUM-INDUCED HYPERPOLARIZATION. YAI, H. Dept. of Physiol., Saitama Med. Sch., Moroyama, Iruma-gun, Saitama 350-04.

Membrane permeability to Cl ions increases during high K-induced hyperpolarization observed on snail neurons. This has been postulated as a result of activity in some inhibitory presynaptic fibers depolarized by high K. Acetylcholine (ACh) also hyperpolarizes some snail neurons through increase of permeability to Cl ions. Cells were chosen which showed these two kinds of hyperpolarizations, and the reversal potentials of them were compared in the same cells. The values were -79.7 ± 6.6 (S.E.M.) mV and -106.5 ± 9.7 mV respectively, being more negative of ACh-induced hyperpolarization than high K-induced one. When all Cl ions were removed from the perfusing solution, both hyperpolarizations were converted into depolarizations in the beginning, and then ACh response disappeared within 15 - 20 min. On the other hand, high K response remained depolarized as far as Cl-free perfusion continued. It is concluded that synaptically mediated hyperpolarization resulted from the increase in membrane permeability to Cl ions dominates the depolarizing effect of high K.

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ON THE INHIBITION OF AMINOPYRIDINE RAPID COOLING CONTRACTION. ANRAKU, M. and HASHIMURA, S. Dept. of Physiol., Fac. of Med., Kagoshima Univ., Kagoshima

The contraction resulted by ACh release from the nerve terminal by the combined effects of aminopyridine and cooling (AP-RCC) is inhibitted by TTX, D-600 and Mn^{++}. These inhibitions were studied on the frog muscle treated with dTC and 4-aminopyridine (30 uM) by analysing the endplate potential which takes place spontaneously and repetitively during cooling (cooling EPP) and the endplate potential elicited by nerve stimulation (EPP). TTX ($0.5\text{-}3\times10^{-9}$M) decreased the frequency of the cooling EPP but not the amplitude of the EPP. D-600 ($0.5\text{-}5\times10^{-5}$M) decreased both of the frequency of the cooling EPP and the amplitude of the EPP although the decrease of the EPP was less. Mn^{++} (0.05-0.5mM) decreased both of the frequency of the cooling EPP and the amplitude of the EPP significantly, but the lowering of Ca^{++} (0.4mM) made the decrease of the former significant leaving the latter less changed. It is concluded that the inhibition of the AP-RCC by TTX was resulted from the membrane stabilizing effect and that those by D-600 and Mn^{++} are from the decrease of the frequency and the amount of transmitter release.

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EFFECTS OF DIVALENT CATIONS ON THE TIME COURSE OF TETANIC POTENTIATION OF THE FREQUENCY OF MINIATURE END-PLATE POTENTIALS IN THE FROG. NARITA, K., KITA, H. AND YASUGI, E. Dept. of Physiol., Kawasaki Med. Sch., Kurashiki

Tetanic stimulation (50 Hz, 2-3 min) of the motor nerve increases the frequency of miniature end-plate potentials (m.e.p.p.s) in saline solutions made without Ca^{2+} but containing 1 mM Mg^{2+}EGTA, or in solutions containing Ca^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+} or Mg^{2+} as a sole divalent cation. The time course for the tetanic potentiation of m.e.p.p. frequency was analyzed by plotting $(F\text{-}F_0)^{-1}$ against $(10^t)^{-1}$, where F is the m.e.p.p. frequency, F_0 the basal frequency before a tetanus and t the duration of the tetanus. When plotted on these coordinates the points obtained in $0Ca^{2+}$-Mg^{2+}EGTA solution fell close to a straight line. As the concentration of Ca^{2+} was raised (up to 0.3 mM), the relation between the two variables changed from linear to concave giving curves of the second order. The same relationship was obtained in Sr^{2+} solution (0.25-0.4 mM). The plot of the data obtained in solutions containing Mn^{2+} (0.2-5 mM), Co^{2+} (1-5 mM) or Mg^{2+} (2-11 mM) gave a straight line irrespective of the concentration of these ions. It is suggested that the action of these divalent cations on tetanic potentiation of m.e.p.p. frequency is similar to that on evoked acetylcholine release.

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A STATISTICAL METHOD FOR TESTING POISSONIAN NATURE OF MINIATURE END-PLATE POTENTIALS ACCOMPANYING FREQUENCY CHANGE IN TIME

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Poissonian nature of spontaneous transmitter release at neuromuscular junction has been examined since it's first observation by Fatt and Katz. In this presentation, an extended statistical method, capable to test the Poisson hypothesis for nonstationary miniature end-plate potentials (m.e.p.p.s) accompanying frequency change in time was introduced. Since the transformation rule of nonstationary Poisson process to stationary Poisson process by time-scaling associated with time course of mean frequency change is known, the first step of the method is estimation of the time course of mean frequency change. The maximum likelihood parameter estimation method is adopted for the specific case of exponential trend in the mean frequency . The next step is time-scaling according to the estimated timecourse of the mean frequency. Then, parameter estimation accuracy is estimated. If expected errors are reasonably small, conventional tests for Poisson process are applied to the transformed m.e.p.p. occurence times. The method widely extended applicable data range for the statistical analysis, since most of observed m.e.p.p. under various experimental conditions show nonstationarity. The validity of the method was demonstrated by applying the method to artificial nonstationary Poisson process with exponential trend in the mean frequency change.

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A STATISTICAL ANALYSIS OF RELEASE OF SPONTANEOUS MINIATURE END-PLATE POTENTIALS IN THE FROG SKELETAL MUSCLE.

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Spontaneous miniature end-plate potentials (m.e.p.p.) were recorded intra- and extra-cellularly at neuromuscular junction of the frog sartorius muscle. The m.e.p.p. occurence times were at first automatically detected with associated m.e.p.p. waveforms. Since detection errors were expected to occur, all detected times were edited by the visual check of associated waveforms. The frequency of the m.e.p.p. in many cases showed to be changing with a slow trend, even though the experimental conditions were kept steady. When such trend was observed in a data set, the time conversion procedure precedingly presented (Yana and Takeuchi) was applied to the data set, and the conventional statistical tests for Poisson process were employed. In the intracellularly recorded data under the physiological range of the external solution, Poisson hypothesis of the m.e.p.p. release was not rejected, whereas under the application of 1-3 mM theophylline, producing some large-sized spontaneous end-plate potentials, showed initial positive departure (within 10 msec from the origin) of the intensity function from the Poisson expectation.

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EFFECTS OF SEVERAL CHEMICAL MODULATORS ON NEUROMUSCULAR TRANSMISSION.

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Effects of thiamine, thiamine antagonists, derivatives of adenine and NAD on the transmitter release processes were investigated. Thiamine, toxopyrimidine and thiamine tetrahydrofurfuryl disulfide had a weak accelerating effect but thiamine strongly inhibited the postsynaptic sensitivity. Pyrithiamine had a weak inhibitory effect at pre- and postsynaptic levels. All of NAD, NADP, adenosine and adenine had a weak depressing effect while nicotineamide had no effect. Pyrithiamine and adenosine had no antagonistic effect on 4-aminopyridine (4AP). Further study was required on the function of the receptors of 4AP. The computer simulation of the mathematical model of presynaptic membrane potential and Ca current suggested the possible existence of a direct modification of the inactivation process of the Ca gate particle with 4AP.

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INTRA- AND EXTRACELLULAR RECORDING FROM A CRAB NEUROSECRETORY SYSTEM.

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The neurosecretory system of the crab eyestalk was isolated intact from Cardisoma carnifex or Podophthalmus vigil and mounted and perfused in a double chamber. Spontaneous activity of the axon tract, recorded over the resistance between the chambers, was always present. Spontaneous, overshooting impulses intracellularly recorded in terminals of the sinus gland can often be correlated with a unit in the extracellular record; the polarity indicates that such activity originates near the somata in the X-organ. Bursting, both spontaneous and evoked, is sometimes observed during intracellular recording from somata, axons or terminals. Terminals have Na- and Ca-mediated action potentials of 5-20 msec duration, as indicated by tests using Na-free, TTX ($3X10^{-7}$ M) or Cd (0.2 mM) saline. TEA (50 mM) increases 1/2 amplitude duration up to 50-fold. Axons have faster (2-5 msec) impulses which are Na-dependent; TEA has little effect. Recordings from somata often have no action potentials or electrotonically decremented impulses. Some have overshooting action potentials; these are both Na- and Ca-mediated. TEA increases their duration about 10-fold. Lucifer yellow injections into terminals reveal that a single axon branches to form numerous dilatations and bulbous terminals. Experiments done at Békésy lab., U. of Hawaii, supported by NIH Grant NS 15453 to I. Cooke.

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ROLE OF THE SHWANN CELL ON POLYNEURONAL INNERVATION OF TEH FAST TWITCH MUSCLE.

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During the elimination of polyneuronal innervation, morphological changes in the end plate were investigated in the triceps of the mouse over periods new born to 30 days old by means of a double staining method (a silver impregnation and ChE) and electron microscope. Under light microscope at the early stage of elimination (2 days old), either the proper axon terminal or the exess axon terminal were characterized by the shape of Y. After that the exess axon terminal changed into the so called retracting bulb. The elimination was accomplished by 10 days old. Under electron microscope, severe constriction of the exess terminal tightly wound by the Schwann processes were observed during the process of the elimination. The terminal was partially stripped off from the basement membrane and retracted into the Schwann cell soma. Large internalization of the cytoplasma membrane closely associated with the processes were also appeared in the bulbus terminal which contained a small number of synaptic vesicles.

These results suggest that the Schwann cell may act on the exess terminal for its reduction and elimination and that the cytoplasma membrane of the bulb may be treated as membrane whorls.

No Wallerian degeneration was observed in the terminals between the basement membrane and the Schwann cell in the end plate.

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STIMULUS-RESPONSE FUNCTIONS OF CUTANEOUS MECHANORECEPTORS IN THE TOAD'S FEMORAL REGION. SAKADA, S., KURIHARA, T. & KATOGI, K.
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By separating mechanosensitive units from the femoral cutaneous nerve innervating the skin of the toad's femur, the receptors composing the units were classified as Rapidly adapting receptor a(RAa), Rapidly adapting receptor b(RAb) and Slowly adapting receptor(SA) according to their responses to the ramp and pulse mechanical stimulations. Whereas RAa receptors respond to the moving phase of the ramp stimulation by 1∼3 impulses, their velocity thresholds are higher than 1 μm/ms and they were insensitive to thermal stimulations, RAb receptors are sensitive to cooling and their velocity thresholds are lower than those of RAa receptors(lower than 1 μm/ms). The adaptation time of SA receptors is 10∼600 s, their amplitude thresholds are higher than RAa and RAb receptors and they are sensitive to cooling. Each single unit is composed by a uniform kind of receptors, such as 5∼18 RAa, 1∼37 RAb or 3∼19 SA receptors. There was no single unit composed of a mixture of these receptors.

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CALCIUM SPIKE IN RAT SPINAL MOTONEURONS. HARADA, Y. AND TAKAHASHI, T. Dept. of Physiol., Fac. of Med., Kyoto Univ. Kyoto

Intracellular recordings were made from motoneurons of the newborn rat (1-14 days old) spinal cord isolated and perfused *in vitro*. After blocking the Na^+ spike by tetrodotoxin (10^{-6} g/ml) or low $[Na]_o$ (10 mM), Ca^{2+} spike appeared either in high $[Ca]_o$ (10-20 mM) or in the presence of tetraethylammonium (TEA: 10 mM). The Ca^{2+} spike produced by depolarization had an overshoot, which was all-or-none in nature and completely and reversibly blocked by Mn^{2+} or Co^{2+} (2-10 mM). The Ca^{2+} spike was also produced by the Na^+ spike in the presence of TEA. In normal condition, motoneuron spike is followed by the afterdepolarization (a.d.p.) and afterhyperpolarization (a.h.p.). The a.d.p. was shown to be enhanced in high $[Ca]_o$ and was abolished in Ca^{2+} free Mn^{2+} saline. In paralell to the a.d.p., the a.h.p. was also dependent on $[Ca]_o$ as well as on $[K]_o$. It is concluded that the Ca^{2+} spike component exists in the motoneurone, which is normally masked by the potential dependent K^+ conductance and appears as the a.d.p. The Ca^{2+} entry might produce the a.h.p. by inducing an increase in the K^+ conductance, thus serving to control the discharge frequency of the motoneuron.

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AUTORADIOGRAPHY OF A SINGLE NEURON WITH [^{14}C]-2-DEOXYGLUCOSE TECHNIQUE ARAI, O. SAITO, N. AND KIMURA, K.* Dept. of Physiol. and The Lab. of Medical Sci. Section of M. E.*, Dokkyo Univ., Med. Sch., Mibu, Tochigi 321-02

The autoradiography with [^{14}C]-2-DG was applied to measurement of quantitative uptake in soma of a single neuron. A single neuron dissected from crayfish stretch receptor was placed in van Harreveld's solution containing approximately 2μCi/ml of [^{14}C]-2-DG under tension applied to maintain a constant firing frequency. The neuron was rinsed and fixed in formalin after the stimulation. The density map of the autoradiogram was processed with the digital image processer(PDP-11/40 mini-computer). The configuration of a soma was identified by the processed pattern of density. The density can be confined to the cell soma. This allows to exclude the level of contamination in surrounding connective tissues. The [^{14}C]-2-DG consumption of the neuron which was measured immediately after stimulation increased linearly and was related to the logarithm of the impulse frequency to the first approximation in the range of 1 to 50 Hz. This relationship agrees with the result of liquid scintillation counting(refer to NEUROSCIENCE LETTERS supplement,1982).

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FIELD POTENTIAL ANALYSIS AND IDENTIFICATION OF ALPHA-MOTONEURONS WITH INTACT DORSAL AND VENTRAL ROOTS. Y. ATSUTA, T. SAKAMOTO* AND S. MORI* Dept. of Orthop., Asahikawa Med. Coll. Asahikawa ; Dept. of Physiol., Asahikawa Med. Coll., Asahikawa*

To study the neuronal activity of alpha-motoneurons of a reflex standing decerebrate animal, it is necessary to identify motoneurons keeping dorsal and ventral roots intact. To do this, we stimulated distal branches of motor nerves imbedded in muscles. This intramuscular stimulation made it possible to antidromically identify motoneurons innervating hindlimb extensors during reflex standing posture. Characteristics of the intramuscular stimulation for identifying motoneurons were summarized as follows. (1) Field potentials recorded within or near the motoneurons pool were biphasic ones consisted of both antidromic and orthodromic components. (2) There were three response patterns of motoneurons. Type 1 motoneurons showed antidromic firing when stimulus intensity exceeded threshold. In type 2 motoneurons, orthodromic excitation with EPSP was first elicited and with an increase of the stimulus intensity, antidromic spike potential appeared. In type 3 motoneurons, EPSP was first elicited followed by the orthodromic spike potential and finally antidromic spike potential occurred with an increase of the stimulus intensity. Most frequently encountered response pattern was type 2(75%). (3) The intensity of intramuscular stimulation for antidromic activation of motoneurons was about twice that of the direct nerve stimulation.

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EFFECTS ELICITED BY THE STIMULATION OF THE DORSAL PART OF THE MIDLINE TEGMENTUM (DTF) OF THE PONS UPON THE SPINAL ALPHA-MOTONEURON. T. SAKAMOTO, K. KAWAHARA, S. MORI AND Y. ATSUTA* Dept. of Physiol., Asahikawa Med. Coll., Asahikawa ; Dept. of Orthoped., Asahikawa Med. Coll., Asahikawa*

Acute precollicular-postmammillary decerebrate cats are capable of maintaining a reflex standing posture due to postural muscle tone developed after decerebration. In such an animal, DTF stimulation (rectangular pulse 0.2ms, 50 pulses/s) gradedly reduced tonic discharges of bilateral hindlimb extensor muscles and the forces exerted at the foot contacts of the hindlimbs depending on the stimulus intensity. Reduced levels of postural muscle tone persisted even after termination of DTF stimulation. Alpha-motoneurons innervating hindlimb extensor muscles (M.gastrocnemius, M.soleus, M.biceps femoris) were antidromically identified by stimulating the nerve endings intramusculally. The changes in the activity of the soleus motoneurons were simultaneously recorded with those of extensor muscle activity and the forces before, during and after DTF stimulation. The time course of the changes in the motoneuron activity was similar to that of observed changes in postural muscle tone : (1) spontaneous discharges of motoneurons were suppressed with hyperpolalization of membrane potentials and (2) generation of antidromic spike potentials were suppressed.

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AFFERENT PATTERN OF INNERVATION TO SYRINGEAL MOTONEURONS(nXIIts)
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Bird vocalization is controlled by four bilateral nuclei; the caudal portion of the hyperstriatum ventrale(HVc), the robust nucleus of the archistriatum(RA), the nucleus intercollicularis(ICo), and the tracheosyringeal portion of the hypoglossal motor nucleus(nXIIts). nXIIts directly innervates the syringeal muscle, and produces vocalization. Different pattern in afferent innervation to nXIIts between the mynah and the pigeon is compared by using HRP method.

HRP injection is confined carefully to an area of unilateral nXIIts. HRP labelled neurons of the mynah appeared in two areas of the brain; the bilateral doso-medial portion of ICo in the midbrain, and the ipsilateral RA in the archistriatum of the forebrain. The neurons of the pigeon were labelled in the bilateral ventro-lateral portion of ICo, while no prominent projection was found in the archistriatum. nXIIts of either bird receives bilateral innervation of ICo. However, the mynah has double innervation to nXIIts whereas the pigeon has not. The difference of the neural substrate correlates with their behavioral pattern of vocalization: the mynah learns vocalization; the pigeon does not learn.

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AN ANALYSIS OF THE PRIMARY AFFERENT DEPOLARIZATION IN EXTENSOR AND FLEXOR OF THE BULLFROG

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The primary afferent depolarization (PAD) of the single fibers in both of the extensor and flexor nerves of the foot joint of the bullfrog was analysed in terms of the input-output relation. In these muscle nerves,the PAD was generated mainly in the less thick fibers,although the thick fiber was not excluded. Considerable number of nerve fibers has been found to generate no PAD. Generaly,the PAD in the flexor was more significant than that in the extensor. Among the afferent nerve fibers,the less thick fibers contributed more significantly to the generation of the PAD than the thick one. Because the thick peripheral nerve was far effective than the thin one to generate the PAD,the unspecific convergence was supposed to play an important role for the generation of the PAD,although some characteristic combination of modalities has been suggested.

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INFLUENCES OF NECK AFFERENTS ON SPINAL ACCESSORY MOTONEURONS.

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Evoked responses in the spinal accessory nucleus of cats were recorded with microelectrodes intracellularly on stimulation of the accessory nerve branches, dorsal neck muscle nerves, neck cutaneous nerves and trigeminal nerve branches. Antidromic spike potentials and EPSPs were produced in the trapezius (TRAP) motoneurons [or the sternocleidomastoid (SCM) motoneurons] in response to TRAP (or SCM) nerve branch of the accessory nerve. When a stimulus was applied to another branch of the accessory nerve, e.g., SCM nerve, it elicited mainly EPSPs with a latency of 2-3 msec in TRAP motoneurons. These findings strongly suggest the existence of sensory components in the accessory nerve branches. Stimulation of the dorsal neck muscle afferents in the splenius and biventer cervicis nerves produced either EPSPs or IPSPs in the accessory motoneurons. The volleys in the splenius afferents usually evoked an excitatory effect upon the SCM motoneurons. Stimulation of the cutaneous afferents in the cutaneous colli nerve and auricular magnus nerve evoked exclusively EPSPs both in TRAP and SCM motoneurons, while the volleys in the trigeminal nerve branches (the infraorbital and lingual nerves) evoked exclusively IPSPs in the accessory motoneurons. This different effect of the cutaneous inputs upon the accessory motoneurons from the neck and face was of interest in connection with the reflex activities of the neck motor system.

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SPINAL DESCENDING PATHWAY MEDIATING SPONTANEOUS TREMOR IN MONKEYS.

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Possible descending pathway in the spinal cord has been studied in monkeys with spon spontaneous tremor after mesencephalic ventromedial tegmental lesion. Based on the previous experiments of systematic recordings of rhythmic discharge in the spinal cord, the anterior funicule was suspected to play a primary role in mediating rhythmic discharge from the higher center. So, selective lesion was made in the spinal cord by platinum microelectrode to test this hypothesis. In one monkey, lesions at C_2 level destroying most of the gray matter did not influence tremor in the ipsilateral upper limb. But successive lesion in C_3 level touching the anterior funicule almost arrested the tremor. In the next monkey, afterlesions in C_2 level destroying anterior funicule and ventromedial gray matter, tremor in the ipsilateral upper limb diminished but persisted in the shoulder. The successive lesion destroying exactly the anterior funicule identified by rhythmic discharge at medullocervical junction arrested the tremor. It was also verified by muscular injection of HRP that the trapezius motoneurons distributed from medullocervical junction to C_3 level. Therefore, it seems likely that several trapezius motoneurons were escaped from the former lesions, thus engendering remained tremor in the shoulder.

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CHANGES IN STRETCH REFLEX EMG AND STIFFNESS OF HUMAN FINGER MUSCLE

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Changes in stretch reflex gain and stiffness of the long flexor muscles of the thumb in man were examined in position control task, by applying ramp stretches to the thumb and observing the EMG activity of the flexor and the net force around the distal joint. Stretch-evoked EMG activity was averaged during the period 35 to 75 ms following the onset of the ramp stretch and stretch-evoked force during the period 75 to 115 ms. The averaged EMG and force were divided by the amount of stretch to give measure of the reflex responsiveness and the stretch-evoked stiffness, respectively. The subjects were asked to maintain a constant thumb position against applied forces.While both the reflex responsiveness and the stretch-evoked stiffness increased with increasing flexing force, they were greater in unstable load position control than in constant load position control at the same flexing force. The reflex responsiveness was much higher in unstable load position control, even at a constant level of EMG activity in the flexor muscle. The reflex responsiveness and the stretch-evoked stiffness increased with the level of cocontraction of the flexor and extensor muslces for a given flexing force.

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RELATIONSHIP BETWEEN ACUPUNCTURE AFFERENT PATHWAY IN SPINAL CORD AND MORPHINE ANALGESIA.

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Acupuncture and 0.5mg/kg intraperitoneal morphine analgesia mesured by rat tail-flick test were antagonized by intrathecal 0.2μg naloxone. Analgesia caused by 0.05μg intrathecal morphine was equivalent to 0.5mg/kg intraperitoneal morphine analgesia, and exhibited individual variation in effectiveness of analgesia just like as intraperitoneal morphine analgesia. Individual variation in effectiveness of intrathecal 0.05μg morphine analgesia was abolished by 250mg/kg intraperitoneal D-phenylalnine. Bilateral lesion of the anterolateral tracts (ALT) or unilateral lesion of the dorsal periaqueduct central gray (d-PAG) abolished 0.05μg intrathecal morphine analgesia but not abolished 0.1-0.2μg intrathecal morphine analgesia. Acupuncture analgesia was abolished by the contralateral ALT lesion which reduced 0.5mg/kg intraperitoneal morphine analgesia. Unilateral and bilateral lesiones of the d-PAG or hypophysectomy similary abolished above dose of morphine analgesia and reduced large doses of morphine analgesia. These results indicate that small doses of morphine (0.05μg intrathecal, 0.5mg/kg intraperitoneal) analgesia was caused by activation of acupuncture afferent pathway in spinal cord. This mechanism might explaine that of the extradural morphine analgesia which long lasts by small dose of morphine. Large doses of morphine prolonged tail-flick latency by direct action on spinal cord.

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EFFECTS OF ACUTE BALLOON COMPRESSION OF THE SPINAL CORD ON THE REFLEX ACTIVITIES OF THE CAT.

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Compression was given by pressure applied to a balloon mounted in the vertebral window (diameter; 1 cm) opened on the L7 spinal cord of the spinal cat. Monosynaptic reflex by stimulation of Biceps nerve frequently showed increase in amplitude at low pressure (50-150 mm Hg). At a little higher pressure (100-250 mm Hg), a transient increase of the responses at the early phase of pressure application was followed by depression. The changes were completely or partly reversible when duration of pressure application was 3-10 min. Depression was enhanced at longer application of pressure. At much higher pressure (200-400 mm Hg), depression of the responses became irreversible. Ascending responses of dorsal column showed no apparent changes before total depression of the reflex. The effect of compression on the reflex recorded at the S1 ventral root (VR) was smaller than that on L7 VR. The influences of compression were also compared among the responses to stimuli applied to different nerves and different influences were observed among monosynaptic reflex, polysynaptic reflex, posttetanic potentiation of the reflex and inhibitory action.

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GENESIS OF SCRATCHING RHYTHM IN SPINAL CATS.

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Electrical (50Hz, 5-10V) as well as tactile stimulation applied to pinna elicited scratching movements of approximately 4Hz in the ipsilateral hindlimb in C_1 spinalized cats. EMGs recorded during rhythmic scratching from hindlimb extensor and flexor muscles such as tibialis anterior (TA) and gestrocnemius(G) exhibited an alternate burst pattern. The scratch cycle consisted of long flexor (F) phase of about 200 ms and short extensor (E) phase of about 50 ms. Fictitious scratching similar to normal scratching rhythm was evoked in the nerves supplying TA and G muscles in immobilized preparations. Intracellular recording revealed that G and TA motoneurons were depolarized during the E and F phases, respectively and most of the motoneurons displayed the spike discharge at frequencies of 20-100 imp/s. In order to analyze the origin of the scratching rhythm, extracellular recordings of the descending impulses were attempted at Th 10 level. Two groups of rhythmic burst discharges were recorded in the ventral funiculus. The first group showed 2-8 spikes during E phase with the frequency of 100-170 imp/s and the second group showed 5-20 spikes during F phase with the frequency of 20-150 imp/s. These results suggested that rhythmic descending inflows from a certain source control the hindlimb motoneurons during scratching.

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ACTIVITIES OF FORELIMB MOTONEURONES DURING FICTIVE STEPPING IN DECEREBRATE CATS.

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We revealed that the repetitive stimulation of the lateral funiculus of the cervical cord (CLF) evoked fictive stepping in the forelimb muscle nerves in immorbilized decerebrate cats. To investigate the central program for stepping, we analized its spatio-temporal patterns and compared them with those of fictive stepping evoked by the stimulation of mesencephalic locomotor region (MLR). The elbow extensor (T. brachii, T) and flexor (Cleidobrachialis, CB), ipsilateral to the stimulation, showed reciprocal bursts as the case of MLR stimulation. Dorsiflexor (Deep Radial nerve, DR) was activated parallel with CB, but had longer duration of bursts. However the patterns of CLF-fictive stepping were odd, in contrast with MLR ones. The discharges of the contralateral nerves (T, CB, DR) never exhibited bursts and showed smoothly-changed excitatoly undulation and rather steep depression at the times of bursts of corresponding nerves of stimulating side. The excitatory undulation was obscure for CB. The Onset of the excitation of T and DR preceded flexor bursts by 200-800 msec. It was infered that the preceding activation of elbow extensor and dorsiflexor might be scheduled by the central program in order to support the animal during swing or flexion.

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THALAMIC AFFERENTS FROM THE PALLIDUM, SUBSTANTIA NIGRA AND CEREBELLUM IN THE SQUIRREL MONKEY(SAIMIRI SCIUREUS): A HRP STUDY.

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Organization of the pallido-, nigro- and cerebello-thalamic projections was examined by retrograde axonal transport of horseradish peroxidase(HRP) in the squirrel monkey. HRP was injected iontophoretically(5-10uA, 5 min) in the thalamic nuclei.

After injection of the enzyme into the lateral part of VApc and the dorsolateral part of VLo, the labeled neurons were confined in the pallidum internum. When the enzyme spread to the reticular thalamic nucleus, several neurons in the pallidum externum were also labeled.

In the case of injection into the medial part of VApc, VAmc and VLm, the labeled neurons were found in the rostrolateral part of the substantia nigra, pars reticularis.

After injection into the ventrolateral part of VApc and VLo, the neurons were located in the caudoventral part of the interposed nucleus and the rostroventral part of the dentate nucleus. The dorsal part of VLo at the frontal plane of its largest extent receive the projection from the interpositus and dentate nucleus, and the ventrolateral part of VLo at this level and the rostrolateral part of VPLo have projections from the interposed and fastigial nucleus. The present study confirmed our previous electrophysiological findings.

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HINDLIMB STEPPING OF A DECEREBRATE REFLEX STANDING CAT.

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During a period in which a decerebrate cat maintained a reflex standing posture, stimulations delivered in the dorsolaterally located structures to the cuneiform nucleus (stepping point) elicited hindlimb stepping on a still surface. Such a stepping point was not identical with a point in the mesencephalic locomotor region (MLR). The forces exerted at the foot contacts of the bilateral hindlimbs exhibited oscillatory waves reflecting a shifting of the weight between them. Regular hindlimb stepping was elicited only during the period in which postural muscle tone of the animal was developed within a certain range. The period of each force oscillation during regular hindlimb stepping was about 0.5s and EMGs recorded from various muscles of the hindlimb showed bursting discharges associated with each of the oscillatory waves. The higher was the existing level of postural muscle tone, the longer was the latency between the stimulus onset and the beginning of hindlimb stepping. In such a case, the period of force oscillation during initial few steps was 0.8 to 0.6s and it tended to approach to 0.5s gradually. These results suggest that postural setting is necessary before initiation of hindlimb stepping. Furthermore they indicate that stimulations of the same stepping point modify the stimulus effects depending on the existing level of postural muscle tone.

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RETICULOSPINAL NEURON ACTIVITIES ASSOCIATED WITH QUADRUPEDAL LEG MOVEMENT OF CATS.

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To analyze the discharge patterns of the reticulospinal (R-S) neurons associated with four-limb movements, the unit spikes of R-S neurons in 18 thalamic cats were recorded. Spikes of R-S neurons exhibited alternating firings during leg movements, not only stepping on the treadmill but also upon passive forward and backward movement by the experimenter's hand. R-S neurons manifested firing patterns associated with reciprocal, diagonal and quadrupedal leg movements; about half of the neurons showed reciprocal patterns upon bilateral forelimb movements. About 15% of the examined R-S neurons showed discharge patterns correlated with quadrupedal leg movements. Remainders showed reciprocal patterns upon bilateral hindlimb movements and upon fore and hindlimb movements in the same side. Results indicate that afferents of individual limbs ascend to the brainstem reticular formation, that integrative action is organized as pattern generation in that region, and that this pattern information is sent to the spinal cord via the reticulospinal tracts.

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FUNCTIONAL PROPERTIES OF THE NEURONAL CONNECTIONS BETWEEN THE MESENCEPHALIC LOCOMOTOR REGION AND THE MID-PONTINE STRUCTURES RESPONSIBLE FOR POSTURAL CONTROL. K. KAWAHARA, S. MORI AND T. TOMIYAMA* Dept. of Physiol., Asahikawa Med. Coll., Asahikawa ; Dept. of Otolaryngol., Asahikawa Med. Coll., Asahikawa*

We have proposed a hypothesis that both excitatory and inhibitory signals descending from the MLR are needed to activate the spinal stepping generator. The excitatory signal was assumed to be relayed through mid-pontine ventral tegmental field (VTF) eliciting augmentation of postural muscle tone. While, the inhibitory signal was assumed to be relayed through mid-pontine dorsal tegmental field (DTF) eliciting suppression of postural muscle tone. In this study, this possibility was investigated. After identifying the MLR, DTF and VTF, the neuronal connections between them were analysed. Antidromic field potentials were recorded in the MLR by stimulating the DTF or the VTF. Some units within the DTF and the VTF were synaptically activated by stimulating the MLR. The activity of these units tended to be modulated depending on the MLR-elicited locomotor patterns. The activity changes were related to both locomotor rhythm and muscle force during locomotion. With an increase of stimulus intensity of the MLR, the muscle force augmented and the activity of the VTF units also increased. With an increase of a belt speed of a treadmill, the activity of the tonically firing DTF unit tended to be rhythmically modulated.

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FACILITATION OF ANTIDROMIC ACTIVITY OF JAW OPENING MOTONEURONS IN THE RAT OHTA, M., SASAMOTO, K. AND MISHIMA, K. Dept. of Physiol., Fac. of Dent., Kyushu Univ., Fukuoka

Electrical stimulation of the contralateral central amygdaloid nucleus or the contralateral frontal cortex markedly augmented the antidromic field potential evoked by stimulation of the mylohyoid nerve in the rat. Intracellular recording demonstrated that this facilitation was due to EPSPs of the mylohyoid-anterior digastric (Myl-Dig) motoneurons which innervate homonymous jaw opening muscles. These EPSPs relieved IS-SD break in some motoneurons and also facilitated antidromic discharge of most motoneurons which did not show IS or M spike response to the same subthreshold antidromic stimulation.

On the other hand, the cortical antidromic pyramidal tract (PT) potential was not potentiated by the thalamocortical excitatory inputs. This is suggested to be due to different geometrical arrangement between Myl-Dig motoneurons and PT cells and we propose that subthreshold ephaptic excitation may be involved in antidromic activation of Myl-Dig motoneurons.

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INHIBITORY EFFECTS OF TOLPERISONE-HCl ON THE RAT JAW MUSCLE ACTIVITIES. FUNAKOSHI,M., NAKASHIMA,M. AND KITAGAWA,M. Dept. of Oral Physiology, Gifu College of Dentistry.

Anesthetized rats with Nembutal (45 mg/Kg) were used. The rat masseter EMG responses to the periodontal mechanical stimulation of the upper incisor were inhibited after intraperitoneal injection of Tolperisone-HCl (20 mg/Kg Muscalm, Nihon Kayaku Co.). Spontaneous discharges of the gamma motor fibers in the masseter nerve and spontaneous discharges of the muscle spindle neurons in the trigeminal mesencephalic tract nucleus were also inhibited by Tolperisone-HCl(20-50 mg/Kg) injected intraperitoneally . These results suggest that a site of action of Tolperisone-HCl as a muscle relaxant may be the gamma motor system in the brain stem.

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EFFECTS OF CUTANEOUS STIMULATION ON JAW OPENING REFLEX OF THE RAT
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Effects of cutaneous stimulation (CS) on jaw opening reflex (JOR) evoked by dental pulp electric stimulation were examined in rats anesthetized with pentobarbital sodium. The reflex EMG's were recorded from the digastric muscles. The CS was single pulse electric one and was given through needles inserted into the skin of the respective face, fore- or hind-paw. Facilitation followed by inhibition of the JOR was induced by the CS. The facilitation was observed for 30 msec after the CS. The inhibition followed and lasted for about 300 msec.

Facilitation or inhibition induced by the CS could be modified by a preceding CS. The modification occurred up to about 200 msec after a preceding one and was similar when that was applied to a different part from the second CS.

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BULBAR RETICULAR UNIT ACTIVITIES DURING FOOD INGESTION IN THE CAT.
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Single unit activities were recorded from 185 spontaneously firing bulbar reticular neurons during ingestion of solid food and milk in 5 chronically prepared cats. Firing frequency was modulated during food ingestion in 40 neurons either tonically or rhythmically. The frequency tonically increased in 9 neurons and decreased in 1 neuron. The rhythmically modulated 30 neurons were classified into 4 groups based on the relation of occurrence of spike bursts with the phase of rhythmical jaw movement: jaw-opening units (22 neurons), jaw-open units (5 neurons), jaw-closing units (2 neurons) and jaw-closed units (1 neuron). Thus, the firing of 90 % of the rhythmically modulated units closely correlated with either the opening movement or the open position of the jaw. By averaging EMG of jaw-closing muscles by spontaneous spikes of 22 jaw-opening and jaw-open units, a decrease was revealed in temporal EMG in 3 neurons and in masseter EMG in 1 neuron 1.7-2.1 ms after the onset of spikes. The latency strongly suggests that the decrease is due to monosynaptic inhibition of jaw closer motoneurons evoked by spikes of the triggering bulbar reticular neurons. We propose that there are neurons in the bulbar reticular formation which participate in generation of masticatory jaw movements by rhythmically inhibiting jaw closer motoneurons during food ingestion.

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Lingually induced PSPs in masseteric motoneurons after axotomy.
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Postsynaptic potentials produced in axotomized masseteric motoneurons (Mass.Mns) by stimulation of the lingual nerve were explored in cats. It has been reported that lingually induced PSPs in normal Mass.Mns were the IPSP-EPSP-IPSP sequence. In many Mass.Mns 30 days after axotomy an IPSP-EPSP sequence was produced by lingual nerve stimulation. In addition, in one-third of explored Mass.Mns 50 days after axotomy lingual nerve stimulation evoked only EPSPs. No reversal of EPSPs produced in Mass.Mns 50 days after axotomy by lingual nerve stimulation was obtained when the cell membrane was hyperpolarized. In Mass.Mns 30 days and 50 days after axotomy the slope of the primary range in the frequency-current relationship showed a high gain in comparison with normal Mass.Mns.

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THE REFLEX PATTERN OF THE SALIVATORY NEURONS TO STIMULATION OF THE TRIGEMINAL SPINAL SUBNUCLEUS CAUDALIS IN THE CAT. MURAKAMI, T., YOSHIHARA, M., ISHIZUKA, K. AND UCHIYAMA, M. Dept. of Oral Physiol., Nippon Dental Univ., Niigata, Niigata

This study was undertaken to define central pathway of the trigeminal-salivary reflex in urethan anesthetized cat. The salivatory neurons innervating submandibular and sublingual gland were classified into response type (56%) and non response type (44%) according to their responsiveness by the electrical stimulation of infraorbital (IO), lingual (LN) and inferior alveolar (IA) nerve. Non response type neurons did not respond to the electrical stimulation of the trigeminal subnucleus caudalis (CA). A 87% of the response type neurons were activated by the stimulation of CA. The latency range of these activity was 3.2-18.5 msec. This result would suggest that there are a few CA neurons which directly project to the salivatory neurons as suggested by the histological report. A 60% of the salivatory neurons activated by the stimulation of CA were activated via neurons of CA in stimulation of trigeminal nerve. Others would be activated via other trigeminal subnucleus in trigeminal stimulation. However, these neurons were also activated by the stimulation of CA. Therfore, these may recieve other afferent than trigeminal nerve.

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ELECTROPHYSIOLOGICAL STUDY OF DIVERGENT AXON COLLATERALS OF MEDIAL VESTIBULAR NUCLEUS NEURONS IN THE CAT. YOKOTA, J-I. and ISU, N. Dept. of Neurophysiol., Inst. of Brain Res., Sch. of Med., Univ. of Tokyo, Bunkyo-ku, Tokyo, and Nat. Aerospace Lab., Chofu-city, Tokyo.

Spikes of secondary vestibular neurons in the horizontal canal system (type I neurons) were extracellularly recorded in the medial vestibular nucleus in decerebrate cats. Axonal projection of these neurons was examined by their antidromic response to stimulation of the contralateral abducens nucleus, the descending MLF, and the spinal cord. Almost all (95%) of secondary vestibular type I neurons which sent their axon collateral to the contralateral abducens nucleus were antidromically activated by descending MLF stimulation at the level of the obex. Nearly half (43%) of these neurons projected to the level of C_1 segment in the spinal cord as well. Systematic tracking for antidromic microstimulation in the contralateral abducens nucleus and spinal gray matter at C_2-C_3 revealed that axon collaterals of single vestibular type I neurons gave off local branches in both the abducens nucleus and the motoneuron pool in the upper cervical cord. The results indicate that in the medial vestibular nucleus there are type I neurons projecting to both the abducens nucleus and motoneuron pool in the neck segments of the spinal cord. It is suggested that these neurons function as common interneurons to mediate co-ordinated movements of eye and head during horizontal rotation.

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OPTOKINETIC RESPONSES OF THE NUCLEUS OF THE OPTIC TRACT NEURONS IN PIGMENTED RABBITS. MAEKAWA, K., TAKEDA, T. & KIMURA, M. Dept. of Physiol. Jichi Med. Sch., Tochigi 329-04

In Japanese pigmented rabbits, neuron activities (n=39) were recorded with tungsten-in-glass microelectrodes in the nucleus of the optic tract (NOT) under anesthesia with N_2O-O_2 and halothane (1-2%). All cells were activated orthodromically from the optic nerve with a short latency and 28 of them also antidromically from the nucleus reticularis tegmenti pontis. Random Julesz or grating patterns were projected and moved (p-p, 30°) on a tangent screen at a constant velocity (0.1-200°/sec). PDP-11 computer was used to construct impulse density histogram. Orientation selectivity was examined in 24 neurons. Ten cells showed tonic excitation when pattern was moved in temporo-nasal direction, and one cell in the opposite direction. Thirteen had optimal orientation in vertical or oblique direction. In the preferred orientation, velocity sensitivity was studied (n=39). While optimal velocities covered a wide range (5-150°/sec), 27 cells had optimal velocity at 10-50°/sec and 10 at 75-150°/sec. Centers of receptive fields (n=25) scattered widely in naso-temporal direction within the visual streak zone. Means of length and width of them were 28° and 15°, respectively. It is shown that a substantial portion of NOT neurons (67%) can convey optokinetic signals of higher velocity (more than 30°/sec) than those previously reported and about half of them have horizontal (temporo-nasal) preferred orientation while the other half have vertical or oblique one.

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INFLUENCE OF ENVIRONMENTAL LIGHT-DARK SCHEDULE AND BILATERAL ENUCLEATION ON SUPRACHIASMATIC NEURONAL ACTIVITY USING A RAT HYPOTHALAMIC SLICE PREPARATION. SHIBATA, S.*, UEKI, S.* and OOMURA, Y.** *Dept. Pharmacol., Fac. Phamaceu., **Dept. Physiol., Fac. Med., Kyushu Univ., Fukuoka.

We have reported that single neural activity in the rat suprachiasmatic nucleus (SCN) under a normal light-dark (LD) schedule was higher during daytime (6.3 ± 3.2 Hz) than during nighttime (4.7 ± 2.4) and the activity from animals kept on a reversed LD cycle was shifted approximately 12 hr. We therefore examined the effects of a DD, LL schedule, or a bilateral enucleation on SCN activity. In enucleated rats, cells of the ventrolateral SCN, a region where mainly optic nerve fibers terminate, fired with a lower frequency (4.4 ± 2.5) during daytime than the frequency seen in normal LD housed rats (6.7 ± 3.3). However cells recorded from dorsomedial SCN, fired (5.6 ± 3.2) with a similar frequency to those in normal LD housed rats (5.8 ± 3.0). The results obtained from DD housed rats were similar to those from enucleated rats. The firing rates of cells from both regions of the SCN in LL housed rats were low during daytime (4.3 ± 2.6) as well as nighttime (4.1 ± 2.2). These results indicate that SCN neurons show changes corresponding to environmental LD schedules, which correlates well with the glucose utilization rhythm of the SCN and N-acetyl-transferase activity rhythm in the pineal body.

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ULTRASONIC VOCALIZATION-RELATED AND RESPIRATORY NEURONS RECORDED FROM THE NUCLEUS AMBIGUUS AND NEARBY STRUCTURE IN RATS. YAJIMA, Y., HAYASHI, Y. AND YOSHII, N. Dept. of Physiol., Hyogo Coll. Med., Nishinomiya, Hyogo, 663.

To identify motoneurons for ultrasonic vocalization which was elicited by electrical stimulation of the central grey matter, extracellular unitary discharges were recorded from rat ventrolateral medulla with glass micropipettes filled with Fast Green FCF dye. Two main types of neurons were distinguishable; ultrasonic vocalization-related units (V units) and respiratory units, related to either expiration or inspiration(E or I units). V units which showed little or no background activity fired with high frequency bursts prior to the sound emission and ceased firing just before the end of the sound. The duration of unitary discharge was related linearly to that of the ultrasound. During ultrasonic vocalization E units increased firing while I units ceased. Most V units were found in or very close to the ventral part of the nucleus ambiguus(NA). E or I units were located further away from the ventral part of NA.

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NEURONAL REPRESENTATION OF SPEECH PARAMETERS IN THE MEDIAL GENICULATE BODY. HASHIMOTO, T. Div. Biocybernetics, Inst. Med. Dent. Engin., Tokyo Med. Dent. Univ., Chiyoda-ku, Tokyo 101.

The neural representation of speech parameters was investigated in the medial geniculate body of guinea pigs. Single unit responses to synthesized speech-like sound stimulation were recorded from unanesthetized animals. Speech-like sounds were produced by a bank of octave band-pass filters, following the "terminal analog" model of speech production. The feature parameters of speech sound based on the voice generating system can be represented by the excitation source and the transfer characteristics of the entire vocal tract. The decoding or feature extraction of these feature parameters may be essential for the neural mechanism of sppech recognition. One medial geniculate neuron was selectively tuned to a synthesized vowel-like sound. The neuron may be specified as a vowel detector, for the resonant characteristics of the vocal tract would be uniquely determined to the source excitation of the quasi-periodic pulse train. Another geniculate neuron was selectively tuned to a synthesized unvoiced fricative. Another geniculate neuron was responsive to a voiced fricative. Thus, the decoding function of speech parameters may be fully developed at the geniculate level of guinea pigs.

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RESPONSES OF VESTIBULAR NEURONS TO STIMULATION OF THE INTERSTITIAL NUCLEUS OF CAJAL.
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Neurons in the medial and descending vestibular nuclei were recorded and responses to stimulation of the interstitial nucleus of Cajal (INC) were studied in cerebellectomized cats under chloralose anesthesia. Stimuli applied in the ipsilateral INC excited 37 % of neurons that did not exhibit spontaneous activity. About 84 % of spontaneously active neurons were influenced by the INC; excitation was seen in 35 %, inhibition in 22 % and excitation followed by inhibition in 27 %. Of the neurons that were excited, 29 % fired monosynaptically (1.2-1.9ms). Not only mono-, but polysynaptic responses had the lowest thresholds within the INC. Collision test between the ipsilateral INC and contralateral vestibular nuclei-evoked EPSPs and modification of interstitial responses by ipsilateral labyrinthine stimulation suggest that responses evoked by INC stimulation were produced by interstitiovestibular neurons rather than an axon reflex mechanism. Interstitial responses were similar among vestibular neurons that had different projection sites; vestibulospinal, contralateral INC projecting and contralateral vestibular nuclei projecting neurons received similar effects. Qualitatively similar results were obtained among different canal responding vestibular neurons. However, canal responding neurons that were excited by contralateral vestibular nerve were activated more frequently by the INC than those that received inhibition.

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DIRECT EXCITATORY PROJECTION OF NEURONS IN THE PRERUBRAL FIELD TO INFERIOR RECTUS MOTONEURONS IN THE CAT.
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Single neurons activated antidromically by microstimulation at the inferior rectus subdivision of the oculomotor nucleus were explored in the medial mesodiencephalic junction of cats anesthetized with ketamine hydrochloride. Of these neurons, those activated with a stimulus intensity less than 30 μA showed antidromic spike latencies ranging from 0.5 to 2.1 msec and, histologically, they were located in the medial part of the prerubral field. Systematic tracking with a stimulating microelectrode in and around the oculomotor nucleus revealed that low threshold foci for antidromic activation were found in the inferior rectus subdivision and its vicinity. The effective sites were interposed by non-effective sites, indicating axonal branching in this region. Other neurons activated with an intensity more than 30 μA also showed similar antidromic spike latencies and distribution of their location. Most neurons responded to vertical rotation. Stimulation of the medial prerubral field produced synaptic activation of inferior rectus motoneurons with latencies ranging from 1.0 to 2.5 msec.

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OLFACTORY RESPONSES OF THE LATERAL HYPOTHALAMIC NEURONS IN THE UNANESTETIZED MONKEY
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Tanabe et al (1975) indicated that the lateroposterior portion of the orbitofrontal cortex (LPOF) in the monkey plays a most important role in odor discrimination. They considered the hypothalamus as one of the relay areas to LPOF. Thus, the most numerous numbers of odors to which neurons in the olfactory bulb (OB), the prepyriform cortex-the medial portion of amygdala (PPF-MA) and LPOF responded were five, three and one respectively. Besides, the numbers of odors which elisited responses decreased as one ascends from OB to LPOF. In the present experiment, we recorded responses to odors of the lateral hypothalamic area (LHA) in nature. Consequently, it has become a question whethere or not LHA is really a relay area between PPF-MA and LPOF. Naito, Kawamura and Takagi's HRP study (unpublished) showed a pathway from the substantia innominata to LPOF, but not a pathway from LHA to LPOF. It is probable, therefore, that the neurons found in LHA concerned with the other functions, for instance a relay function to the limbic system, as was suggested by Kogure et al (1978).

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REFFERRED SENSATION LIKE PHENOMENON IN THALAMIC VB-NEURONS. AIKAWA, S., OHTA, K., AKITA, H., MEGURO, Y., WATANABE, T. AND KOBAYASHI, S.* Dept. of Physiol., Sch. of Hygenic Sciences, Kitasato Univ., Sagamihara, Kanagawa and Dept. of Physiol., Seishin-Igaku Inst., Itabashi-ku, Tokyo*

Neuronal discharges in ventrobasal thalamic nuclei of immobilized cats were recorded extracellulary. Out of 229 VB-neurons responded to stimuli applied to the receptive fields of body surface, 26 neurons responded to heart beats too. These surface receptive fields were distributed in whole body.

In simultaneous recording by multi-micro electrode technique, one unitary discharge was synchronized with heart beats and the other was not, besides the receptive fields of these neurons were adjacent on body surface.

After Nembutal injection (12.5 mg/kg i.v.) the background activity were inhibited, but discharges synchronized with heart beats were remained.

It is suggested that the refferred sensation like phenomenon participate in simple neuronal pathway.

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PERIODONTAL MECHANOSENSITIVE NEURONS IN THE TRIGEMINAL NUCLEUS OF THE RABBIT
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The unitary activities of the periodontal mechanosensitive neurons were recorded from the trigeminal sensory nuclei, mainly from the trigeminal spinal tract nucleus, in the urethane-chloralose-anesthetized and immobilized rabbits. One hundred and two units in responses to the pressure stimulation applied to the ipsilateral mandibular incisor labiolingually were classified into three groups, "sustained", "transient on" and "transient on-off" units. The units of 16% were sustained type, which had low threshold to the pressure stimulation and medium frequency spontaneous discharge. In two of them the unit activities were found to be inhibited by the mechanical stimulation of the perioral skin. Units of 61% were transient on type, which had high threshold and low frequency spontaneous discharge. The remaining 23% were transient on-off type, which had medium threshold and high frequency spontaneous discharge. The post-excitatory inhibition was observed in most of transient on and on-off units. According to Ness(1954) the majority of the units recorded from the primary dental afferents in the rabbit were sustained type, and the high frequency spontaneous discharge units were few in number. The present findings, taking together with those of Ness, may suggest that the response pattern and spontaneous activitiy of the primary dental afferent fibers is modified at the level of trigeminal nucleus.

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FUNCTIONAL DIFFERENTIATION OF TRIGEMINO-THALAMIC PROJECTION SYSTEMS.
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In order to clarify function of the projection system from the trigeminal subnucl.caudalis to the thalamus and the cortex, effect of trnsection of the spinal tract at level of the obex on cortical surface potentials evoked from trigeminal inputs was studied in chloralose-anesthetized cats. As inputs, facial skin, the masseteric and pulp nerves were stimulated electrically. Cortical responses induced by trigeminal nerve stimulation were composed basically of initial positive(P1) and negative(N1) potentials. Innocuous facial stimulation evoked the largest P1 in SI with the shortest latency(ca. 3.0msec). Noxious masseteric and pulp stimulation evoked the largest P1 in SII. The largest N1 in SI were induced by facial and pulp stimulation, while the largest N1 in SII were by masseteric stimulation. Spinal tractotomy abolished dominantly N1 from noxious inputs, while innocuously induced P1 and N1 were unchanged appreciably. Therefore, it can be assumed that the trigeminal subnucl. caudalis might be mainly involved in induction of N1 potencial from noxious inputs.

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PROPERTIES OF NEURONS IN TRIGEMINAL SUBNUCLEUS CAUDALIS PROJECTING TO SUBNUCLEUS INTERPOLARIS.

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Single unit activities were recorded from neurons in the subnucleus caudalis (Vc) of the spinal trigeminal nucleus and the adjacent reticular formation of the cat, which responded antidromically to stimulation of the subnucleus interpolaris (Vi) but not to stimulation of the subnucleus oralis (Vo). Their responses to non-noxious and noxious facial stimuli were compared with those of Vc-neurons projecting to either the main sensory nucleus (Vms), Vo or thalamus, and with those of non-projection neurons projecting neither of them. Of 80 Vc-neurons projectiong to Vi, 72 were low-threshold mechanoreceptive neurons, 5 were wide dynamic range neurons, 2 were nociceptive specific neurons and one showed background activity which were inhibited by cutaneous stimulation. They were located mainly in layers IV, V and VI. Mean antidromic latency was 1.47 ms and was longer than those of Vc-neurons projecting to Vms or Vo. The latency of first spike induced by electrical stimulation of receptive field (RF) and the size of RF were significantly larger than those of non-projection Vc-neurons. Trigeminal tractotomy at the level of the obex either increased or decreased RF size of 7 trigeminothalamic Vi-neurons. These results indicate that Vc-neurons projecting to Vi have characteristic physiological properties and exert excitatory or inhibitory influences to Vi.

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ROLE OF SPINAL TRIGEMINAL NUCLEUS IN THE SUPPRESSIVE EFFECT ON THE JAW OPENING REFLEX INDUCED BY ELECTROACUPUNCTURE STIMULATION IN RAT

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The aim of the present study was to identify the site of action of electroacupuncture in the jaw opening reflex pathway. We examined the electroacupuncture effects on three responses involved in the reflex (1) the jaw opening reflex to stimulation of the oral and facial regions, (2) evoked responses in a secondary neuron site in the spinal trigeminal nucleus, and (3) jaw opening responses to stimulation of the secondary neuron site. Electroacupuncture stimulation almost suppressed the responses involved in the path of the reflex evoked by high threshold afferents ,but it scarcely affected those evoked by low threshold ones. From a comparison of the suppressive degree of the responses, it is suggested that electroacupuncture suppression of the jaw opening reflex was induced mainly on the activity of the spinal trigeminal nucleus. In conclusion, it can be said that electroacupuncture stimulation is almost effective on the activity of the spinal trigeminal nucleus in the reflex arch.

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EFFECT OF STIMULATION OF NUCLEUS RAPHE MAGNUS ON THE NOXIOUS THALAMIC RELAY NUCLEI IN CAT.

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The single cell noxious responses from cat medial thalamus were recorded extracellularly using tungsten microelectrodes and the effect of stimulation of the Nucl. raphe magnus on these units was examined. The thalamic noxious responses were recorded mainly from Nuclei Pf, Spf, and CL. Although Nucl. raphe magnus is well known to inhibit noxious responses strongly at the spinal cord level, our data revealed that stimulation of the Nucl. raphe magnus only mildly inhibited the thalamic noxious responses. Besides, the spontaneous discharges of the thalamic noxious cells were markedly enhanced by the stimulation of the Nucl. raphe magnus. This result has led us to the hypothesis that the Nucl. raphe magnus might play a role as a descending inhibitory system to the noxious responses at the spinal cord level, and at the same time, play a role of an activating system on the spontaneous discharges at the thalamic noxious relay nuclei in order to maintain normal physiological functional activity of the central nervous system, and keep the animal in alert conditions.

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NEURONS IN SPINAL TRACT NUCLEUS OF TRIGEMINAL NERVE RELAYING NOCICEPTIVE INPUT TO SUBNUCLEUS RETICULARIS VENTRALIS MEDULLAE OBLONGATAE.

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Neurons responding to noxious stimulation of the trigeminal nerve territory are found within subnucleus reticularis ventralis medullae oblongatae (SRV), which lies ventral to the trigeminal subnucleus caudalis and subnucleus reticularis dorsalis medullae oblongatae. The present study attempts to identify neurons relaying trigeminal nociceptive input to SRV. After electrophoretic injection of HRP into SRV, retrogradely labeled neurons were found ipsilaterally in subnucleus interpolaris, and bilaterally in subnucleus oralis. Neurons within subnucleus interpolaris were confined to the rostral half of the subnucleus. Electrical stimulation of SRV also antidromically activated neurons in subnucleus oralis. These neurons were excited by either mechanical stimulation of the cornea, electrical stimulation of canine tooth pulp afferents or noxious mechanical stimulation of the face. These findings indicate that neurons relaying trigeminal nociceptive input to SRV are located within the ipsilateral subnuclei oralis and interpolaris and contralateral subnucleus oralis.

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VESTIBULO-AUTONOMIC RELATED NEURON ACTIVITIES IN RETICULAR FORMATION.

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Chloralose-urethane anesthetized cats were immobilized by myoblock under artificial respiration. The postganglionic sympathetic-renal nerve potential (RN) was recorded with bipolar silver electrode, and the field potential in reticular formation (RF) was recorded with microelectrode. The conditioning stimuli were applied to vestibular nerve or cerebellar cortex and the testing stimuli were applied to peroneal nerve or vestibular nerve. It is known that a convergence site by the excitation of vestibular nerve, peroneal nerve and cerebellum was presumably reticular formation, therefore, the recovery curve of testing response was a help to know the mode of convergence. It is reported that vestibulo-autonomic responses originate from the medial vestibular nucleus (MVN). The RN and RF responses were compared before and after lesion to the ipsilateral MVN. Before the lesion, both the latency and the amplitude of evoked potential are quite similar in bilateral RF, and also the unitary response in bilateral RF did not show difference. In many cases of lesion, the responses of RF remained unchange, but RN reflex discharges were decreased. In the case of lesion including in sites of MVN and its fring, RF and RN responses were disappeared. From these results, it can be thought that the vestibulo-sympathetic inhibitory action does not relate to only MVN.

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PHYSIOLOGICAL PROPERTIES OF NEURONS IN THE ROSTRAL PART OF THE SOLITARY TRACT NUCLEUS IN DECEREBRATED RATS.

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To study the effects of descending fibers from the higher taste centers on the solitary tract nucleus (NTS) neurons, the properties of gustatory (G) and mechanoreceptive (M) neurons were examined in the NTS of decerebrated albino rats. Among the 30 G and 21 M units studied, the receptive fields (RF) could be located in 15 G and 21 M units. Five G and 4 M units had an RF(s) exclusively on the tongue, 6 G and 14 M units on the palate, and the remaining 4 G and 3 M units on both the tongue and palate. These findings showed a marked contrast to the fact that 96% of the G units in the intact rats had an RF solely on the tongue. The G units of the decerebrated rats, with their RF only on the palate, gave much weaker discharges in response to a smaller number of four basic taste stimuli, compared to the units with an RF on the tongue. On the other hand, the mean magnitude of the responses of the latter units was nearly equal to that in the intact rats. The present findings suggest that the higher taste centers have suppressive effects on the taste and mechanical inputs from the palate to the NTS.

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RECIPROCAL CONNECTIONS BETWEEN THE PARAVENTRICULAR NUCLEUS AND THE NUCLEUS TRACTUS SOLITARIUS: AN ELECTROPHYSIOLOGICAL STUDY IN RATS. KANNAN, H. AND YAMASHITA, H. Dept. of Physiol., Univ. of Occup. Environ. Health Sch. Med., Kitakyushu 807

Neural connections between the paraventricular nucleus (PVN) and the nucleus tractus solitarius (NTS) were studied electrophysiologically in urethane-anesthetized male rats. Stimulation of the NTS area evoked antidromic spike in 66 of the 415 units recorded within the PVN or close to it. Antidromic spike latencies ranged 8-60 msec. Of the 33 tested units which were antidromically activated following NTS area stimulation, 24 showed inhibition lasting 40-205 msec and 3 facilitation lasting 30-60 msec following the antidromic spike. Subthreshold stimuli for the antidromic spike also produced the inhibition and the facilitation. Stimulation of the NTS area orthodromically excited 23 and inhibited 9 of the 45 PVN neurosecretory neurons tested. Stimulation of the PVN area evoked antidromic spike in 8 of the 180 units recorded within the NTS or close to it. Antidromic spike latencies ranged 30-40 ms. Forty-five units were orthodromically excited and 3 inhibited following the PVN area stimulation. These results suggest the existence of reciprocal connections between the PVN and the NTS via monosynaptic pathways. These connections may be involved in the coordination of neuroendocrine and autonomic responses to peripheral visceral stimuli.

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FORMATION OF NEW CORTICO-RUBRAL SYNAPSES IN THE CAT FOLLOWING CROSS-INNERVATION OF FLEXOR AND EXTENSOR NERVES : AN ELECTRON MICROSCOPIC STUDY. MURAKAMI, F.*, KATSUMARU, H.* AND TSUKAHARA, N*,**. National Inst. Physiol. Sci. Okazaki 444* and Dept. of Biophysical Eng., Fac. Eng. Sci., Osaka Univ., Toyonaka 560**.

An electron microscopic study of cortico-rubral synapses was performed to find out a morphological correlate for previously reported altenation in time to peak of cortico-rubral e.p.s.p.s following cross innervation of flexor and extensor nerves of adult cats. Rubrospinal neurons identified by antidromic activation from C_1 spinal segment but not from L_1 spinal segment were stained by intracellular injection of horseradish peroxidase (HRP). Sensori-motor cortex was ablated to give rise to terminal degeneration in the red nucleus. HRP-filled cells were found in the dorso-medial portion of the nucleus and electron micrographs of degenerating terminals (DT) in the area around each HRP-filled neurons, 1.2×11^6 sq. μm, were taken and the dendrites in contact with DT were measured. Analysis of a total of 234 DT found in the area of 1.2×10^7 sq. μm from 5 cats indicated that many degenerating terminals make contact with dendrites with diameter of 3 - 6 μm and somata, whereas most of the cortico-rubral terminals normally make contact with finer dendrites (<3μm). We concluded that new cortico-rubral synapses were formed on proximal portion of soma dendritic membrane of red nucleus neurons as a consequence of cross-innervation.

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MEMBRANE PROPERTIES AND CHEMOSENSITIVITIES OF RAT DORSAL RAPHE NEURONS. UCHIYAMA, H., INOKUCHI, H., HIGASHI, H. AND NISHI, S. Dept. of Physiol., Kurume Univ. Sch. of Med., Kurume

Intracellular recordings were made from neurons contained in the dorsal raphe nucleus (DRN) in tissue slices (300 μm in thickness) transected from rat's brain. DRN neurons showed resting membrane potentials of -40 to -60 mV, input resistances of 60 - 400 MΩ, and membrane time constants of 10 to 30 msec. Single focal stimuli elicited fast EPSPs which had a reversal potential of about zero mV. Fast EPSPs were insensitive to nicotinic or muscarinic blocking agents, while they were abolished in the presence of l-glutamate (1 mM). Nullification of glutamate-depolarization by an anodal current did not restore the depressed EPSP. A train of repetitive stimuli caused in some neurons a slow depolarization which was accompanied by an increased membraned resistance. Noradrenaline (NA), dopamine (DA), acetylcholine, l-glutamate, homocysteic acid or aspartate applied by superfusion depolarized DRN neurons. NA- and DA-induced depolarizations were characteristic in that they were associated with an increased membrane resistance. Serotonin and enkephalin caused hyperpolarizations. GABA and glycine always decreased membrane resistance with or without causing a small depolarization.

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DIFFERENCE IN PROPERTIES BETWEEN THE TWO COMPONENT WAVES EVOKED IN THE MAMMILLARY BODY OF CAT.

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Population response was recorded in the mammillary body of cat under the Nembutal anesthesia. The response was evoked by a single shock to the fornix body or septum and composed of three components (wave 1, 2 and 3). Wave 1-2 complex was evoked in separation from wave 3; the former was by the stimulation of the fornix column and the latter by the shock to the stria medullaris. In the present experiments, wave 1-2 complex or wave 3 was evoked solely to compare the properties of individual waves. Double shock of 5 ms interstimulus interval at threshold intensity remarkably increased the amplitude of wave 3 (about six-fold increase compared to that of wave 2). Following the tetanic stimulation (10Hz for 10 s), the amplitude of wave 2 did not show potentiation. As concerns wave 3, however, spontaneous waves similar to wave 3 appeared repetitively just after the stimulation and continued for about 20 s.

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NEURONS IN THE VENTROLATERAL MEDULLA OBLONGATA WITH ASCENDING PROJECTIONS TO THE PREOPTIC AND ANTERIOR HYPOTHALAMIC AREA.

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We have identified at least two types of neurons ('fast' and 'slow' cells) in the ventrolateral medulla oblongata with ascending projections to the preoptic and anterior hypothalamic area (POA/AHA) by antidromic stimulation in urethane-anesthetized female rats. We report here the results of qualitative analysis of these neurons by making use of clonidine and 6-hydroxydopamine (6-OHDA). Intravenously administrated clonidine produced a dose dependent inhibition of the spontaneous firing of the slow cells tested (n=25) but had no effect on the fast cells tested (n=6). 6-OHDA (10μg in 2μg) was injected directly in 0.5 mm caudal to the site of medial forebrain bundle stimulation, which activated antidromically both types of neurons. Within 1 h after 6-OHDA, antidromic responses of 16 out of 25 slow cells could no longer be obtained, but those of the residual 9 slow cells remained unaffected. The antidromic responses of the fast cells (n=6), on the other hand, was not affected at all. In conclusion, results of the present study indicate that: (1) 6-OHDA-sensitive slow cells are A1 noradrenergic neurons, (2) 6-OHDA-resistant slow cells are C1 adrenergic or non-catecholamicergic neurons and (3) fast cells are non-catecholaminergic neurons.

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GLUCOSE RESPONSIVE NEURON IN RAT HYPOTHALAMIC SLICES IN VITRO.

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The ventromedial nucleus (VMH), dorsomedial nucleus (DMH) and lateral area (LHA) of the hypothalamus are considered to play an important role in feeding and body weight regulation. It was suggested in the in vivo experiment that the glucoreceptor neuron in VMH and glucosensitive neuron in LHA are related to these controls. Here, we studied effects of glucose to VMH, DMH and LHA neurons, and the interaction among those using rat hypothalamic tissue slices. Results were summarized as follows: 1) The response to glucose (5.5-20 mM) was divided into three patterns; excitation (E), excitation followed by inhibition and inhibition (I). 2) Of 50 VMH responding neurons (50/256, 19.5%), the majority were E-type neurons. Of 45 DMH responding neurons (45/103, 43.7%), three types of neurons had a comparable population (each nearly 15%). Of 24 LHA responding neurons (24/59, 40.7%), the majority were I-type neurons (23.7%). 3) Activity of the VMH glucoresponsive neuron tended to increase in glucose in low Ca/high Mg medium. 4) A minor modification in the ratio of glucose response were obtained in the VMH and DMH when each nucleus was isolated from the other nucleus. These results suggest that the glucose response in the VMH, DMH and LHA are due directly to properties of these neuron membranes.

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ROLE OF CATECHOLAMINE IN THE MONKEY ORBITOFRONTAL CORTEX NEURONS DURING FEEDING BEHAVIOR. AOU, S., OOMURA, Y.*, NISHINO, H.**, INOUE, M., INOKUCHI, A. AND MIZUNO, Y. Dept. of Physiol., Fac. Med., Kyushu Univ., Fukuoka, *Dept. of Humoral Control, National Inst. Physiol. Sci., Okazaki, **Dept. of Physiol., Toyama Med. and Pharmaceu. Univ., Toyama.

To elucidate prefrontal neuronal mechanisms involved in motivated behavior and the role of subcortical catecholaminergic, cholinergic and opiate systems, effects of iontophoretic application of noradrenalin (NA), dopamine (DA), acetylcholine (ACh) and morphine on the single unit activity of monkey orbitofrontal cortex neurons during a bar press feeding task was investigated. The majority of cells sensitive to NA showed a decrease in activity during the bar press period. Iontophoretic application of an adrenoceptive blocker suppressed this change in activity. In some cases morphine also affected this type of neuron. Cells which increased in activity during the bar press period also showed an excitation to application of DA. There was no apparent relationship between the sensitivity to ACh with an excitation and the firing pattern during the feeding task. These findings suggest that the catecholamine system of the orbitofrontal cortex plays an important role in the neural regulation of feeding behavior and motivation-related modulation.

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CHOLINERGIC MECHANISM IN THE MONKEY PREFRONTAL CORTEX DURING OPERANT FEEDING BEHAVIOR. INOUE,M., OOMURA,Y.* NISHINO,H.**. AOU,S., SIKDAR,S.K., HYNES,M. AND KATAFUCHI,T. Dept. of Physiol , Fac. Med., Kyushu Univ., Fukuoka, *Dept. of Humoral control, National Inst. Physiol. Sci., Okazaki, **Dept. of Physiol., Toyama Med.and Pharmaceu. Univ., Toyama.

Unit activity in the monkey prefrontal cortex was recorded during operant feeding behavior and the effects of ACh and the stimulation of substantia innominata(SI) were examined. 60 % of the tested neurons were excited by iontophoretically applied ACh, whose effect was mimicked by methacholine and blocked by atropine. Seventeen ACh sensitive neurons were driven by SI stimulation with a latency of at least 17 msec and this effect was also blocked by atropine. 4 % of the neurons were suppressed by ACh, one of which was also suppressed by SI stimulation. A correlation between the effect of ACh and the responses to a particular operant feeding behavior event could not be found, suggesting that cholinergic system exerts nonspecific excitatory control on these neuronal activities. The exitatory responses to a event were, however, attenuated, following the blockage of ACh effect by atropine. In some neurons the simultaneous application of ACh facilitated the response. In conclusion, the cholinergic system which originates from the SI effects operant feeding behavior by facilitating neuronal responses.

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FEEDING CONTROL BY NEWLY FOUND ENDOGENOUS HUNGER AND SATIETY SUBSTANCES. OOMURA, Y., SHIMIZU, N., SAKATA, T.*, ARASE,K.*, FUJIMOTO, M.*, FUKUSHIMA, M*. and TSUTSUI, K*. Dept. Physiol. and *Dept. Internal Med., Fac., Med., Kyushu Univ., Fukuoka 812.

Since endogenous factors which contribute as physiologically effective signals of hunger, and the elicitation of feeding behavior still remain uncertain, we have examined the effects of newly found organic acids, present in the blood of the fasted rats during various hunger stages. The level of 2-deoxytetronate (2-DTA) $CH_2(OH)\cdot CH(OH)\cdot CH_2\cdot COOH$, 3-deoxytetronate (3-DTA) $CH_2(OH)\cdot CH_2\cdot CH(OH)\cdot COOH$, and 3-deoxypentonate (3-DPA) $CH_2(OH)\cdot CH(OH)\cdot CH_2\cdot CH(OH)\cdot COOH$, increased gradually and peaked at around 36 hr during fasting. After intra 3rd cerebral ventricule application in normal rats 2-DTA suppressed food intake continuously for 24 hours on all tested rats, whereas 3-DTA transiently elicited feeding, in one fifth of the rats. 3-DPA elicited short-lasting food intake in two thirds of the rats tested, with a latency of 10-20 min. Control 0.15M NaCl had no effect. Iontophoretically applicated 2-DTA depressed glucose-sensitive neuronal activity in the rat lateral hypothalamus (LHA), while 3-DPA caused excitation. Due to a high correlation between activity of LHA glucose-sensitive neurons and feeding behavior, and consistency of long-term chronic LHA neuronal activity offer microinjection of the organic acids, it is postulated that they are endogenous hunger or satiety substances.

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NEURONAL ACTIVITY OF THE HYPOTHALAMUS DURING SEXUAL BEHAVIOR IN THE MALE MONKEY. Yoshimatsu, H., Aou, S., Katabuchi, T. and Oomura, Y. Dept. Physiol., Fac. Med., Kyushu Univ., Fukuoka 812.

The preoptic area (POA) and other hypothalamic regions play an important role in animal sexual behavior. We have recorded single neuronal activity in the POA, the dorsomedial hypothalamic nucleus (DMH) and the posterior hypothalamus (PH) of a partially restrained male monkey during actual sexual performance. A pattern common to POA neuronal activity was characterized by high activity preceding sexual behavior, a slight reduction after contact of the female and a further reduction following ejaculation. The time for recovery of the neuron was synchronous with the behavioral refractory period of the monkey. In some POA neurons, the activity changed in relation to initiation of each mating act and ejaculation. Other while, increased neuronal activity in the DMH and decreased neuronal activity in the PH were found to be synchronized to each mating act which included mounting, intromission and thrusting without changing before or after sexual behavior. These findings suggest that the POA is involved in the sexual motivation, the DMH and the PH in the performance of mating act.

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THE INFLUENCES OF NOXIOUS HEATING OF THE SCROTAL SKIN AND MORPHINE ON THE LATERAL HYPOTHALAMIC GLUCOSE SENSITIVE NEURONS. SIKDAR,S.K., SHIMIZU,N., and OOMURA,Y. Dept.Physiol., Fac.Med., Kyushu Univ., Fukuoka 812.

In order to understand the neural mechanism of pain induced anorexia, and specify the role of opiates in the lateral hypothalamic area (LHA), we studied the effects of noxious radiant heat stimulation of the scrotal skin and the microiontophoretic application of Morphine on the LHA glucose sensitive neurons, in urethane anaesthetized rats. Radiant heat stimulation was done using a halogen lamp and the scrotal skin temperature was raised from the basal level to 38-52°C. For differentiating the pain from the heat influences, the effects of : a)stepwise increase of scrotal skin temperature (max.42°C for heat, 43-52°C for pain), b)varying the diameter of the illuminated spot on the scrotum (10mm. for heat & 2mm. for pain), and c)strong tail pinch, were examined. Thirty nine neurons in the LHA were studied for the above mentioned effects. Twelve neurons were glucose sensitive and 27 non-glucose sensitive. All the 12 glucose sensitive neurons were found to be inhibited both by noxious heating of the scrotal skin and Morphine, except for one whose activity did not change with noxious heat. Such a high correlation of pain and Morphine inhibitions of glucose sensitive neurons suggests, that the pain induced inhibitions are possibly mediated through the release of endogenous opiates.

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DIRECT INHIBITION OF RAT LATERAL HYPOTHALAMIC NEURONS BY GLUCAGON AND CALCITONIN IN VIVO. SHIMIZU, N., OOMURA, Y., MIYAHARA, S., HATTORI, K. and INOKUCHI, A. Dept. of Physiol., Fac. Med., Kyushu Univ., Fukuoka 812.

It has been reported that peripheral administration of glucagon or calcitonin inhibits food intake. Recent radioimmunoassay and immunocytochemical studies revealed high levels of gut glucagon in the hypothalamus. It is possible that glucagon and calcitonin might act on the neurons in the lateral hypothalamic area (LHA), which is linked to the control of food intake. The present study was designed to clarify this feature. Electroosmotically applied glucagon to LHA neurons suppressed activity in 86% of the glucose-sensitive neurons (N=43), while it inhibited 19% of the non-glucose-sensitive neurons (N=62). Calcitonin inhibited 64% of the glucose-sesitive neurons (N=14) but only 14% of non-glucose-sensitive neurons (N=21). It had no effect on 81% of the remaining non-glucose-sensitive neurons tested. Ouabain or phenoxypenzamine blocked the inhibitory effect caused by glucose or noradrenalin respectively but did not affect the inhibitory action of calcitonin. Intracellular records revealed that both glucagon and calcitonin hyperpolarized the membrane potential by about 15 mV, reducing the conductance to almost 25% of the control value. The present results indicate that suppression of food intake by glucagon and calcitonin could be induced by directly inhibiting the activity of glucose-sensitive neurons.

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THE EFFECTS OF AN ANORECTIC DRUG, MAZINDOL, ON NEURONS OF THE VMH NUCLEUS; INTERACTION WITH THE NORADRENERCTIC SYSTEM. HYNES,M., MIYAHARA,S., HATTORI,K., and OOMURA,Y. Dept. of Physiol., Fac. Med., Kyushu Univ., Fukuoka 812.

Mazindol, an anorectic drug which suppresses feeding in both humans and animals was tested for its effects on single neurons of the central nervous system. This was done through iontophoresis and extracellular recordings of neurons in the rat. The neurons of the ventral medial nucleus of the hypothalamus (VMH), an area believed to be involved in satiation, were studied in detail. In the VMH, Dopamine (DA), Noradrenaline (NA), α and β adrenergic blockers were tested simultaneously. Mazindol produced two distinctly different types of responses on neurons of the VMH. One, an inhibitory effect strongly corresponding to the incidence of inhibitory responses by NA, was seen in 16% of the neurons tested. This response was blocked by an α adrenergic blocker. The other effect, seen in 59% of the neurons tested, was a strong excitatory-inhibitory (E-I) sequence, or at lower doses, a slightly less potent excitation. The biphasic E-I sequence was more prevalent. The full E-I sequence could not be blocked by either an α or β adrenergic blocker, however the excitatory component was blocked by the β blocker, propranolol, and enhanced by the α blocker, phenoxybenzamine. This excitatory effect of Mazindol in the VMH and inhibitory effect of glucose sensitive neurons of the LHA, may underly the anorectic effect of orally administered Mazindol.

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VARIOUS NEUROPEPTIDE EFFECTS ON VENTROMEDIAL HYPOTHALAMIC NEURONS. Shiraishi, T., Dept. of Physiol., Sch. of Med., Tokai Univ., Isehara 259-11 and Simpson, A.D., Dept. of Physiol., Sch. of Med., Showa Univ., Shinagawa, Tokyo 142 Japan.

Effects have been reported for several neuropeptides applied electroosmotically to neurons in the lateral hypothalamus (LHA) while unit activity was monitored, and gastric acid secretion was occasionally monitored simultaneously. Results suggested that some neuropeptides (bombesin, neurotensin, enkephalin) while having no direct effects when used alone, could modulate the effects of other factors on chemosensitive neurons in the LHA. Effects on gastric acid secretion could be correlated with LHA unit activity.

In this study, we observed effects of various previously unreported neuropeptides on neurons in the ventromedial hypothalamus (VMH), which is known as the satiety center. The effects were either simply reciprocal to those on LHA neurons (TRH, CCK, Somatostatin), or more complex (substance-P, enkephalin).

In general, these various neuropeptides have shown more complicated patterns of effects in the VMH than in the LHA.

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OSMOSENSITIVE NEURONS IN THE SUPRAOPTIC AND PARAVENTRICULAR NUCLEUS OF THE RAT HYPOTHALAMUS. YAMASHITA, H. AND INENAGA, K. Dept. of Physiol., Univ. of Occup. Environ. Health Sch. Med., Kitakyushu 807

Research on antidiuretic hormone secretion from supraoptic (SON) and paraventricular (PVN) magnocellular neurons was focused on mechanisms of osmoreceptors in the hypothalamus. We studied the osmosensitivity of SON and PVN neurons in constantly perfused rat hypothalamic slices. About 50% of the SON and PVN neurons responded to osmolarity changes in the perfused solution. Whereas only 20% of the hypothalamic neurons in other areas showed response. An increase in the solution's osmolarity increased spontaneous activity of all SON neurons and decreased spontaneous activity in 75% of the PVN neurons. After blockage of synaptic input with Ca free high Mg solution, some neurons still responded to osmotic stimuli. Firing thresholds for osmotic stimuli were between 305 and 308 mOsm/kg. Intracellular recordings showed a slow depolarization followed by an increase in spontaneous activity accompanied by large synaptic noises in SON neurons. Hyperpolarization was seen in PVN neurons. These results show that magnocellular neurons in SON are osmoreceptor neurons. However, functions of the magnocellular neurons in PVN in relation to water balance regulation are not clear.

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FACILITATORY EFFECT OF REPETITIVE PRESENTATION OF US ON ELICITATION OF UR AND ON AN ACQUISITION OF PAVLOVIAN CONDITIONING

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The eye-blink behavioral response was observed in 11 cats with different context of paradigms to clarify the role of repetitive presentation of glabella tap as an unconditioned stimulus (US). Eye-blink response (UR) was significantly suppressed after 150 repetition of US in a day, phenomenon well known as 'habituation'. However, this suppression became weaker as US-repetition progressed day by day. After 10 days of US-repetition, spontaneous eye-blink rate increased significantly. The acquisition of Pavlovian conditioned response was accelerated in the cats which received repetitive presentation of US 7 days prior to the conditioning. Those results suggest that repetitive presentation of US has a facilitatory effect as well as an inhibitory one on the eye-blink response. The time course of this facilitation is in good accordance with neuronal excitability increase of descending motor pathways (Woody et al., Brons et al., Matsumura et al.).

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STIMULUS-RESPONSE RELATIONS OF THE TOOTH PULPAL EVOKED RESPONSES IN THE RAT TRIGEMINAL SENSORY COMPLEX.

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The stimulus response functions obtained by recording the field potentials in the trigeminal sensory complex (rostral and caudal part of the spinal trigeminal nucleus and trigeminal main sensory nucleus) were investigated in lightly anesthetized Wistar albino rats. The relations of the power functions were seen only in the lower range of the stimulus intensity. The value of the power was 1.58 in the main sensory nucleus, 1.92 in the rostral part of the spinal trigeminal nucleus and 2.41 in the caudal part of the spinal trigeminal nucleus. In higher stimulus intensity, the relation gradually saturated with increasing the stimulus intensity. The domain of the stimulus intensity between the threshold and the saturation was higher in the caudal part of the spinal trigeminal nucleus than in the rostral part of the nucleus. Domain of that in the main sensory nucleus was overlapped with that of both rostral and caudal parts of the spinal trigeminal nucleus.

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IONIC MECHANISMS OF THE SPONTANEOUS QUIESCENCE OF Ca SPIKES IN CEREBELLAR PURKINJE CELL DENDRITES IN VITRO. OKAMOTO, K. AND SAKAI, Y. Dept. of Pharmacol., National Defense Medical College, Tokorozawa, Saitama 359, Japan.

Mechanisms underlying the spontaneous quiescence of Ca spike firing in the dendrites of Purkinje cells in guinea pig cerebellar slices were investigated by means of intradendritic recording. The following 4 possibilities were examined as factors contributing to the electrical quiescence: 1) voltage- and calcium-dependent K conductances, 2) inhibitory inputs from interneurons, 3) electrogenic ionic pumps, and 4) cyclic nucleotide systems. The results obtained under varied Ca concentrations suggested a partial contribution of the increase of a Ca-activated K conductance. Studies under varied external Cl^- concentrations and in the presence of antagonists(TAG, strychnine, picrotoxin and bicuculline) indicated that inhibitory inputs from interneurons were not responsible for the electrical quiescence. Blockade of ionic pumps by ouabain or by the absence of external K ions abolished the quiescent period. Application of di-butyryl cAMP, dibutyryl cGMP or noradrenaline tended to prolong the quiescent period, while histamine or theophylline increased the magnitude of spontaneous hyperpolarization. These results suggest that the spontaneous quiescence of Ca spikes in cerebellar Purkinje cell dendrites may be induced by a Ca-activated K conductance and also by metabolic processes.

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EFFECTS OF MECHANICAL PERTURBATIONS DURING LOCOMOTION ON LIMB MOVEMENTS, PURKINJE CELL ACTIVITY AND INTRACELLULAR POTENTIALS IN DEITERS' NEURONES. UDO, M. AND MATSUKAWA, K. Div. Neuromuscular Skills, Fac. Health and Sport Sci., and Dept. Biophys. Engnr., Fac. Engnr. Sci., Osaka Univ., Toyonaka, Osaka

During locomotion of cats decerebrated at the precollicular and premammillary level, kinesiological analysis of limb movements, single unit recording from Purkinje cells (P-cells) and intracellular recording from Deiters' neurones (D-neurones) were performed, while mechanical perturbations (taps of 50-550 gr-wt) were applied at the paw dorsum of the left forelimb (LF). In response to the LF taps during stance phase of the LF, the ongoing LF stance phase was shortened and the ongoing swing phase of the right forelimb (RF) was shortened correspondingly. This maintained the bisupport phase in which both forelimbs were in stance. A notable response to this LF tap was earlier onset of the RF extensor activity than in unperturbed steps. D-neurones controlling the RF extensors and P-cells connected to these D-neurones were identified by their antidromic and orthodromic activations (Udo et al. Exp. Brain Res. 41, 1981). In response to the LF tap, simple spike discharges of the P-cells were depressed and the D-neurones showed depolarizing potentials. These responses of P-cells and D-neurones occurred in a close time relation to the earlier onset of the RF extensor activity, suggesting the cerebellar contribution to interlimb coordination during locomotion.

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AXONAL REGENERATION OF AXOTOMIZED CEREBELLOTHALAMIC PROJECTION NEURONS IN ADULT CATS. KAWAGUCHI, S., MIYATA, H. AND KATO, N. Dept. of Physiol., Inst. for Brain Res., Fac. of Med., Kyoto Univ., Kyoto

In contrast with the current concept of abortive regeneration of axons in the mammalian central nervous system, we reported the occurrence of axonal regeneration of axotomized cerebellothalamic projection neurons in kittens (Neurosci. Lett., 25, '81). The present experiments tested whether or not such axonal regeneration occurs in adult animals. In 19 cats, the decussation of the brachium conjunctivum (BCX) was transected midsagittally by a trilateral tungsten wire in a manner similar to the previous study. After 25-66 days, the cerebello-cerebral projections were examined electrophysiologically by recording cerebello-cerebral responses and morphologically using anterograde axonal transport of HRP. In 3 cats, axonal regeneration was proved to occur since fibers anterogradely labeled with HRP crossed the midline where the BCX had been completely transected and since responses were evoked contralaterally in the frontal motor and parietal association cortices by stimulation of the cerebellum. In these animals, gliosis near the lesion was sparse. By contrast, in the remaining 16 cats, labeled fibers faced a dense glial scar and passed hardly through the scar which appeared like an impenetrable barrier for growing axons.

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ROLE OF CEREBELLAR FLOCCULUS IN EYE MOVEMENTS REVEALED BY LESION WITH KAINAC ACID IN PIGMENTED RABBITS NAGAO, S., Dept. of Physiol. Fac. of Med., Tokyo Univ., Tokyo, Japan

The horizontal ,vestibulo-ocular reflex (VOR) and optokinetic reflex (OKR) were examined in pigmented rabbits before and after lesion of flocculus or nodulus-uvula. VOR was induced by sinusoidal rotation of head (0.1Hz) and OKR was induced by sinusoidal rotation of striped screen. Continuous rotation of the head (±5°) while fixing the screen caused an increment of VOR gain of 0.2 (in average) in 4 hours. When the rotation of the screen (±5°) was in the opposite direction of that of the head (±5°), VOR gain was increased by 0.3. When the screen was rotated (±5°) in the same direction, VOR gain was decreased by 0.16. However, when the screen rotation (±10°) was larger than the head rotation (±5°), VOR gain stayed unchanged. A significant phase lead of VOR was observed in this particular situation. OKR gain was increased when screen was rotated (±2.5°, 0.3Hz) continuously around the stationary animals. Bilateral flocculectomy with local injection of kainic acid reduced gains of VOR and OKR. This also abolished the adaptive changes of VOR and OKR. Removal of nodulus-uvula caused gain increase and phase lead in both VOR and OKR. But no change was observed in the adaptability of VOR or OKR.

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PROJECTION FROM THE ALAMINAR SPINAL TRIGEMINAL NUCLEUS TO THE CEREBELLAR FLOCCULUS VIA THE CAUDAL PART OF THE DORSAL NUCLEUS OF THE RAPHE IN CATS. KAWASAKI, T., SATO, Y. Dept. of Physiol., Fac. of Med., Toyama Medical and Pharmaceutical University, Sugitani, Toyama.

Unitary activities were recorded extracellularly in the caudal part of the dorsal nucleus of the raphe (DNR) which was identified as one of the origins of cerebellar floccular afferent projection in the previous studies. Out of seventy three units recorded in DNR, fourty five responded orthodromically to electrical stimulation of the ipsilateral magnocellular division of the alaminar spinal trigeminal nucleus (5SM) (latency, 1.4 ± 1.08 msec) and twenty eight, to stimulation of the ipsilateral parvocellular division of the alaminar spinal trigeminal nucleus (5SP) (latency, 1.9 ± 1.10 msec).

The units responded to stimulation of the ipsilateral 5SM were not activated antidromically by stimulation of the cerebellar flocculus. On the other hand, out of twenty eight responded to stimulation of the ipsilateral 5SP, seven units were activated antidromically by stimulation of the contralateral cerebellar flocculus (latency, 1.3 ± 0.3 msec). These findings suggest that there exist projections from the spinal trigeminal nucleus of the cerebellar flocculus via the caudal part of the dorsal nucleus of the raphe.

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ANALYSIS OF NEURONAL ACTIVITIES IN THE CEREBELLAR CORTEX OF THE MUTANT ROLLING MOUSE NAGOYA IN VITRO. OHNO,T., TAMAKI,M* AND OGUNI,E** Dept. of Physiol., Inst. of Basic Med. Sciences, Dept. of Neurol., Inst. of Clinical Med* and School of Med**, The Univ. of Tsukuba, Niihari-gun, Ibaraki

The rolling mouse Nagoya (RMN) is a mutant mouse characterized by a severe gait disturbance which has been suggested to be of cerebellar origin, and abnormalities in the concentration of glutamate (Gl), GABA and noradrenaline (NA) in the cerebellum has been reported. In this study, by using slice preparations, responses of cerebellar cortical neurones of RMN to electrical stimulation of the white matter (WM) and to iontophoretic application of Gl, GABA and NA were investigated, and were compared with those of the normal mouse. Spike discharges of neurones were recorded extracellularly from the Purkinje cell layer of the anterior lobe. In RMN, neurones fired simple spikes spontaneously at frequencies of 5 to 60 Hz, and the stimulation of WM induced a facilitation of simple spike discharge with short latencies followed by an inhibition, as in the normal mouse. Iontophoretic application of Gl enhanced the firing frequency, while that of GABA and NA reduced it. The dose-response relationship in RMN was similar to that in the normal mouse. It is concluded that, at least qualitatively, there is no difference between RMN and the normal mouse in the response of cerebellar cortical neurones to stimulation of WM and to iontophoretically applied Gl, GABA and NA.

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OUTPUT CHARACTERISTICS OF PYRAMIDAL TRACT CELLS IN THE PARIETAL CORTEX OF THE CAT.

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The distribution of collateral branching of PT cells in the parietal cortex (area 5) to various subcortical structures and the effect of stimulation of area 5 on spinal motoneurons were investigated using the cat. PT cells in area 5 were found to be mainly composed of slow PT cells. Axon collaterals to the following structures were found in 83 PT cells tested; ipsilateral (3-20 %) VA, LP, RN, MRF, PN, IO, BRF and LRN, and contralateral (3-10 %) IO, BRF and LRN. PT cells activated antidromically following stimulation of ipsilateral corticospinal tract at C_1 were very small in number. In pyramidal cats with removal of area 6, 4 and 3-1-2 and with section of callosal fibers, stimulation of area 5 evoked EPSPs of relatively long latency (15-20 msec) in the majority of slow and fast motoneurons examined, EPSPs or IPSPs of relatively short latency (8-12 msec) being observed in some cases. Stimulation of the ipsilateral area 5 produced only EPSPs of relatively long latency. Experiments with decorticated cats, in which axons of slow PT cells could be activated selectively, revealed that slow PT cells in area 5 exerted excitatory effects on contra- and ipsilateral slow and fast motoneurons, while fast PT cells in area 5 exerted inhibitory and excitatory effects on contralateral slow and fast motoneurons respectively.

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STRUCTURAL CHANGES ON THE PYRAMIDAL NEURONS IN COBALT-INDUCED EPILEPTOGENIC FOCI OF CAT'S BRAIN.

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Acute and chronic focal epilepsy was produced in cats (6 to 12 months) by the topical application of metallic cobalt (Co.) powder on the sigmoid gyrus. Morphological study with Rapid Golgi staining tissues were done lightmicroscopically.

In the Co.-induced lesion and in immediately ajacent region to Co.,neuronal depopulation and abnormalities of dendrites were found. Such abnormalities were nodular changes with loss of spine along the shaft of apical and basal dendrites of pyramidal neurons. Shortning of apical dendrites,reduction of dendritic branches and angular distortion of apical shaft were also noticed. These changes were similar to those in human epileptogenic foci (Scheibel et al. 1974) and other experimental foci in monkey (Westrum et al. 1964) or in rat (Willmore et al. 1980). In two experiments,several apical dendritic shafts of pyramidal neurons in layer 3,4 and 5 in the mirror area (sensory-motor) had changes of spine density;such as local increases in number, and increase in size.

The present data indicated that abnormalities on dendrites were relates to generation of epileptogenicity,especially increasing of spine on apical shaft were probably related to maintain chronic seizure.

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NEURONAL RESPONSES IN THE SUPPLEMENTARY AND PRECENTRAL MOTOR CORTEX TO MOTOR INSTRUCTIONS.

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Monkeys (Macaca Fuscata) were trained to press a key with the hand in response to a tactile signal (vibration of a plate attached to the palm) or an auditory signal (tone burst of 1000Hz). Prior to the occurrence of the triggering signals, instruction signals were given to the animal by tone bursts of two different frequencies. The 100Hz tone signal told the animal that it should respond to the forthcoming tactile signal but ignore the auditory triggering signal of 1000Hz, while the 300Hz tone signal meant that it should respond to the 1000Hz triggering signal, ignoring the tactile signal. 22 % of 323 neurons recorded in the supplementary motor cortex exhibited activity changes in response to the instruction signals and 57 % of the instruction related response was different depending upon whether the instruction was 100Hz or 300Hz. Thus activities of a number of neurons in the supplementary motor cortex were different depending upon the modality of the sensory signal to which the animal was prepared to respond. In contrast, 6 % of 180 precentral motor cortex neurons exhibited instruction related activity. Most of their activity was not different regardless of whether the instruction called for tactile or auditory triggered movements.

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FRONTAL CORTEX CONCERNED WITH EYE MOVEMENT IN THE CAT.

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The frontal cortex concerned with eye movement was investigated by electrical stimulation in encéphare isolé cat. In addition to the mesial wall of the hemisphere under cluciate sulcus and the presylvian sulcus, the coronary sulcus was included in the cortical region concerned with eye movement. This region, which predominantly dominates the contralateral eye movement, has not yet been reported. Stimulation of this coronary oculomotor area evoked ipsiversive deviation or centering. In case of the ipsiversive deviation, adduction of the contralateral eye movement was more striking than conjugate deviation of the eyes. That is, the eye on the side being stimulated showed a slight ipsilateral deviation later than the contralateral obvious deviation. If the stimulation was intensified, enophthalmos occured. The latency was as short as 18-20 msec (average 19 msec) and the eye movement was often evoked by only two train pulses. This region was also characterised by evoked contralateral blink with latency of 13.5 msec.

In our present study, it is considered that the descending pathway of the coronary oculomotor area project on the brain stem adjacent to the oculomotor nuclei without passing the thalamus or superior colliculus.

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FIRING PATTERNS OF THREE TYPES OF VISUAL TRACKING NEURONS IN THE POSTERIOR PARIETAL ASSOCIATION CORTEX OF THE MONKEY.

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Visual tracking neurons in the posterior parietal association cortex (area 7a or PG) are activated during smooth pursuit eye movement and show directional selectivity. Monkeys were seated in a primate chair fixed to a turn table which provided horizontal rotation around the vertical axis. Visual tracking neurons were classified into three groups. Neurons which did not respond to rotation of the chair in the dark were classified as type A(26/53). Those which responded to rotation of the chair in the dark were classified into type B or C: type B showed the same preferred direction of rotation as visual tracking(19/53), and type C showed the preferred direction opposite to visual tracking(8/53). When the target and the chair were rotated together, by which the vestibulo-ocular reflex was suppressed, most of the visual tracking neurons(type A and B) responded to movement of the target in the same way as in visual tracking, in spite of eye movement being suppressed. When the monkey fixated the stationary target during sinusoidal rotation, type C neurons were clearly modulated, but the responses of type A and B neurons were much smaller than those during visual tracking. The results suggest that visual tracking neurons in PG are likely to play a role in perception of movement of the target relative to space and the body.

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RECEPTIVE FIELD PROPERTIES OF SOME VISUAL NEURONS OF THE POSTERIOR PARIETAL ASSOCIATION CORTEX OF THE MONKEYS.

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Functional properties of visual neurons of the posterior parietal association cortex were studied in alert monkeys using both moving real objects and visual stimulation on the rear screen. 114 neurons in nine hemispheres of five rhesus monkeys were identified as passive visual (PV) neurons. Twenty-six of them were activated preferentially by movement of visual objects in depth rather than in fronto-parallel plane (horizontal, vertical, etc). Large majority of them (23/26) were activated by approaching movement, whereas a few (3/26) of them were activated by receding movement. We tested the effect of changing size of the retinal image upon eight of these depth-movement-sensitive PV neurons using rear screen. Six neurons whcih preferred approaching movement were activated by enlargement of the square, whereas two neurons which preferred receding movement were activated by diminution of the square. The opposite movement of two edges, aparting from each other, was almost as effective as enlargement of the whole square, and the change of the orientation or the length of these two edges did not vary the response of these neurons. "Response areas" of these neurons examined by size-change stimulation were fairly large, often covered one quadrant of the contralateral visual field.

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SOME PROPERTIES OF IPSPs RECORDED FROM IN VITRO SLICES OF CAT'S VISUAL CORTEX. KATO, H. AND OGAWA, T. Dept. of Physiol., Akita Univ. Sch. Med., 1-1-1 Hondo, Akita-010.

IPSPs recorded from visual cortical neurons in layer II or III in response to stimulation of the white matter were investigated using thin slices of brain. 93% of neurons(107 out of 134 cells studied) responded to single shocks with EPSPs followed by hyperpolarizing IPSPs. Bath application of bicuculline(4 μM) blocked an increase in membrane conductance associated with IPSPs and reversed in hyperpolarizing IPSPs to depolarizing potentials on which multiple spikes were superimposed. Replacement of Cl^- ions in the medium with propionate abolished IPSPs and also revealed long lasting depolarizing potentials. The mean reversal potential of IPSPs was -73.0 mV(n=7). This figure was very close to that obtained for hyperpolarizing potentials produced by local application of GABA(10 μM)(-76.4 mV, n=4). GABA responses were found to be similar to IPSPs in many ways.

These findings strongly suggest that IPSPs are mediated by GABA with the involvement of Cl^- ions.

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MONKEY PREFRONTAL NEURON ACTIVITY DURING REVERSALS OF A COLOR DISCRIMINATION GO/NO-GO TASK. KOMATSU,H. and KUBOTA,K. Department of Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama City, Aichi, 484

Activity changes of monkey lateral prefrontal units were compared between two conditions in which different responses were associated with the same color stimuli. Each condition consisted of two pairs of one of colors (green or red) and one of responses (GO or NO-GO). Conditions were reversed whenever six correct responses continued. Two kinds of differential activity to green and red color stimuli were found. In Type I unit (n=8), the activity change depended on the following response, so the color with larger activity change was reversed between two conditions. In Type II unit (n=14), the difference of the degree of the activity change between two colors is larger in one condition than in the other. It is suggested that prefrontal neuron activities are influenced not only by intratrial task parameters (colors and/or responses) but also by intertrial task parameters (two kinds of conditions).

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FURTHER STUDY ON THE CLARE-BISHOP CORTICAL NEURONS RELATED TO LENS ACCOMMODATION. TAKEHIKO BANDO, NOBUHIKO YAMAMOTO, SHIGEHUMI KAKIMOTO and NAKAAKIRA TSUKAHARA, Dept. Physiol., Yamanashi Med. Sch., Tamaho, Nakakoma, Yamanashi 409-38, and Dept. Biophy. Engineering, Fac. Engineering Sci., Toyonaka, Osaka 560, Japan.

In the previous study, we suggested that the accommodation-related (acc) neurons in the Clare-Bishop (CB) area of the cat have the output pathway independent of the cerebellum, based on the effect of the reversible block of the cerebellar output.

In the present report, the destination of axons of CB acc neurons was studied by using the electrophysiological method. Acc neurons were defined if they discharged in temporal correlation with the spontaneously-occurring lens accommodation. Eleven out of 21 acc neurons were activated antidromically from the lateral posterior nucleus of the thalamus (LP), the pretectum (PT) and/or superior colliculus (SC). The antidromic spikes were identified by 1) activation with stable short latencies and 2) stable responses to high frequency train pulses (200-500 Hz). In all of 11 units, antidromic spikes from LP, PT and/or SC collided, suggesting that axon collaterals of the same CB neuron were activated from these structures. The recording sites of antidromically activated units were located in the same region of the CB area as 31 other acc units.

It is concluded that the accommodation-related CB neurons send their axons directly to the dorsal midbrain.

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THE IPSP MEDIATED BY INTERNEURONS IN THE RABBIT ANTERIOR OLFACTORY NEUCREUS (AON) NEURONS TAZAWA, Y., MORI, K. AND TAKAGI, S. F.
Dept. of Physiol., Sch. of Med., Gunma Univ.

The stimulation of the lateral olfactory tract (LOT), ipsilateral olfactory bulb (IOB) or anterior commissure (AC) elicits two types of IPSPs (fast-IPSP and slow-ipsp) in AON neurons in the urethane anesthetized rabbit. In the present study, we examined properties of the fast-IPSP and the neuronal pathways which are responsible for the fast-IPSP. When 3M KCl-filled micropipet was used, the fast-IPSP reversed its polarity into a depolarization. At the peak of the fast-IPSP, the input resistance of AON cells decreased by 50 to 75%. These results indicate that the fast-IPSP is generated mainly by an increase in chloride conductance. When the stimulas intensity was adjusted to just subthreshold or the threshold for generating the fast-IPSP, spatial facilitation of the amplitude was observed between the AC-evoked fast-IPSPs and the LOT- or IOB-evoked fast-IPSPs. This observation suggests that fast-IPSPs are mediated by common interneurons.

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NEOCORTICAL RESPONSES TO ODORS IN THE PUPPY IMAMURA, K., ONODA, N., ARIKI, T. AND IINO, M. Dept. of Physiol., Sch. of Med., Gunma Univ.

In order to study the functional development of the olfactory area, single unit responses of orbitofrontal cortex to odors were examined in the paralysed puppy (0.8-3.0 kg) in the pre-closure period of the skull within 3 month ages. Animal products (urine, feces), dry dog food, and 8 pure chemicals were used as odor stimuli. These odors were applied with artificial air intake which was produced by applying negative pressure pulses to the postnassal cannula. Twenty-four units responded to at least one of these odors were identified in the restricted region of the orbital gyrus from histological procedures. Out of 24, 7 units (29.0%) responded to odors of pure chemicals and 21 units (87.5%) did to those of animal products. As compare the results obtained from the puppy with those from the adult dog, each response probability to the own urine odor and the odor of γ-undecalactone was significantly lower in the former than in the latter ($P < 0.05$). The odor patterns stimulating the neocortical neurons were probably affected by aging.

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RESPONSE PROPERTIES OF NEURONES OF CAT'S SOMATOSENSORY CORTEX SI TO DENTAL MECHANICAL STIMULATION

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Discharges of the dental mechanosensitive neurones (DM-neurone) were recorded extracellularly in the somatosensory cortex (SI) of the cat by delivering the mechanical stimuli to canines. Projection area of the four canines'was localized topographically in the anterior coronal gyrus as an inverted image. Properties of the dental mechanosensitive neurones were analyzed by varying several parameters of the stimulus. When the incremental force of a constant gradient was applied, a phasic response was observed in most of the neurones. Although total numbers of the impulse discharges which observed at incremental phase of the stimuli were increased with increase of force levels, the maximum frequency of the phasic response was not affected with magnitude of stimulus force. The maximum frequency, however, was varied remarkably by the change of stimulus gradient. These data indicated that the DM-neurone seems to act a some physiological role for the perception process of the mechanical sensation of the periodontal membrene.

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STATISTICAL ANALYSIS OF HIPPOCAMPAL UNIT ACTIVITY IN UNANESTHETIZED RABBITS.
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By means of statistically assessing inter-spike-intervals and spike heights, two hundreds and four hippocampal units in unanesthetized rabbits were categorized into the following three types, i.e. SB, CB, and S type. (1) SB type units (N=66) showed predominantly rhythmically-recurring spike bursts. They changed their firing pattern to simple spike discharge pattern in response to sonic stimuli. And they were located in deeper layers of the hippocampus. (2) CB type units (N=109) exhibited complex bursts as well as simple spike discharges at a slow rate (7.61 ± 2.94/sec). Eighty-nine per cent of these units were suppressed for a period of 229.21 ± 72.89 msec following single shock stimulation of the contralateral hippocampus. A negative correlation could be found between the durations of suppression and the mean frequencies of this type units (r=-0.8061). (3) S type units (N=29) exhibited only simple spike discharges which were high in rate (12.98 ± 5.73/sec) and relatively regular. They did not show any spike bursts, nor any suppression by stimulation of the contralateral hippocampus.

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TEMPORAL PATTERN OF AROUSAL REACTIONS IN CAT MOTOR CORTICAL NEURONES.
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Motor cortical neurones in cat encéphale isolé or pretrigeminal preparations respond to EEG arousal with characteristic combinations of excitation, inhibition, disfacilitation and disinhibition. Our earlier hypothesis maintains that cascade transmissions proceed initially from the cortical surface to depths during phasic EEG arousal and later from depths to surface during tonic arousal, through the neuronal chains consisting of at least three cells connected in series with all possible excitatory and inhibitory combinations. The present study supports this hypothesis by providing the following three measures of temporal response patterns. (1) The onset of phasic arousal measured in neurones by the latency of initial responses to juxta-threshold midbrain reticular stimulation was earlier in superficial than in deep layer cells. (2) The onset of tonic arousal estimated by the time lag between the onset of relatively strong EEG arousal and the transitory point from initial to late neuronal responses was earlier in deep than in superficial layer cells. (3) In the neurones excited in both phasic and tonic phases of EEG arousal, early and late excitatory surges were observed with two separate peaks in superficial layer cells, but appeared to be fused into a continuous depolarization in deep layer cells.

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ON CEREBRAL RESPONSES EVOKED BY STIMULATION OF THE FASTIGIAL NUCLEI IN THE CAT.
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Stimulation of the cerebellar fastigial nuclei induced responses bilaterally in the medial part of the precruciate cortex in approximately one-third of cats anesthetized with Nembutal. The responses were similar to those elicited by stimulation of the cerebellar interpositus nucleus with respect to the laminar distribution of potentials.

Presumable relay cells for the responses were detected in the ventrolateral and ventromedial nuclei of the thalamus. Thalamic neurons mostly responded with repetitive spike discharges to stimulation of the fastigial nuclei, exhibiting predominantly EPSPs. Conditioning stimuli applied to the mesencephalic reticular formation enhanced the probability of spike generation but suppressed repetitive discharges of thalamic neurons following stimulation of the fastigial nuclei. A suggestion was made of a modification of fastigio-thalamic transmission under the influence of Nembutal.

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SPATIAL DISTRIBUTION OF MITRAL CELL INHIBITION IN THE CARP OLFACTORY BULB
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Field potentials in the carp olfactory bulb and intracellular potentials of the mitral cells were analysed in the carp. Electrical shocks were applied to a part of the output pathways of the bulb (either the lateral or medial olfactory tract) or of the input pathways (either the lateral or medial part of the olfactory nerve). Spatial distribution of component waves in the field potentials was different between the lateral and medial parts of the olfactory bulb when the olfactory bulb was partially activated after such shocks. Distribution of the mitral cells showing inhibitory potentials also differed between these two parts of the bulb. It was suggested that the lateral and medial parts of the olfactory bulb are controlled by inhibitory neurons in the respective parts of the bulb.

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NEURONAL RESPONSES TO ACOUSTIC STIMULI IN MONKEY PREFRONTAL CORTEX.
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In alert monkeys, many prefrontal neurons located in the superior dorsolateral area were activated by acoustic stimuli. The neurons were more responsive to natural stimuli than to pure tones, but their responses were little influenced by loudness level: hand-tapping, key-jingles, striking two metal or wooden chips and voice evoked vigorous response irrespective their loudness but tone pip evoked only weak response.

The neurons were tuned to the stimulus direction. Their activation occurred when stimulus sources were placed in a limited arc around the animal. The arc to evoke the response was usually less than 45° with most effective direction in the middle of the arc: this was usually opposite to the recording hemisphere and lateral or posterolateral to the animal.

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ODOR DISCRIMINATION IN THE PYRIFORM CORTEX OF THE DOG
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This work was undertaken to find the correlation between the functional properties indicated by single unit response to odors and anatomically proved layer structures in the pyriform cortex (PC). Extracellular unitary responses to eight pure chemical odors were studied in the PC of the paralyzed dog. Based on their neuronal properties, we classified PC units into three groups: superficial units, deep units, border area units. Most PC units showed a facilitatory response. Deep PC units responded to a smaller number of odors than superficial PC units did. Therefore it was concluded that olfactory information would be processed selectively across a vertical plane in the PC. Many possibilities were suggested on this selective mechanism such as the chemotopic convergent input from superficial to deep PC units or the inhibitory mechanism via inhibitory interneurons or the contributions of association fiber, commissural or centrifugal inputs.

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ELASTICITY OF THE FAST AND SLOW FIBERS OF THE CAT EXTRAOCULAR MUSCLES.

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Extraocular muscles are consist of slow and fast fibers. In this study, we examined the elasticity of these fibers and discussed the mechanical roles in the eye movements. Inferior oblique (IO) and retractor bulbi (RB) muscles of the cats were used. Slow fibers take part of 15-20% and 0-5% in tension of IO and RB respectively. Tension-length curves of IO were more steep than those of RB, and decreased to about a half of the maximum tension when IO were stretched to 120% in the length. The tension recovery after the quick shortening by the vibrator during the tetanus were slower in IO than in RB. The recovery curve of the IO showed fast and slow phases. The amplitude of the tension by the sin wave vibrations during the tetanus (P) were larger than those during the resting (Po). The amplification of the P/Po were 2-2.5 in IO while 1.5 in RB. These results suggest that slow fibers have higher elasticity than fast fibers, and play roles in the fixation of the eye ball.

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CONDUCTION VELOCITY OF FROG MUSCLE FIBER MEASURED BY UNIDIMENSIONAL TOPOGRAPHY.

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Action potential elicited by intracellular stimulation of the frog satorius muscle was recorded from 12 or 16 electrodes attached on the surface of the muscle. The conduction velocity (C.V.) was measured from unidimensional topography calculated and displayed by computer (abscissa: location of recording electrodes, ordinate: latency). The slope of equi-potential distribution on the topography reveals the C.V. And electric properties were also obtained by passing hyperpolarizing current to the fiber, whose C.V. was obtained. The C.V. decreased when the medium temperature was lowered below normal condition, 20°C ($Q_{10} \fallingdotseq 2.0$). In many cases, C.V. was constant along the muscle fiber. But in a few cases, C.V. varied during conduction along the fiber. Decrement of C.V. near the tendon was most frequently observed. Stimulation at near the center of the muscle fiber displayed a bidirectional conduction like that of the nerve stimulation. Central and deep fibers tend to have slower C.V. than peripheral ones. The relationship between the C.V. and some electric constants were studied. The mean values of constants were as follows: C.V. = 1.90m/s, Diameter = 126.5μm, Space constant (λ) = 3.09mm, Time constant (τ m) =25.9ms. Among these constants, The C.V. were proportional to the fiber diameter. But the correlation between C.V. and λ or τ m was not obvious.

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ELECTROMYOGRAPHIC ANALYSIS OF THE INHIBITORY PHASE OBSERVED IN THE MASSETER MUSCLE.

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The purpose of this study was to analyse the masseter electromyographic inhibitory phase induced by electrical or mechanical stimuli applied to oro-facial area. Several types of inhibitory phase were distinguished in the massetericus, which is one of the jaw closing muscles. Similar inhibitory phase was observed in the EMGs during tooth tapping or clenching in man and voluntary jaw closure movement in rat. The present results revealed that (1) Latency and duration of the masseter inhibitory phase converged to a certain range. (2) The threshold value of the digastric activity in jaw opening reflex was somewhat larger than that of the appearance of masseter inhibitory phase. (3) When pulse train stimuli were applied to periodontal tissue (0.1 ms-duration, 2 ms-intervals) with a constant intensity of about 1.5 times threshold for evoking the digastric reflex, latency and duration became longer with increasing the number of pulses of stimulation.

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SACCADIC EYE MOVEMENTS TO TARGETS PRESENTED AT FIXED AND RANDOM TIME INTERVALS.
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When a human subject makes a saccadic eye movement from one target to the other distanced more than 10° each other, frequently he can not fixate the target by an initial saccade (IS) and uses a corrective saccade (CS). CSs were mostly forward (69-93%), indicating that IS undershoots when a subject fixate to a distant target. Frequency of occurrence of CSs was studied as an index for precision in saccadic eye movements using two paradigms; 1) targets were presented at fixed (FI) and 2) random time intervals (RI). Under FI paradigm, CS occurred less frequently at shorter time intervals (percent for occurrence of CS was 58.3 at 0.5 Hz and 38.7 at 1.2 Hz), while there was no such difference under RI paradigm (40.3 at mean frequency of 0.5 Hz and 36.3 at 1.2 Hz). In addition, in FI paradigm ratio of backward CS to forward CS increased at shorter intervals (0.66 at 0.5 Hz, 1.10 at 1.2 Hz). These results suggest that IS can be made in greater precision in FI paradigm, particularly at shorter intervals. The increase in the ratio of backward to forward CS is consistent with the view that saccades were made in a more predictive way at shorter intervals under FI paradigm.

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THE CONDUCTION VELOCITY OF MUSCLES MEASURED BY UNIDIMENSIONAL TOPOGRAPHY IN MAN.
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For the purpose of accurate measurement of conduction velocity, 12 electrodes were linearly placed each with 1cm interval on the surface of muscles. Direct electric stimulation of the muscles were performed. Unidimensional topographs were displayed horizontally and superimposed vertically along post stimulus time scale (by 2ms) on a color TV monitor. The amplitude of potential was divided into 11 levels. Positive potentials were indicated as reddish tint and negative ones as bluish. The slope of bluish area corresponds with conduction velocity of negative potential. Recording conditions were kept constant throughout the experiments. Conduction velocity of brachioradialis, bicepsbrachii and sternocleidmastoid muscles in man was 3.9 ± 0.41, 3.6 ± 0.22, 3.6 ± 0.37 m/s respectively.

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EMG ACTIVITY IN SOLEUS MUSCLE DURING FORWARD INCLINATION.
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When a human subject was instructed to incline his body in a ballistic manner, a silence occurs in EMG activity of soleus prior to the onset of the inclination. Soleus activity is resumed before the inclination attains the final value. We already reported that resumption of soleus activity was determined by the angle of ankle rotation (θ). The angle for resumption of soleus activity (θr) was constant for inclinations from different initial values to the same final value. In the present study we found that θr remained unchanged even when a subject inclined against an external force. A subject was asked to incline himself on a platform which was pulled in a ballistic way (20 cm in 1 sec). The pull was applied at various timing to the instruction for inclination (a light signal). θr was constant (6.8°) with (0.15 sec before - 0.3 sec after the light signal) or without the pull of the platform, although the time for resumption of soleus activity measured from the onset of the silence varied greatly (0.24-0.58 sec) under these conditions. These results suggest that θr represents the motor command for control of body posture and that soleus activity is determined by difference between the motor command (θr) and the angle of ankle rotation (θ) signalled by proprioceptive and exteroceptive (somatic and labyrinthine) inputs.

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BODY INCLINATION AND SOLEUS MUSCLE ACTIVITY WITH DISCRIMINATIVE AND NONDISCRIMINATIVE WARNING. SUZUKI, N. AND UCHIDA, T. Dept. of Physiol., Kyoto Pref. Univ. Med., Kyoto

When human subjects incline his body in response to a light signal, a silence occurs in the soleus activity, prior to the onset of body inclination, the lead-time (LT) being constant in every trial (150ms). We examined the case when a warning sound preceded the imperative signal at random or fixed intervals. Both the latency of the silence (LS) and the reaction time of the inclination (RT) were reduced (LS was 200 ms and 150 ms, RT 350 ms and 300 ms), although their difference, that is, LT remained almost constant. Next we examined the case when a discriminative signal (positive signal was 1 KHz tone and negative one 1.05 KHz). Both LS and RT were prolonged considerably (230 ms and 380 ms), while LT remained constant. These findings indicate that the time spent for excecution of postural control was invariant, althought the time for perception of the warning signals was variable in these three conditions (with non-discriminative and discriminative warning signals and without warning signal).

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PATTERN OF LEG MUSCLE ACTIVITY IN EXECUTING FAST BODY SWAY IN MAN. R. Hayashi,A.Miyake,S.Watanabe.Inst.Equilibrium Res.Gifu Univ.Sch.Med.,Gifu

In the triphasic pattern of EMG activity in soleus (Sol.) and tibialis anterior(TA)muscles which accompanies fast body sway,in bending posture (N),to forward (F) around ankle joints in response to a signal,the first discharge cessation in Sol. and followed discharge in TA provides the force for the forward movement, while following cessation in TA and re-discharge in Sol. provide the deceleration force (Hayashi et al. 1981). In this paper, this pattern was studied under ischemia of feet produced by tourniquets, surrounded just above ankles and inflated to 450 mmHg. Forty minutes ischemia decreased pain and vibration sensation at toes and magnitude of M-wave in the abductor hallucis less than 30% of control. The movable range of body sway,in this condition,decreased to 75%of control in both cases with eyes open and closed. When the subject swayed fast his body from N to F after 40 min. ischemia, he was unable to stop his body at F and falled down.The triphasic pattern of EMG activity was observed in the same manner except larger variance in deceleration phase. We suggest somatic sensation from toes contributes to postural control.

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FREQUENCY ANALYSIS(FFT) ON QUIET PERIOD IN MYOELECTRIC SIGNALS OF THE MASSETER AND THE TEMPORALIS. HAMBA,M., NAGATA,A.*, MIYAZAKI,M.**& MURO,M.*** Dept.Physiol.,Showa Univ. Sch. Dent.,Tokyo * Bio-dynamics Lab.Facul.Sci.Tokyo Metropolitan Univ** Dept.Physical Ed, Waseda Univ.Tokyo ***Bio-dynamics Lab.Tokyo Coll.Pharm. Tokyo

The occurrence of quiet period (silent period,QP) in activities of the masseter and the anterior part of temporalis of nine human subjects with normal occulusion was investigated during clenching movement and during percussion of the bony structure of the mandible. Power spectra(PS) analysis were carried out by means of Fast Fourier Transform about myoelectric signals of these muscles for the period of each 50 ms before and after QP occurrences. In the clenching experiment of the ramp-wise method, QP(duration 10-70 ms) were observed(44%) almost simultaneously in four muscles, at both experimental conditions of the high accelerative movement and of low bite force levels. PS"AFTER QP" were distributed with high energy in higher frequency bands(200-480 Hz), while PS "BEFORE QP" were characterised in low frequency bands(20-180 Hz) with low energy. It was assumed by these frequency analysis, that the recruitment of phasic motor units(high frequency bands of PS) were changed into high rate coding in the EMG with QP occurrences. In the experiment of mandible tap-percussion,QPs were also observed,but PS of "BEFORE QP" and "AFTER QP" were distributed in the same frequency bands in this case.

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STRUCTURE AND FUNCTION OF THE TENDON ORGAN-LIKE NERVE ENDINGS ON THE RAT JAW CLOSING MUSCLES.

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The existence of tendon organ-like nerve endings was observed on the jaw closing muscles in the rat by means of the methylene blue staining method. These nerve endings extend from the lateral aponeurosis of pars superficialis of m. masseter to the medial surface of the origin of m. pterygoideus medialis by way of the medial surface of m. temporalis, and are innervated by branches of n. buccalis, the fiber diameters of which are 8.8-12.4μm. Electron microscopically, the axon terminals are situated among the collagenous fibers and elastic fibers around the cord-like structure consisting of cells resembling epithelial cells, and are covered by capsular layers which are considered to be perineural cells in origin. Electro-physiologically, these nerve endings show the peculiarities of stretch receptors like those of Golgi tendon organs. Therefore, it was considered that these nerve endings are involved in the jaw movement control. The same nerve endings were also observed in mice and golden hamsters.

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SOMATOSENSORY EVOKED POTENTIALS INDUCED BY SUBCUTANEOUS DEEP STIMULATION
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Subcutaneous deep tissues near the human ulnar periost were stimulated by a rectangular electric pulse of 0.5 msec in duration and of 1 Hz in frequency. A pair of electrodes used were of teflon-coated stainless steel needles with a diameter of 0.2 mm, each tip of which was left uncovered. They were inserted onto the periosteum with an interelectrode distance of 2 to 3 cm. Topographic fields of the averaged somatosensory evoked potentials(SEPs) were obtained by monopolar recordings against the connected auricular electrodes and also against the electrode placed on the root of the nose. Bipolar recordings were also carried out. The SEPs were seen only on the scalp contralateral to the stimulated forearm. A small positive or positive-negative wave with a latency of about 50 msec was followed by slow deflections of P-75 and N-140. P-50 or P-N-50 has an amplitude maximum over the postcentral area. Since this component disappeared by a subcutaneous injection of 1% lidocaine, it is supposed to be induced by nerve fibres conveying periostal pain.

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CORTICAL EVOKED POTENTIALS ASSOCIATED WITH SUBJECTIVE ASSESSMENTS OF TACTILE AND NOXIOUS STIMULI IN MAN.
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Somatosensory potentials(SEPs) evoked by electric stimulation of the right index finger were recorded monopolarly from the scalp in 8 human subjects. Five and 6 stimulus intensities within the range weaker and stronger than pain threshold were employed, respectively. Of 3 principal positive waves in a SEP, P25-36 and P44-55 were localized at C_3, the remaining P160-240 at vertex. Amplitudes of P25-36 and P160-240 were most strongly related to stimulus intensity of the weaker and stronger range, respectively, when compared the index of power function with others. Based on the psychophysical function generated under similar stimulus conditions, highly significant positive correlation was obtained only between amplitude of P160-240 and subjective painfulness with stimulus intensity partialed out, whereas amplitudes of P25-36 and P44-55 were correlated significantly with stimulus intensity of lower range when psychological factor was partialed out. The earlier SEP waves thus may reflect to sensory signal processing while the later wave appears to concern pain evaluating system.

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AN EXPERIMENTAL STUDY ON THE EFFECT OF INFERIOR COLLICULUS LESIONS UPON THE AUDITORY BRAIN STEM RESPONSE.
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The relationship of activity of the inferior colliculus to each component of the ABR was investigated, using an electrical coagulation method in rats.

The ABR seems to be a composite of slow and fast waves, and the central nucleus of the inferior colliculus plays a more important part for the slow wave than for the fast waves. Little effect was present on the shape and latency of the fast waves of the ABR when the destruction was limited to the central nucleus. When the lesion was extended to or located in the lateroventral part of the inferior colliculus, where the nerve fibers of the lateral lemniscus enter the inferior colliculus, potentials 5 and 6 were abolished, but potential 4 remained with a slightly prolonged latency.

These findings suggest that the structures peripheral to the inferior colliculus play an important role for generation of potential 4 and the inferior colliculus, particularly its lateroventral part, is essential for generation of potentials 5 and 6.

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PROPERTIES OF PHOTICALLY EVOKED LID POTENTIAL CHANGES, WITH SPECIAL REFERENCE TO THE ELECTROOCULOGRAM. HOSHINA, Y., OZAKI, T., SUGA, S., TAKEO, T. and NIKARA, T.* (Dept. of physiol., Hirosaki Univ. Sch. of Med., Hirosaki and Lab. of Physiol., Sch. of allied med. sci., Hirosaki Univ., Hirosaki*.)

In the present paper were investigated from the standpoint of their origin the slow components of the lid potential changes elicited by flash stimulation. The average evoked potential changes were obtained with the summation technique by the signal processor (7T08, San-Ei Sokki, Tokyo). The average responses of the ERG, EOG, evoked MV and EEG responses were also obtained simultaneously. In awaking normal subjects, there was observed the late slow component in addition to early rapid and slow components of the average lid potential changes, which were elucidated to appear corresponding to the electromyographic potentials of the orbicularis oculi and b wave of the ERG, respectively. Under the condition of occulded eyelid, the early rapid and late slow components disappeared. These components were not effected by the application of mydriatic agents. In moderately sleeping subjects or nembutalized rabbits, the early rapid and late slow components were considerably decreased or disappeared in amplitude. In the subject with an artificial eyeball, the early and late slow components were not observed in the side of the artificial one. From the above-mentioned results, it seems likely that the late slow component of the photically evoked lid potential changes is closely related to the eye movement.

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NONLINEAR CHARACTERISTICS OF VISUAL EVOKED RESPONSES IN LATERAL GENICULATE BODY AND VISUAL CORTEX OF THE CAT

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The aim of this paper was to clarify the nonlinear topological characteristics of visual evoked responses to the frequent flash-light stimulation and to elucidate the mathematical models of EEG generating mechanism. Evoked potentials were recorded simultaneously from the lateral geniculate body (L.G.) and visual cortex (Cx) in 17 cats. Photic stimuli were given in stepwise in increasing and decreasing order with constant stimulus-pause intervals between 1 and 30Hz. In most cases,"resonance phenomena",such as the increase of amplitude ,the jumps of peak latency and of the phase of harmonics,were observed in specific frequency bands. The plots of total power and the amplitude of the 2nd harmonics at each stimulus frequency showed hysteresis curves and revealed the same shapes in Cx as in L.G.These facts may suggest that there exist a close relationship betweeen L.G. and Cx ,and some high nonlinear structures (other than van der Pol type) in the oscillation generation systems of EEG of the cat.

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DIFFERENCE OF RESPONSE PATTERNS BETWEEN LATERAL GENICULATE BODY AND VISUAL CORTICAL NEURONS TO FREQUENT FLASH LIGHT IN THE CAT.

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Single unit analysis in response to the flash light stimulation was investigated in the lateral geniculate body (LGB) and visual cortex (Cx). They were classified into X cell and Y cell in LGB and simple cell and complex cell in Cx according to receptive field properties. Each cell was examined by the flash light with the frequency from 1 to 25 Hz. Data were stored in the magnetic tape, and analyzed the pulse density histogram to the stimulus frequency (frequency tuning curve) used by computer. LGB cell, especially X cell showed various type of the tuning curve; a simple decay type, a flat type and re-increased type. On the other hand, Cx cells, both simple and complex cells, showed a simple decay type. When these response patterns were compared with the evoked field potential in the LGB and Cx, it could be found a very good correspondence between them.

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Average of occurrence rates of alpha blocking was 35.0% in ten subjects by fifty flash stimulations given in train of eight stimuli of 1/2 Hz. As the stimulations were not always effective, we examined favorable conditions for the occurrence of alpha blocking. Ten students sit in a dark shield room facing to a stroboscope placed at 30 cm apart from the face. EEG was recorded monopolarly, from which alpha waves were selected by Ormsby filter and converted into digital values by 4 msec sampling rate. Examined conditions were (1) increase or decrease of the slope of the waves, (2) if the waves were convex or concave and(3) their amplitudes. Number of the trials in which alpha blocking occurred (Na) in fifty trials and number of the alpha blocking trials with one of the above-mentioned conditions (Nx(t)) at t (time after the stimulation ranging from 0 to 176 msec) were counted. The ratios of the two numbers (Rx(t)=Nx(t)/Na) of increasing slope, convex form and middle amplitude were large during the period from 40 to 60 msec after the stimulation. From this facts the latency of the arrival of the information about the conditions of the alpha wave to the mechanism of alpha blocking is estimated and the difference between this latency and the latency of alpha blocking can be considered as the time necessary for the alpha blocking mechanism to function.

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THE DEACTIVATING SYSTEM FOR EEGS. FUJITA, Y., TAKEUCHI, T., HARADA, H. AND MATSUZAWA, I. Dept. of Physiol., Nippon Med. Sch., 1-1-5, Sendagi, Bunkyo-ku, Tokyo, Japan

Effects of stimulation of the area including nucleus raphe magnus on hippocampal EEGs of rabbits were studied in acute experiments lightly anesthetized with Nembutal. High frequency stimulation of the area (0.4 msec pulses at 50-100/sec) produced EEG spikes in both hippocampi, in effect suppressing the theta rhythm. But they were not necessarily synchronized. They were 2-3 times the background EEGs in magnitude. Low frequency stimulation (2-10/sec) yielded essentially the same results. Thus the area could exert deactivating effects, for the theta rhythm, i.e., the hippocampal arousal pattern, was suppressed by its stimulation. Effects of stimulation of the area upon pyramidal cells were then intracellularly studied. Hyperpolarizations were the shortest latency responses elicited by the stimulation. Thus the EEG spikes induced by stimulation of the area are probably the extracellular counterparts of the hyperpolarizations of pyramidal cells. If so, the EEG spikes are essentially the same as the interictal spikes of the kindled hippocampus (Jpn. J. Physiol., 1981 (31) 879-889.

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THE TEMPORAL RELATIONSHIP BETWEEN EVOKED POTENTIALS AND MULTIPLE UNIT ACTIVITY IN FREELY BEHAVING RATS. SHIMURA, T. and SHIMOKOCHI, M. Dept.of Behav.Physiol., Fac. of Human Sciences, Osaka Univ., Osaka-565

Multiple unit discharge (MUA) and evoked potential (EP) were simultaneously recorded from the same microelectrode in freely behaving rats. 1) In general, the waveshape of visual EP closely resembles the poststimulus time histogram of MUA, as was obtained in anesthetized animals. In the auditory EP, however, this relationship is not so obvious, especially in the later components. 2) As the arousal level lowered, EP always increased in amplitude. This augmentation is not due to a change in probability of firing, but to decrease in background discharges of neurons: the height of amplitude of EP is negatively correlated with that of the background MUA level. 3) When paired flashes are presented with an interval of several hundreds millisecond, the EP to the second flash always shows simpler configuration, longer latency, and smaller amplitude (short-term habituation). In this case, the background MUA unchanged but the probability of firing decreased: the amplitude of EP is positively correlated with the probability of firing of neurons.

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CIRCADIAN AND DIURNAL LOCOMOTOR RHYTHM AFTER PINEALECTOMY.
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Pineal organ of lamprey, Lampetra japonica, was suggested to be highly associated with the circadian locomotor rhythm from the previous studies on pinealectomized animals. In addition to this, the present study demonstrates that the pineal of this species might control also the daily locomotor rhythm seen in the LD cycle. The locomotor activities of the intact animal in the light phase (50-80 lux) were suppressed except for the short period (lasting usually within 30 minutes) at the beginning of the phase. Frequent movements started within 10 minutes after the onset of dark phase and lasted for more than half period of the phase. Enucleation of lateral eyes did not alter this pattern of locomotor rhythm. However, pinealectomy completely reversed this type of activity, i.e. frequent and continuous movement in the light and suppressed movement in the dark phase. Thus, the pineal of this animal may have excitatory in the dark and inhibitory effect in the light on the locomotor activities. This might be related in the electrical activities of the pineal organ firing in darkness.

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EFFECTS OF SCN LESIONS ON THE CIRCADIAN WHEEL-RUNNING AND SLEEP-WAKING RHYTHMS IN THE CHIPMUNK. SATO, T. and KAWAMURA, H. Physiol. Psychol. Lab., Dept. Neurosci. Mitsubishi-Kasei Institute of Life Sciences. Machida-shi, Tokyo.

The role of the suprachiasmatic nucleus (SCN) as an oscillator of circadian rhythms was examined in a diurnal rodent, the siberian chipmunk (*Tamias sibiricus*). Circadian rhythms were measured by means of continuous recordings of wheel-running activity or polygraphic recordings of ECoG, EMG and EOG. Polygraphic recordings indicating sleep-waking transition were simultaneously analysed by an automatic analyser. In the LD condition, animals showed strict diurnal activity patterns with the onset of wheel-running or waking 1-2 hours before the onset of the light period. After transition to LL (ca, 10 lux), most of the animals showed freerunning rhythms with periods slightly shorter than 24 hours. After SCN lesions, these freerunning rhythms had completely disappeared till the end of recordings lasted up to 45 postoperative days. Furthermore, these animals had lost the ability to entrain the LD schedule. On the other hand, lesions of the loci other than the SCN did not abolish freerunning rhythms in the LL and entrainments to the LD cycle. In addition, direct retinal projection to the SCN was histologically confirmed using HRP as an anterograde tracer. These results strongly suggest that, in diurnal chipmunks, SCN plays a role as a circadian pacemaker similarly as in nocturnal rodents.

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VENTROMEDIAL HYPOTHALAMUS AND THE SUPRACHIASMATIC NUCLEUS IN THE REGULATION OF CIRCADIAN RHYTHMS. INOUYE, S. T. Laboratory of Neurophysiology, Mitsubishi-Kasei Institute of Life Sciences, Machida-shi, Tokyo.

In addition to the occurrence of dissociation or splitting phenomena in the circadian rhythms, multiplicity of the circadian pacemaking system is suggested by anticipatory activities which precede the feeding time under restricted daily feeding schedule. In search for physiological mechanisms underlying these anticipatory activities, effects of ventromedial hypothalamic (VMH) lesions were investigated using the rat. Day and night differences in the frequency of overall movement were greatly reduced after the VMH lesions, but small differences still existed. However, anticipatory increase in motor activities before the time of food-delivary was disappeared when the bilateral VMH were ablated. On the other hand, circadian rhythms in multiple unit activity of the suprachiasmatic nucleus were essentially not influenced by the VMH lesions.

Taking previous reports into consideration, these results indicated the possibility that the ventromedial hypothalamic nucleus might be a relay structure which convey the circadian information from the suprachiasmatic nucleus to the feeding behaviour, which, in turn, regulated many other physiological states.

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ECOG SLEEP-WAKING RHYTHMS AND BODILY ACTIVITY IN THE CERVEAU ISOLÉ RAT.
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In the rat with a high mesencephalic transection isolating both the raphe nuclei and locus coeruleus from the forebrain, Electrocorticogram (ECoG) and Electromyogram (EMG) of neck muscles were continuously recorded. Normal sleep-waking ECoG changes with a significant circadian rhythm reappeared in 4 to 9 days after transection. EMG indicating body movements was completely independent of the ECoG sleep-waking rhythms. These results suggest that in the forebrain a mechanism inducing ECoG changes in transition from the slow wave sleep to waking and vice versa is localized. In these rats, we made large bilateral electrolytic lesions either in the preoptic areas or in the posterior hypothalami. After the preoptic area lesions, the amount of waking time per day increased by 36.3% of that before lesions, whereas after the posterior hypothalamic lesions, the total amount of waking time per day markedly decreased showing continuous slow wave sleep pattern.

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DAILY VARIATION OF SLEEP AND WAKING IN THE LABORATORY CATS UNDER L-D 12:12 AND CONSTANT DARK SCHEDULE
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Daily variations of sleep-waking in 17 male adult laboratory cats(1-3years,3500-4500gr.)were observed polygraphically under repeated L-D 12:12 (07:00-19:00, light period)and subsequent constant dark schedule. Brain electrical activity (bilateral F-C, C-O,Hippocampus, RF), EOG,EMG,ECG and Brain Temperature were continuously monitored during these observations. Three sleep stages were difined on the bases of above mentioned information that were Awake, Slow Wave Sleep and Paradoxical Sleep.

Pattern percentage of each sleep stage during L-D 12:12 schedule indicated that there shared much more total sleep time (TST) in the lighted period than in the dark period: Aw,38.9±4.7%, TST,60.2±5.9% in light period Aw,53.6±6.1%, TST,46.1±2.8% in dark period. In the constant dark schedule Awake time incresed in 07:00-19:00 and 19:00-07:00 period: Aw,48.0±6.9%, TST,52.0±6.3% in the former., Aw,53.5±11.7%, TST,46.5±5.5% in the latter.

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CIRCADIAN SLEEP RHYTHMS OF THE RATS ADMINISTERED WITH METHYLMERCURY CHLORIDE.
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Polygraphic recordings of circadian sleep rhythms of adult male rats were made before and after oral administration of methylmercury chloride (MMC) at a dose of 15 mg MMC/kg body weight/day for two consecutive days. The MMC-dosed rats exhibited an increase of slow wave sleep (SWS) only during the dark period, a decrease of light-phase paradoxical sleep (PS) and an increase of dark-phase PS. A phase of the circadian PS rhythm was delayed by 2-4 hrs after the MMC administration, whereas the circadian SWS rhythm was still entrained synchronously to a light/dark cycle. The increased amounts of dark-phase SWS retrurned gradually to the original values. The delayed phase of the circadian PS rhythm was still observed even after the dark-phase SWS had returned to the normal one. In an attempt to elucidate possible mechanisms of the delayed phase of the circadian PS rhythm, noradrenalin (NA) concentrations in several brain regions of both MMC-dosed and control rats were determined. In the morning, NA levels of MMC-dosed rats were higher in the pons + medulla and the midbrain + diencephalon than those of control rats. This relationship of the NA levels tended to be reversed at the midnight.

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EEG AND SLEEP PATTERN IN RATS WITH IRON-INDUCED CORTICAL FOCUS.

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In one group of Wistar rats, a 2.5 μl of 0.2 M $FeCl_3$ solution was injected with a microsyringe into the left sensorimotor cortex (experimental rats). Control rats were injected saline instead of ferric solution. Seven to 9 days after the microinjection, all the animals were undergone polygraphic recording during 12 h of light period(7:00-19:00). On their EEG, three states of wakefulness, slow wave sleep and paradoxical sleep were descriminated and difference in sleep pattern between experimental and control groups was distinguished. In EEG of experimental rats, isolated spike or spike and wave complex were observed during the period of wakefulness and slow wave sleep. As the most characteristic EEG finding, sleep spindle-like activity was observed during paradoxical sleep period in some of experimental rats. The averaged number of this activity was 0.92±0.75 per min during paradoxical sleep. This figure was significantly larger than that of control animals. In the animals which showed spike and wave complex in EEG, total sleep time was shorter and the percentage of paradoxical sleep to total sleep time was the lowest.

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Effect of α-endorphin on sleep

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Intraperitoneally injected α-endorphin changed a sleep latency longer 2-3 fold than the control as a dose-dependent manner in mice. The optimal doses were 1-3 nmole per body (per 0.1-0.3 ml water), although there were no significant difference of the sleep latency in a range of 4-6 nmole per body (per 0.4-0.6 ml water) between two groups. A daily amounts of paradoxical sleep and slow wave sleep were not affected by α-endorphin.

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RELATION BETWEEN CENTRAL MECHANISM OF ANIMAL HYPNOSIS AND ACUPUNCTURE ANALGESIA.

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The central mechanism of animal hypnosis was investigated in correlation with that of acupuncture analgesia by measuring the duration of animal hypnosis(DAH) induced by inversion of rabbits.

DAH and analgesia during inversion were not influenced by naloxone, an opiate antagonist. DAH was prolonged by stimulation of the dorsal periaqueducal central gray or by administration of morphine and this effect was abolished by naloxone or by the lesion of acupuncture afferent pathway such as the lateral septum or the anterior hypothalamus.

DAH was shortened by methysergide, a serotonergic blocker, and it was extremely prolonged by tetrabenazine, a monoamine depletor.

The lesion of lateral portion of the fourth ventricle in the dorsal pons shortened DAH.

From these results, it was concluded that the central mechanism of animal hypnosis was different from that of acupuncture analgesia, but was modified by acupuncture afferent pathways and hence by endogenous morphine like factor which was liberated by activation of acupuncture afferent pathway.

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EFFECTS OF ELECTROCONVULSIVE SHOCK ON SLEEP AND MONOAMINES IN THE BRAIN
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Electroconvulsive shock (ECS) was given to rats every 6 hrs, 4 times. Slow wave sleep appeared within 30 min after ECS and its amount was same as in the control group. Paradoxical sleep (PS) was slightly suppressed for 3 hrs after ECS and in this period the amount of PS remained constant regardless of the frequency of ECS and administrated time. Thereafter the amount of PS returned to the level of the control group, but no rebound phenomenon was observed. Occasionally, before the appearance of PS after ECS incomplete PS lasted for only several seconds and its latency fluctuated according to the circadian rhythm of PS. Rebound phenomenon was not observed even with ECS every 3 hrs, 5 times.
The amount of noradrenaline and dopamine in the brain was not changed after ECS. Serotonin increased 30 min after ECS and decreased 4 hrs after ECS in the midbrain and thalamus. 5-hydroxyindoleacetic acid increased in the same part of the brain 4 hrs after both single and repeated ECS.

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EFFECTS OF L-DOPA ON SLEEP-WAKEFULNESS PATTERN AND BRAIN AMINE METABOLISM IN PORTACAVAL SHUNTED RATS.
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Rats with portacaval shunt(PCS) is often used for a study of hepatic encephalopathy. Changes of durinal(9:00-19:00) sleep-wakefulness pattern and brain amine metabolism in rats with PCS were previously reported. In this report, the effects of L-dihydroxyphenylalanine(L-dopa) on the sleep-wakefulness pattern and the metabolism were studied to elucidate the mechanism of these changes.

1) On the 14th or 16th day after PCS, latency of paradoxical sleep(PS) was prolonged and the amount of PS was significantly decreased. However, these alterations were eliminated when L-dopa(100 mg/kg, iv) was infused cotinuously for 4 hours(9:00-13:00) on the 16th day after PCS. 2) After the continuous administration of L-dopa, the increased concentration of aromatic amino acids in the brain after PCS was not lowered. However, levels of noradrenaline and dopamine were significantly increased in various parts of the brain. Serotonin was slightly decreased and 5-hydroxyindoleacetic acid tended to be lowered to the normal level in the brain.

The relationship among brain amine metabolism, sleep-wakefulness pattern and effects of L-dopa in rats with PCS was discussed.

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EOG AND HEAD MOVEMENT DURING VISUAL PURSUIT IN THE MENTALLY RETARDED CHILDREN.
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In the mentally retarded children, their developmental handicap is partially reflected on the eye movement in a visual pursuit, but a role of the head movement in it should be altogether considered. The method of the present experiment was almost the same as in the last report (1981), that is, the task for a subject was to track visually a sinusoidally or step-like moving target in the horizontal direction. In order to detect the head rotation, a chin-stabilizer was enabled to move laterally and its displacement was transduced into an electric current. Subject groups were those of mildly retarded children(retarded), normal children and adults (normal).

Frequency response characteristics were not essentially different between the head-fixed and head-unrestrained conditions in each group. The head movement was more unstable and irregular than the eye movement and the time relation to the target was also varied. In the head-unstrained condition, however, attenuation of the EOG amplitude in higher tracking frequencies was noticed in children, especially in the retarded. The attenuation in the low or middle frequency may be due to the compensatory head movement. In the retarded group, the target was often followed rather by the head movement than by the EOG. From these, a large dependence on the head movement in the retarded is suggested.

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SPEECH-PHYSIOLOGICAL STUDIES ON THE "T" SERIES IN JAPANESE LANGUAGE.
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I have made uninterrupted observation on six Tokyo-accent children without any defects in their speech-organ at their period of changing dentition from shedding of milk central incisors to eruption of lateral incisors by recording on Palatogram and Sonagram. I have compared the results of the observation with those on pronunciation of "T" series by infants and adults. The comparison showed that in the Palatogram of children at the period of changing dentition, the pronunciation of "Ta", "Te", and "To" was completed in their Palatogram at a comparatively early stage.

But the pronunciation of "Tshi" and "Tsw" showed self-revision by their tongues and showed lack of stability.

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CHANGES IN LEARNING PERFORMANCE IN RATS WITH SPONTANEOUSLY HYPERTENSION
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We examined learning performance to use a physiological conditioning of operant type. Spontaneously hypertensive rats(SHR) are much sensitive and hyperactivity to strong stress such as a footshock, so we subjected the more natural learning schedule than negative reinforcement.

One was a brightness discrimination learning test, and the other was a Fixed-Ratio 50 schedule using a Skinner box.

SHR was superior in the lever-press response on the brightness discrimination learning test, but the correct response ratio($R+/R^{+}+R-$) was inferior to those of Wistar Kyoto strain. SHR reached a high level of performance in the Fixed-Ratio 50 schedule, shorter in the interreinforcement interval and postreinforcement pause, and higher in response rate per minute than those of WKY.

The data clearly indicate that SHR has a high level of response, but a low level of discrimination ability in physiological conditioning.

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CHANGES IN LEARNING PERFORMANCE OF TRYPTOPHAN DEFICIENT FEEDING RATS.
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Newborn rats were fed on tryptophan deficient diet. The retardation of body and brain growth was remarkable. The physical growth recovered under the tryptophan supplemented therapy to tryptophan deficient diet. Three month old rats of try. deficient and try. supplemented therapy were subjected to a brightness discrimination learning test using an operant procedure.The try. deficient rats did not exceed 80% on correct response ratio until 30th session, whereas normally and try.supplemented rats scored more than 85% on the 20th session. Fixed-Ratio 50 schedule also run to these 3 group rats. Interreinforcement interval and postreinforcement pause prolonged session by session on normal and try. supplemented rats for 20 trials. On the contrary, try. deficient rats did not prolong in FR-50 schedule. The activity of these rats, response/minute showed remarkably high rate in try. deficient rats.

These behavioral changes of try. deficient rats correlated with the retardation of serotonergic nervous system.

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LATERAL HYPOTHALAMIC NEURAL ACTIVITY AND TASTE STIMULATION IN CONSCIOUS RAT YAMAGUCHI,K.*,HARADA,S. AND KASAHARA,Y. Dept.of Psychol., Fac. Let., Kagoshima Womens Col.* & Dept. of Oral Physiol.,Dent.Sch.,Kagoshima Univ., Kagoshima.

The present study was undertaken to investigate the effect of taste stimuli on neural activities of lateral hypothalamus (LH). Anesthetized 25 rats were chronically implanted manipulating microelectrode to the LH and experiments were carried on unrestrained unanesthetized condition under the 23 hours water deprived state. As a indication of taste stimulation, licking patterns were also recorded on the neuron activities simultaneously. The taste solutions used in this experiment were tap water, 0.3M & 0.5M NaCl and 0.5M Sucrose. These solutions were presented to the subjects at random and changes of neural activities of LH during tasting these solutions were recorded. Results obtained 25 units out of 100 units were summarized as follows; Taste sensitive LH neurons which increased firing rate were 17. On the contrary, 8 units were decreased or disappeared by taste stimulation. Among other things, the most evident phenomenon was the pre-phasic increase of firing rate before licking and this pre-phasic increase of firing rate seemed to reflect the motivational aspect.

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REGIONAL DIFFERENCE OF HISTAMINE-ELICITED CYCLIC AMP ACCUMULATION IN INCUBATED SLICES FROM RABBIT CEREBRAL CORTEX WITH A DOMINANT FOCUS. HATTORI,Y., MORIWAKI,A., INABA,K.*, PAVLYGINA,R.A.** and HORI,Y. Dept. of Physiol., Okayama Univ. Med. School, Okayama 700, Dept. of Cell Biol., Hyogo Univ. of Teacher Educat., Yashiro, Hyogo 673-14* and Institute of Higher Nerv. Act. and Neurophysiol., Butlerov Str., Moscow 117485**.

In unanesthetized rabbits, a dominant focus(DF) was unilaterally formed in the right motor cortex by passing a weak anodal direct current (0.6-30μA). A criterion for DF formation was more frequent flexion of left forelimb than the right. Animals were sacrificed after confirming DF formation. The cerebrum was quickly extirpated, and the cerebral cortex was dissected into four parts; anterior and posterior halves of the right and left cortices. Slices prepared from each cortical quadrant were incubated in Krebs-Ringer bicarbonate buffer with or without 0.1 mM histamine for 15 min after 50 min-preincubation. In the right anterior cortical quadrant, histamine-elicited accumulation of cyclic AMP was larger than in the left anterior quadrant. In the posterior quadrants on both sides, cyclic AMP accumulation was almost in the same level as in the right anterior quadrant or in a little lower level.

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MULTIPLE UNIT ACTIVITY RELATED TO OPERANT LEARNING OF EYE MOVEMENTS IN ENCEPHALE ISOLÉ CATS. IKEGAMI, S. and KAWAMURA, H. Lab. of Physiol. Psychol., Mitsubishi-Kasei Institute of Life Sciences, Machida-shi, Tokyo.

In encéphale isolé cats under local anesthesia, operant conditioning of vertical eye movements was achieved in total darkness by reinforcement of lateral hypothalamic (LHT) stimulation, when visual feedback associated with eye movements was eliminated. Neural mechanism involved in the motor learning was studied by recording multiple unit activity (MUA) in intermediate layer of superior colliculus (SC), area 17 of visual cortex (VC) and mesencephalic reticular formation (MRF). Discharge patterns for 2.8 sec before and after onset of eye movement were averaged every 16 eye movements using a signal processor. EOG was averaged similarly. Averaged responses obtained from the SC and VC indicated slightly specific enhancement of discharge rate associated with the movement onset in the control session. This enhancement increased markedly during the conditioning session and decreased to the control level during the extinction. However, it did not so much increased when LHT stimulation was given non-contingently with respect to eye movements. Responses in the MRF showed facilitation to LHT stimulation, which were related to general arousal. These results are in agreement with our earlier finding on evoked potentials in the SC and VC (Brain Res., 169, 1979) and suggest that corollary discharge may play an important role for acquisition of the operant conditioning.

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BIMODAL EFFECTS OF 2-DEOXY-D-GLUCOSE ON HYPOTHALAMIC NEURONS, AND ON SHORT AND LONG TERM FEEDING. Shiraishi, T., Dept. of Physiol., Sch. of Med., Tokai Univ., Isehara 259-11 Japan.

Microinjections or electroosmotic applications of 2-deoxy-D-glucose (2-DG) were made in the lateral hypothalamus (LHA), and 2-DG was perfused into the IIIrd ventricle (i.c.v.) or injected intraperitoneally (i.p.). Respective changes in gastric acid secretion, LHA unit activity, or feeding behavior were then observed. Electroosmotic application induced four types of LHA unit activity change. Gastric acid secretion usually increased after microinjection of 2-DG into a specific LHA region, and occasionally upon its electroosmotic application in the same region. Hypophagia after carefully controlled lesions in this LHA region verified the involvement of these neurons in feeding control. Applications of 2-DG, either i.p. or i.c.v., induced hyperphagia within 4 hr which was then followed by hypophagia for at least 96 hr. Low frequency EEGs were concomitant with the lethargy and ataxia which followed i.p. injection. After i.c.v. injections, the low frequency EEG and the lethargy and ataxia were not evident. Present results in connection with prior reports indicate that 2-DG has a long term bimodal effect on feeding which is mediated basically through central neurons. Hypophagia after peripheral application of 2-DG appeared to be caused at least as much by concomitant traumatism as by effects on neural control of feeding.

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UNIT ACTIVITY IN THE BASAL GANGLIA DURING BAR-PRESS FEEDING BEHAVIOR IN MONKEYS. NISHINO, H., ONO, T., FUKUDA, M., SASAKI, K. AND MURAMOTO, K-I. Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01.

Unit activity in the head of the caudate nucleus (CD) and the globus pallidus (GP) of the monkey was recorded during bar-press feeding behavior and relations between neuronal activity and feeding events were investigated. The feeding behavior consisted of 3 stages: i) 1st stage, at the sight of food, ii) 2nd stage, during bar press, and iii) 3rd stage, during ingestion. Results were: 1) Of 350 CD neurons 47(16%) responded in the 1st stage and one third of them responded selectively at the sight of food with different responses depending on food nature. 2) Thirty-five(10%) neurons responded in the 2nd stage with poor correlation with bar-press movement. Some of these responses disappeared during simultaneous iontophoretic application of α-aminoadipate or dopamine and during bilateral prefrontal cooling. 3) Sixty-two(18%) of the neurons responded in the 3rd stage. 4) Of 317 GP neurons, 11(7%) responded in the 1st stage, and a few of them responded selectively at the sight of food. 5) In the 2nd and 3rd stages, however, 146(45%) and 96(30%) of the neurons responded respectively, and most of these were correlated with arm, hand or mouth movement. The results suggest that the CD which receive massive inputs from association cortices is related to sensory and motor integration while the GP is predominantly concerned with motor function.

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MODIFICATION OF RUBRAL UNIT ACTIVITIES ASSOCIATED WITH CLASSICAL CONDITIONING IN THE CAT. ODA, Y., KUWA, K., MIYASAKA, S. AND TSUKAHARA, N. Dept. of Biophysical Eng. Fac. of Engineering of Science, Osaka University.

We examined unit activity of the red nucleus (RN) cells before and after establishment of classical conditioning of forelimb flexion in the cat. After pairing electric shocks to the cerebral peduncle , as the conditioned stimulus (CS), and to the forelimb skin, as the unconditioned stimulus (US), an initially ineffective CS gave rise to elbow flexion. It was confirmed by using the computer system for automatic delivery of CS-US and estimation of positive conditioned responses (CR). (If CS was applied to the interposito rubral fibers, there was not any acquisition of CR.) Unit discharges of RN cells were recorded extracellularly in the alert conditioned and control cats. Control cats involved two cats where CR was extinguished, one cat which received pairing CS with US at random intervals and two cats which received no trainig. More than half of RN cells responded to CS in the conditioned cats with the minimum latency of spike ranged 3.5-4.0 msec. In the control cats RN cells usually did not respond to CS above spontaneous firing rate. The firing rate of RN cells during 11 msec following the onset of CS in the conditioned cats was significantly higher than those in the control cats ($p<0.001$).
The present results indicated that the modification of the RN cell activities indeed occurred after the establishment of classical conditioning mediated by the cortico-rubro spinal pathway.

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LONG-LASTING EFFECTS OF THE SLEEP-PROMOTING SUBSTANCE.
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Under continuous intracerebroventricular infusion of saline solution, male rats regularly exhibited 394.0±12.5 min slow wave sleep (SWS) and 80.1±6.2 min paradoxical sleep (PS) during the light phase (L, 0800-2000), while 219.6±6.8 min SWS and 28.7±4.0 min PS during the dark phase (D, 2000-0800). A 10-h infusion (1900-0500) of the sleep-promoting substance (SPS) caused a 53 % increase in SWS and a 31 % increase in PS during the D phase. SWS was unchanged during the following L phase, but a 20 % increase was still observed during the D phase. By contrast, PS largely decreased during the following L phase (70 %) and increased dramatically during the D phase (99 %). The rises and/or falls in both SWS and PS returned to the baseline levels during the next L and D phases. It is suggested that daily requirements for total PS lie within a fixed range (ca. 110 min) under the control of a circadian balance mechanism, that SPS may exert long-lasting effects to promote SWS, and that a rebound PS during the recovery night may be attributed to a requirement for a daily PS balance.

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RADIOACTIVE 2-DEOXYGLUCOSE UPTAKE INTO BRAINS OF MONKEYS PERFORMING INTRACRANIAL SELF-STIMULATION TASK. MATSUNAMI,K.,*FUNAHASHI,S. AND KUBOTA,K. Dept. of Neurophysiol., Primate Res. Inst., Kyoto Univ. Inuyama & *Dept. of Physiol., Nara Medical Collage, Kashihara, Nara.

(^{14}C) 2-deoxy-d-glucose (2-DG) uptake into brains of two monkeys was studied in relation to intracranial self-stimulation (ICSS). Substantia innominata was stimulated to elicit ICSS effects, which were observed in both monkeys. Using these ICSS as rewards, monkeys were trained to move a handle with wrist movement from a central start position to a left or right target position. Monkeys were rewarded with ICSS when they succeeded to keep the handle in the target position for 0.5 second. On the experimental run, 2-DG was injected into the vein through an implanted cannula. After 45 minutes' continuation of the task, monkeys were killed to make autoradiographs from sections of brains. In this study, 2-DG uptake into the "reward system" was investigated. Remarkable to moderate 2-DG uptake was observed in caudate nucleus, substantia nigra, locus coeruleus and raphe nuclei. But it could not be determined in this study alone whether it was ICSS specific or motor specific.

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NEURONAL INTERACTION IN THE RETICULAR ACTIVATING SYSTEM AS ASSESSED FROM THE CROSS-CORRELOGRAM. EGUCHI, K. AND SATOH, T. Dept. Physiol., Fac. Dent. Med., Aichi-Gakuin Univ., Nagoya 464.

Temporal cross-correlation of firing of neuron pairs simultaneously recorded in the cat's brain stem was measured to investigate the mode of operation of the reticular activating system during different states of sleep and wakefulness. None of 125 neuron pairs recorded from the midbrain reticular formation(MRF) showed a cross-correlogram suggesting cascade connection between two neurons. However, 41 pairs showed weakly synchronized firing which often occurred periodically with the mean interval of 1.23 sec. This periodicity was most accentuated during slow wave sleep. The neuronal circuit which can produce the observed periodic synchronization would be the shared inhibition. No positive relationship was found between the MRF neurons and the neurons in the dorsal raphe nucleus or the solitary tract nucleus. The non-specific thalamic neurons showed quite often a weakly correlated firing with the MRF neurons; The delay between the correlated firing being 10 - 900 msec.

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THRESHOLD ILLUMINATION FOR ENTRAINMENT OF THE CIRCADIAN LOCOMOTOR RHYTHM IN THE GIANT LAND SNAIL, ACHATINA FULICA. OGASAWARA, T. and TASAKI, K. Dept. of Physiol., Tohoku Univ. Sch. of Med., Sendai

Entrainment and phase-shifting of the circadian locomotor rhythm in the giant land snail, Achatina fulica were examined in 24 hr cycles of light of various intensities. Intact and eyeless snails can show the entrained nocturnal behaviors in 300 lux light cycles and can exhibit free-running rhythms in continuous 300 lux light. The rhythms in intact snails were also entrained to 3 and 0.7 lux light cycles and followed 6 hr phase advance of the cycles. Splitting of active phase was observed in the cycles such that one active phase occurred in the latter half of the light period and the other in the latter half of the dark period. In continuous 3 lux light, one of the two splitting peaks almost disappeared and the other freeran. Animals whose one eye had been surgically removed also showed the nocturnal behaviors in 0.7 lux light cycles and followed 6hr phase advance of the cycles. Eyeless animals, however, became inactive and expressed ultradian rhythmicity in small activity. Eyeless animals showed the nocturnal behaviors in 30 lux light cycles, but tended to increase total activity in the light period of 3 lux light cycles.

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FOSTER MOTHER ENTRAINS THE CIRCADIAN RHYTHM OF BLINDED INFANT RATS TAKAHASHI,K. AND DEGUCHI,T. Dept. of Medical Chemistry, Tokyo Metropolitan Institute for Neurosciences

To investigate the role of mother rat in entraining the circadian rhythm in pups, three different rhythms were measured in blinded pups optically enucleated on day 1 and raised by either natural mother or foster mother with the reversed rhythm to that of the natural mother. The rhythm of serotonin-N-acetyltransferase activity in the pineal gland was determined at 4 and 6 weeks after birth. Forty-eight h patterns of blood corticosterone levels were determined once a week for consecutive 4 weeks starting at the 4th week. Locomotor activity was recorded continuously after weaning at 3 weeks until the end of the 8th week. All of these rhythms were in phase during the period examined. The phase of the rhythms was identical between blinded pups raised by natural mother and by foster mother under the same lighting condition, indicating that the foster mother acted as an entrainer of the circadian rhythm of blinded pups.

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NA,K-ATPASE INHIBITOR IN BOVINE CENTRAL NERVOUS SYSTEM

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Bovine hypothalamus was extracted with acetone-HCl solution and the extract was treated with petroleum ether. In the lyophilized extract, we found inhibitory activities against Na,K-ATPase and ouabain-binding. The inhibitory activities were eluted in small molecular fractions by Sephadex G-15 gel filtration. These inhibitory activities were not due to metal ions since the contents of metal ions in the Sephadex fractions were not parallel to the inhibitory activities. The inhibitory activities were also adsorbed to anion exchanger in basic pH after adsorption to cation exchanger in acidic pH, suggesting that the inhibitory substances acted as cation or anion in different pH. That the inhibition of ouabain-binding was competitive type was shown by scatchard plot analysis. The activities survived after treatments of trypsin, boiling but slightly decreased after carboxypeptidase A treatment. By thin layer chromatography, it appeared that the inhibitory activities were not due to a single substance.

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ISOLATION OF CNPASE FROM SERUM AND RED BLOOD CELL MEMBRANE.

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The enzyme 2',3'-cyclic nucleotide 3'-phosphohydrolase (EC 3.1.4.37. CNPase) is largely present in the myelin of the central nervous system and has been isolated from bovine cerebral white matter by the extraction of ammonium acetate - Triton X-100 and successive column chromatographies. We found that CNPase existed in serum and red blood cell (RBC) membrane on many kinds of mammals at considerable amount. CNPase from the serum of rabbit was isolated by successive column chromatographies. The specific activity of this isolated enzyme was about 100 u moles/min/mg protein. CNPase from the RBC membrane of rabbit was isolated by the procedures for the CNPase purification from bovine cerebral white matter. The specific activity of this isolated enzyme was about 1,500 u moles/min/mg protein. The enzymes obtained from the serum and the RBC membrane were basic and hydrophobic protein having pI 8.6 - 8.8. Optimum pH for the activity of these enzymes was 5.6 - 6.0. Km values were 2.04 mM and 1.98 mM respectively using 2',3'-cAMP as a substrate. These properties were very similar to those of the myelin CNPase.

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BIOSYNTHESIS OF PROTEINS IN PERIPHERAL NERVE MYELIN

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In vitro synthesis of myelin proteins in chick embryo peripheral nerve were studied. Myelin prepared from the chopped tissue after incubation with radioactive leucine showed much higher incorporation than that from the homogenized tissue. At the stage of myelination radioactive leucine was more actively incorporated into myelin proteins than adult. Incorporation of radioactive leucine into the myelin continued up to 24 hours incubation. When these proteins were separated on SDS polyacrylamide gel electrophoresis, peaks of radioactivity were found to correspond to myelin specific proteins, such as P0 and BP. Proteins of high molecular weights (more than 30,000 dalton) also showed a considerable incorporation of radioactivity. At 19 chick embryonic days, specific activity of the BP protein was higher than that of the P0 protein in vitro experiments. These results may indicate the different localization and role of two proteins in myelin formation.

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NEUROCHEMICAL STUDIES ON MYELINOGENESIS IN ORGANOTYPIC CULTURE FROM RODENT CEREBELLA.
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We attempted to analyze the biochemical organization of myelin sheath in organotypic cerebellar culture. In the organotypic cultures of rat and mouse cerebella from the 14th day of gestation to the 2nd day of postnatal life, myelinogenesis in all cultures examined promptly started on the 9th day of postnatal life.

The glycoprotein synthesis in the explants of cerebellar culture from newborn mice were found from the 7th day of postnatal life before the onset of myelin formation. The synthesis of protein and lipid and the content of galactocerebroside were being increased during the myelinogenesis in the explants. The contents of myelin specific proteins, such as myelin basic protein (MBP),2¦3'-Cyclic nucleotide 3'-phosphohydrolase (CNPase) and proteolipid proteins were increased along the maturation of myelin sheath.

By the addition of EAE serum to the culture from the begining of incubation, myelinogenesis did not occured accompanyed with the lowering of synthesis of protein and lipid and the activity of CNPase. The addition of 2% anti-galactocerebroside serum to the culture medium, myelin formation was completely inhibited, and glycoprotein synthesis and CNPase activity were also suppressed. When 10% fresh anti-MBP serum was added to the culture, myelinogenesis and increases of protein synthesis and CNPase activity were clealy diminished.

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NEUROCHEMICAL STUDIES ON THE DYSMYELINATION OF THE SNELL PITUITARY DWARF MICE.
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We attempted to delineate the events resulting in the poor myelination in the brain of the Snell dwarf mouse(dw/dw), and to devise an immunohistochemical method for identifying the mutant neonate. Differences in the brain weights of the dw/dw and normal littermates(+/?) first became apparent on the 10th day of age, and from this time on no further increase in the weight of the dw/dw mouse brain was recorded. Increase in CNPase activity was found to be suppressed in the cerebrum and brain stem throughout the developmental stage, but not in the other parts of the brain. The yield of isolated myelin decreased by 58% in the mutant mouse, and CNPase activity was equivalent to that of control myelin. Differences in DNA content per cerebrum from the dw/dw and +/? mice first became apparent on the 10th day of age. Henceforth, the dw/dw mice showed no further increase, although the +/? mice continued to increase. [^{3}H]-Thymidine incorporation into DNA fraction in vivo on the 7th day of age, when glial cell proliferation in the cerebrum is most active, was suppressed to about 50% of the control level in all parts of the dwarf brain. These findings indicate that the poor myelination found in the mutant cerebrum is a dysmyelination due to reduced oligodendroglial proliferation caused by lack of circulating growth hormone.

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DEVELOPMENTAL CHANGE IN DNA CONTENT IN GUNN RAT'S BRAIN
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The DNA content, a marker to growth of cell, and glutamic acid decarboxylase activity, a marker to GABAergic terminal, were measured in Gunn rats, which possess an automosal recessive gene for jaundice or kernicterus, during postnatal growth. It is the aim, whenever possible, to correlate these changes with the structural and physiological maturation of the brain. Male heterozygous (phenotype, normal) and homozygous (phenotype, jaundiced) Gunn rats raised in this laboratory were used. The brains were analyzed at 1, 2, 3 and 4 weeks after birth and adult age. Under ether narcosis, the rats were decapitated, whole brains were immediately removed, and divided into 4 regions: the cerebellar cortex (hemisphere), neostriatum, pallidum and substantia nigra. Immediately after excision, tissues were weighed and homogenized with 0.1 ml of 0.1% Triton X-100 solution for the analysis of DNA by the thiobarbituric acid assay and of GAD by the radiochemical tracer experiment. In the cerebellar cortex, neostriatum and substantia nigra from the animals with kernicterus, the growth of cell is delayed,but by the adult stage it is normal in the cerebellar cortex and neostriatum,at least as indicated by DNA content to mg protein. In the cerebellar cortex from the animals with kernicterus, GAD activity to mg DNA is lower than in the normal animals. However, it is difficult to see how the difference of the developmental changes correlates with defects.

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EFFECT OF CYTOSINE ARABINOSIDE ON DEVELOPING RAT BRAINS WITH REFERENCE TO CHANGES IN CEREBELLAR DNA AND MONOAMINE LEVELS. TAMARU, M., HAYAKAWA, Y. AND MATSUTANI, T. Dept. of Dev. Physiol., Inst. for Comprehensive Med. Sci., Fujita-Gakuen Univ., Sch. of Med., Toyoake, Aichi 470-11.

On four successive days from 4 days of age, 30mg/kg/day(s.c.) of cytosine arabinoside (ara-C) was administrated to a group of newborn rats, while the same amount of physiological saline was given to control animals. At 60 days of age, the weight and concentration of DNA of cerebellum were reduced to one third of control. The RNA concentration also reduced by 80% of control, resulting in 2.7 fold increased in the RNA/DNA ratio. This suggested that a relative increase of large cells, such as Purkinje cell, resulting from damaged production of small granular cell due to ara-C poisoning. Concentrations of norepinephrine(NE) and 5-hydroxytryptamine(5-HT) in the cerebellum were 3 and 4 fold higher than control, respectively, suggesting abnormal formation of monoaminergic synapses accompanying the damaged proliferation of granular cells. Ara-C(280mg/kg;i.p.) treatment on the 15th day of gestation caused no significant change in levels of DNA, RNA, NE and 5-HT of cerebellum, though the cerebellar weight slightly reduced. These findings indicate that ara-C poisoning at the earlier postnatal stage causes on elevatios of monoamine concentrations accompanying tissue malformation in the cerebellum similar to that in the cerebrum poisoned prenatally which previously reported by us.

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THE INFLUENCE OF THE RESTRICTION OF DIET ON THE BRAIN WEIGHT AND DNA CONTENT IN THE BRAIN OF RATS TREATED PRENATALLY WITH METHYLAZOXYMETHANOL. NAKAHARA, T., NAGAYOSHI,M. AND MATSUTANI,T. Dept. of Dev. Physiol., Inst. for Comprehensive Med. Sci., Fujita Gakuen Univ., Sch. of Med., Toyoake, Aichi 470-11, Japan

Changes in cerebral DNA content as well as reduction of brain weight were studied in methylazoxymethanol(MAM)-poisoned microencephalic rats placed under restriction of diet during developmental period, and the relationship between the time of undernourishment and the influence on brain development was also analyzed. Undernourishment was performed by halving the ration for dams during prenatal period, and by doubling the number of suckling per mother rat after birth. DNA content per cerebral hemispheres and brain weight of undernourished offspring at age of 21 days were about 70% of those of normally-nourished animals in the MAM-poisoned group, and 80% in the control group. The values for undernourished MAM-rats were about 43% of those for normally-nourished controls. This reduction of cerebral DNA content and brain weight was shown to be more strongly influenced by prenatal malnutrition in MAM-poisoned animals, while in unpoisoned controls it was shown to be more significantly related to postnatally malnutrition. These findings suggest that when brain development in rats is prenatally impaired by mitotoxic effect of MAM-treatment, prenatal malnutrition may exert a more significantly detrimental effect on the course of impaird cerebral development.

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RNA AND POLYAMINE METABOLISMS IN THE GOLDFISH RETINA DURING OPTIC NERVE REGENERATION KOHSAKA, S. AND*AGRANOFF, B.W. Department of Physiology, Keio University School of Medicine, Shinjuku-ku, Tokyo 160. *Neuroscience Laboratory, The University of Michigan.

Previous work in this laboratory on the goldfish retina following crush of its optic nerve has demonstrated unilateral enhancement of RNA precursor metabolism. An earlier change is a coordinate increase of 30-40% in kinases for uridine, UMP and UMP appearing on Day 4, with a gradual return to normal values by Day 21. Uptake studies reported here confirm a selective stimulation of the uridine salvage pathway. Autoradiographic experiments were performed to determine what specific cell is responsible for the enhanced RNA precursor metabolism. Retinas obtained from 3 days and 5 days post-crush (PC) fish were incubated with labeled uridine for 15 min and were submitted to emulsion autoradiography. At Day 3, the ganglion cell is not labeled, while by Day 5, overlying clumps of grains are clearly visible. This result indicates that the enhancement of RNA metabolism is localized to the ganglion cells.

Ornithine decarboxylase (ODC) activity increased in the goldfish retina, brain and kidney several days following unilateral optic nerve crush. Activity in both retinas followed a similar time course, and elevation being first detectable 3 days PC, maximal on Day 5 and returning to the control levels by Day 7. Sham operation or behavioral stress did not lead to a significant increases in ODC activity in the brain and retina.

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NEUROCHEMICAL STUDIES ON LYSOSOMES IN THE FETAL RAT BRAIN

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In control animals, an age-dependent variation of lysosomal enzyme (L.E.) activity was observed ; the brain had higher activities of beta-glucuronidase (β-glu.) and acid DNase (DNase) in fetuses than in adult animals (eight weeks after birth), and the liver had a higher activity of β-glu. in adult animals. In the kidney, activities of DNase and acid phosphatase were higher than those of the brain or liver. No difference between control female and pregnant animals was observed in the activities of DNase or β-glu. On day 14,15 and 16 of gestation, 15,30 or 60 mg/Kg body weight/day of Dexamethasone (Dex) was intraperitoneally given to pregnant rats of Wistar -Imamichi strain in two divided dose. In treated groups, 20 mg/Kg body weight of MAM was given intraperitoneally on day 15 of gestation, control groups received the same amount of physiological saline. On day 17 of gestation, animals were sacrificed, L.E. activities were assayed using homogenate as the enzyme source. While Dex acted in vivo as a stabilizer of lysosome menbrane in the liver of dams whether or not they were MAM-treated, in fetal brain it showed lysosome menbrane stabilizing effect only when they were MAM-poisoned. The mechanism of these effects will be explored in next stage of our study.

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DEVELOPMENTAL CHANGES OF NEURON-SPECIFIC ENOLASE AND S-100 PROTEIN IN THE CEREBRAL HEMISPHERES, BRAIN STEM AND CEREBELLUM OF RAT.

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Developmental change of the levels of neuron-specific enolase(NSE) and S-100 protein in the cerebral hemispheres (CH), brain stem (BS) and cerebellum(Ce) of rats was studied with a highly sensitive enzyme immunoassay. The concentration(μg/g wet wt.) of S-100 was higher in BS and Ce than in CH at the early stage. No such regional difference was found for NSE. On the 30th day after birth, the value of S-100 was 21.9 in CH, 20.3 in BS and 39.0 in Ce, and that of NSE was 231.3 in CH, 227.5 in BS and 313.8 in Ce. The ratio of total NSE to total S-100 reached to the adult value on the 30th day after birth in each of the three brain regiones, being high at the prenatal stage, decreasing after birth till 9th day, then increasing till 21st day, and again decreasing to the low adult level. The first peak of this ratio may represent the prenatal proliferation of neurons in the brain, and the second peak, which corresponds to the postnatal period for functional and morphological rapid development of the nervous tissues, may indicate the vigorous differentiation of the neurons.

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NEURONAL PLASTICITY OF THE BRAIN FROM THE MUTANT MICE.

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Weaver mutant mouse is characterized by the absence of granule cells in the cerebellum and reeler is characterized by the inversion of cell layers in both cerebellum and the cerebrum. Immunohistochemical staining by the antiserum against myelin basic protein was performed to visualize how the myelinated fiber arborization was altered. In the control cerebellum, myelinated fiber arborizes radially from the white matter to the granular layer (GL) to form synaptic contact with granule cells. In the weaver, the myelinated fibers send their processes to surround the Purkinje cells. In the reeler, densely packed intermingled fibers coursed in three different directions ans surrounded the Purkinje cells which located in the white matter. In the control cerebrum, the myelinated fibers from the corpus callosum radiate vertically up to the surface. In the reeler, however, the myelinated fibers climbed up in an oblique fashion as bundles to the surface and radiate in a diffuse manner. 2-Deoxyglucose method was applied to visualize the metabolic maps in the mutants. In the weaver, considerable incorporation was observed in the Purkinje cell layer, while intense incorporation was found in GL of the control and the nervous that lacks Purkinje cells. The fiber arborization was found to be much altered accompanying the alteration of 2-deoxyglucose incorporation.

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MOLECULAR BASIS OF THE ACTION OF NERVE GROWTH FACTOR.

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Mouse 2.5S nerve growth factor (2.5S NGF) stimulates the differentiation of sympathetic or sensory neurons, and keeps them alive. In this report, the molecular mechanisms of NGF action on the immature rat superior cervical ganglia was studied by the analysis of RNA' and protein' synthesis, in vitro.

Data confirmed that NGF significantly stimulates uridine uptake and RNA synthesis, but does not stimulate thymidine uptake and DNA synthesis. Furthermore, data suggested that; 1. NGF either stimulate the turnover rate of RNA synthesis and degradation, or increase the number of "channel" by which uridine is transported into neuron from the culture medium.

2. the stimulation of RNA synthesis does not regulate by the amount of uridine uptake, even if uridine uptake is stimulated by NGF.

3. NGF may act stimulative on RNA synthesis as a trigger of the sequential process.

4. the stimulation of protein synthesis is depended on the stimulation of RNA synthesis.

5. NGF may act on the synthesis of m RNA.

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EFFECTS OF TUNICAMYCIN ON THE METABOLIC REGULATION OF ACH RECEPTORS IN CULTURED SKELETAL MUSCLE CELLS.

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We have previously reported that skeletal muscle cells of newborn rats cultured under aneural conditions can synthesize two forms of surface acetylcholine receptors (AChRs) with different isoelectric points (pI∿5.1 and 5.4, as complexes with radiolabelled d-bungarotoxin). In the present study, we examined effects of tunicamycin (TM), a specific inhibitor of protein glycosylation, on the metabolism of AChRs in these cells. TM (5μg/ml for 6h) completely inhibited the incorporation of [^{3}H]mannose by the cells into TCA-precipitable fraction. AChRs synthesized under such conditions are metabolically degraded at a rate approx. 3-times faster than control. When analyzed by isoelectric focusing, only a single major form of toxin-AChR complex was detected with pI∿5.8. These results suggest that glycosylation may play an important role in the differentiation of AChRs into the two forms.

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Characteristics of digitonin-solubilized muscarinic acetylcholine receptor from rat brain.

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Muscarinic acetylcholine receptor (mAChR) was solubilized from a membrane fraction of rat striatum by 1% digitonin. The solubilized preparation was labelled with ^{3}H-quinuclidinyl benzilate (^{3}H-QNB) and separated from free ^{3}H-QNB with gel filtration on sephadex G-50 chromatography. In the present study, molecular properties of mAChR have been studied with the solubilized striatal preparation. The ^{3}H-QNB binding components in fractionation by sephadex G-50 gel filtration were prevented by 1 μM atropine, 1 mM oxotremorine and 10 mM carbachol. The Scatchard plots of specific ^{3}H-QNB binding to the 1% digitonin solubilized preparation revealed a one class of binding sites with a K_D of 1.21 nM and a B_{max} of 1546 p moles/g protein. The inhibition of ^{3}H-QNB binding to the solubilized preparation by cholinergic ligands was to be in the following order; atropine was most potent, followed by oxotremorine and carbachol. The binding of muscarinic ligands both in homogenate and in the solubilized preparation was influenced by 5'-guanylylimdodiphosphate and the binding curve of ^{3}H-QNB was displaced towards higher concentration of oxotremorine. Upon SDS polyacrylamine gel electrophoresis, the solubilized mAChR exhibited several protein and one radioactive band for the receptor was obtained and that molecular weight was estimated as 80,000.

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UNIT ACTIVITY IN RAT LATERAL HYPOTHALAMUS DURING REWARD BEHAVIOR. MURAMOTO, K-I., ORIKASA, S., 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 effect of catecholamine (CA) and opioid on intracranial self-stimulation (ICSS) behavior in the posterior lateral hypothalamus, and changes of both the CA level and lateral hypothalamus (LHA, feeding center) neuron activity were investigated in rats. Results were: 1) Intraventricular administration of amphetamine (200 nmol) and a low dose of morphine (0.5 nmol) facilitated the ICSS, while a high dose of morphine (10 nmol) inhibited and naloxone (10 nmol) had no effect. 2) Norepinephrine (NE) and dopamine (DA) in the cerebrospinal fluid and epinephrine (E) in the plasma increased significantly during ICSS. 3) Much of the LHA neuron activity was inhibited by NE intraventricular administration, but was not significantly changed by DA intraventricular administration. 4) More than half of the neurons whose activity were inhibited in the ICSS were also inhibited in feeding behavior. These results suggest that inhibition of LHA neuron activity in both ICSS and feeding behavior might depend on the common NE reward system.

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REGIONALLY DIFFERENT EFFECT OF ADENOSINE ON CYCLIC AMP LEVELS IN INCUBATED SLICES OF RAT CEREBRAL CORTEX WITH A CHRONIC EPILEPTIC FOCUS. HATTORI,Y., MORIWAKI,A., INABA,K.*, NISHIDA,N., YASUHARA,H. and HORI,Y. Dept. of Physiol., Okayama Univ. Med. School, Okayama 700 and Dept. of Cell Biol., Hyogo Univ. of Teacher Educat., Yashiro, Hyogo 673-14*.

A chronic epileptic focus without showing generalized convulsions was induced by a microinjection of 5 µl of 0.1 or 0.2 M $FeCl_3$ solution into the left sensorimotor cortex of rats. Epileptic activities appeared in ECoG ca. 1 week after the injection. Animals were sacrificed after confirming the appearance of spike discharges. The cerebral cortex was dissected into four parts, sliced and incubated in a buffer with or without 0.5 mM adenosine. In animals showed the isolated spikes in ECoG, adenosine-elicited accumulation of cyclic AMP was invariably smaller in the anterior cortical quadrant on the contralateral side of dominant spike appearance. In the other three cortical quadrants, cyclic AMP accumulation was almost in the same level. In animals showed bilateral spike and wave complex, adenosine-elicited accumulation of cyclic AMP was largest in the left anterior quadrant which included the site of microinjection and smallest in the right posterior quadrant.

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EXTERNAL IONIC CONCENTRATIONS AND PINEAL PHOTORECEPTOR MEMBRANE POTENTIAL
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Photoreceptor cells in the pineal organ of lamprey, Lampetra japonica, are hyperpolarized by light stimulation accompanied with a decrease of membrane resistance. To investigate the ionic mechanism of the receptor potential, we measured the resting membrane potential and the amplitude of hyperpolarization to a criterion light stimulus in the isolated pineal organs by changing external ionic concentrations. Decrease of external K^+ (1/10 of normal Ringer's solution) had only slight effect on both potentials. Same tendency was observed in the case of Cl^-. However, decrease of external Na^+ (1/5 of normal Ringer's solution) produced significant effects on the potentials. The resting membrane potential decreased from 34.9 ± 1.1 mV (mean $\pm$ S.E., n=37) to 24.0 ± 1.1 mV (n=33) and the amplitude of hyperpolarization decreased from 17.8 ± 1.0 mV (n=37) to 5.8 ± 0.5 mV (n=33). In addition, the hyperpolarizing response showed significant slow time course in the solutions of low external Na^+ concentration.

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PHOTORECEPTOR COUPLING IN THE PINEAL ORGAN OF LAMPREY.
TABATA,M. AND MORITA,Y. 1st Dept. of Physiol., Hamamatsu Univ. Sch. Med. Hamamatsu 431-31

The photoreceptor coupling of the pineal organ of lamprey was demonstrated with the electrophysiological and the electronmicroscopic studies. Two adjacent photoreceptor cells were recorded simultaneously with glass microelectrodes separating within 30μm. Current injection to the one cell (P1) produced potential changes in the other cell (P2). Identical results were obtained when current was injected to the P2 cell and potential was recorded in P1 cell. The maximum hyperpolarization to the criterion current intensity (-1.0nA) was 6.9mV. No measurable latency difference was observed between the onset of current injection in P1 and the elicited potential in P2. The coupling ratio between cells was estimated to be 0.6. With electronmicroscopic studies, gap juctions were often observed both in the basal processes and the inner segments of the photoreceptor cell. These results indicate that photoreceptor cells of the pineal organ of lamprey are electrically coupled. This was discussed in relation to the functional meaning of the pineal which electrically active mainly in the dim light.

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AQUATIC GASTROPOD RETINA AND DUPLICITY THEORY.
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For studying the functional and structural duplexity the retina of 14 species of aquatic gastropod were examined electrophysiologically and electron microscopically. In the retinas of all species except Haliotis discus and Turbo cornutus, two components were found in the intensity-amplitude curve of the ERG and the dark adaptation curve, suggesting the presence of the dual system as discovered in our previous study on the terrestrial gastropod retina. Repeated examination of Haliotis and Turbo retinas failed to demonstrate the existence of the functional duality, only showing a simple sigmoid in the intensity-amplitude curve of the ERG and a monotonously decreasing threshold curve during dark adaptation. This clearly indicates a single photoexcitation process in the retina of these two species. An electron microscopic examination of the retinas of all species but those of Haliotis and Turbo revealed segregation of photoreceptor cells into two types according to the density of photic vesicles in the nuclear layer of the retina. Thus the duplicity theory can be extended not only to the terrestrial gastropod but also to the aquatic gastropod except those belong to the lower evolved species.

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INFLUENCE OF THE LIVER FAT ON THE CHOROIDAL RIBOFLAVIN IN FROGS

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The liver, choroid and a piece of leg muscle were isolated from a bull frog. The fat in the liver and flavins in the choroid, muscle and liver were determined. The flavin contained in each choroid of 76 frogs ranged from 30×10^5 to 350×10^5 mol/g. Necessary conditions for the choroidal flavin being more than 150×10^5 mol/g were examined and found to be

1) flavin concentration in the muscle $\rangle 0.15 \times 10^{-5}$ mol/g,

2) 4.0 % $\rangle$ fat content in the liver $\rangle$ 2.6 %,

3) 0.037 $\rangle$ wet weight of the liver / body weight $\rangle$ 0.020.

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CEREBRAL PHOTOSENSITIVE NEURONS IN THE ORB WEAVING SPIDERS, *ARGIOPE BRUENNICHII* AND *A. AMOENA*. YAMASHITA, S. AND TATEDA, H. Dept. of Biol., Fac. of Sci., Kyushu Univ., Fukuoka 812.

1. Frequency of efferent impulses in the optic nerves of the orb weaving spiders *Argiope bruennichii* and *A. amoena* increased by illumination of the brain in which certain neurons must therefore be light activated.
2. After blocking the synaptic action by adding 2 mM-Co^{2+} to the physiological saline, cerebral illumination elicited efferent optic nerve impulses, showing that the efferent neurons were directly sensitive to light. The maximum of the spectral sensitivity occurred at 420-4420 nm.
3. Frequency of efferent optic nerve impulses decreased during illumination of the eyes. The spectral sensitivity for the inhibition was measured, and the peak was obtained at 480-540 nm with a smaller peak at 360 nm.
4. Frequency of efferent optic nerve impulses increased transiently following the light striking the eyes became dim. When the light striking the eyes and the brain became dim simultaneously, responses to dimming of light enhanced as compared when the light striking the eyes alone became dim.

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EFFECT OF INTRACELLULAR INJECTION OF TEA ON THE PROLONGED DEPOLARIZING AFTERPOTENTIAL IN FLY PERIPHERAL PHOTORECEPTORS

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A strong blue light induces a prolonged depolarizing afterpotential (PDA) following the light coincide receptor potential in a fly peripheral (Rl-6) photoreceptor when the light is given to the cell with 100% rhodopsin. A red light and a blue light which is given after the PDA do not cause PDA. After intracellular injection of TEA, however, the cell responds to both the red light and the second blue light with an enhanced receptor potential and PDA. In the present work it is shown that part of the PDA results from conductance increase of some ion of which reversal potential is around the resting membrane potential. Based on these findings, it is suggested that the PDA cancellation by red light is caused by the interruption of the transduction process rather than an inhibition by metarhodopsin to rhosopsin photoconversion.

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ROLE OF PERIPHERAL AND CENTRAL PHOTORECEPTORS IN VISUAL PATTERN DISCRIMINATION OF THE FLY

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It was suggested that the receptive field patterns of the photoreceptors contribute to the pattern discrimination through electrophysiological and behavioural studies (Mimura, 1981). This study was conducted to identify which photoreceptors among R1 to R8 subserve the pattern discrimination, on the basis of the phototactic choice behaviour in some of the photoreceptor mutants (*ora*, *sev* and *sev;ora*)of *Drosophila melanogaster*. Wild type *Drosophila* showed almost the same pattern discrimination as seen in the flesh-fly. From the phototactic behaviour in the mutants, it was concluded that the peripheral photoreceptors R1-6 mainly subserve the pattern discrimination, and the central photoreceptor R7 plays a subsidiary role but R8 does not contribute.

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PROJECTIONS OF RETINULAR AXONS INTO THE BRAIN IN THE LEPIDOPTERAN LARVA.

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A larval Papilio butterfly has six stemmata (I-VI) on each side of the head. Each stemma has seven retinular cells organized in two tiers, distal and proximal. Stemmata I-IV have three distal cells (R1-3) and four proximal cells (R4-7). Stemmata V and VI have four distal cells (R1-3, R6) and three proximal cells (R4, R5, R7).

Projections of receptor axons were examined by intracellular cobalt injection. Six axon bandles from individual stemmata entered their respective optic neuropils. The optic neuropil was divided into three regions, distal neuropil, intermediate chiasma region, and proximal neuropil. Distal cells had short axons which ended at the distal neuropils and had no spine. In stemmata II-IV, proximal cells had long axons which extended through the distal neuropils to the chiasma region (R6) or furthere to the proximal neuropils (R4, R5, R7). The long axons had spines at more or less all regions of the optic neuropils. In stemmata V and VI, proximal cells had also long axons extending to the proximal neuropils, but the axons had spines at only distal and proximal neuropils.

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PROPERTIES OF INVERTEBRATE PHOTORECEPTOR CELLS FOLLOWING RESORATION OF MEMBRANE POTENTIAL CAUSED BY A PHYSIOCOCHEMICAL METHOD.

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When the micropipettes filled with potassium ferricyanide were inserted into photoreceptor cells in ommatidia of Japanese horseshoe crab, Tachypleus tridentatus, the membrane potentials of injured cells within ommatidia were often restored and further the negativity of micropipettes increased by application of divalent Fe, Co, Ni and Mn ions into the external media.

These changes were usually accomplished within 5 to 20 min after application of these divalent cations at the concentration of 10 to 20 mM. These changes seem to be due to the formation of poorly soluble complex salts between the micropipettes and injured cell membrane caused by their insertion and also at their tips. The order of these effects is likely dependent upon the solubility of complex salts.

Responses to light, resting potential, changes in the response amplitude by high K-Ringer solutions, electrogenic Na pump and discrete potential waves were almost same as those observed in cells following successful insertion of micropipettes filled with potassium salt solutions. These evidences show that this treatment to injured cells does not give any significant effect on the photoelectrical transduction mechanism.

These effect could not be obtained by the reverse application of potassium ferricyanide and Fe and Co salt solutions in micropipettes and external media.

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THE CORRELATION BETWEEN CYCLIC GMP LEVEL AND ERG IN OCTOPUS

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Effect of internal cyclic GMP on the visual transduction was studied using isobutyl methylxanthine (IBMX), which is known as a inhibitor of phosphodiestrase (PDE). The recovery of photoresponse after bright flash was affected largely by the addition of IBMX. Phosphatidylinositol (PI) metabolism, which was assesed by the incorporation of radioactive terminal phosphate of ATP, was also influenced by IBMX; ^{32}P incorporation of DPI and TPI was decreased, and that of PA was increased. These changes were interpreted advantageously by introducing a convenient parameter, calcium charge φ, of plasma membrane. From the results it was thought that Ca^{2+} can satisfied several requirements for the internal transmitter which is presumed to mediate between photon absorption by rhodopsin in microvilli membrane system and the permeability decrease in invertebrate visual transduction process.

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X-RAY MICROANALYSIS OF FREEZE-DRIED FROG RETINA SECTIONS

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Bullfrog retinas were shock-frozen at liq.N_2 temperature. The frozen retinas were sliced from receptor side at temperature below -120°C to prepare thin sections (below 1000 A°). The thin sections were freeze-dried at -80°C over night. The freeze-dried sections were analyzed by X-ray energy spectrometer (Kevex 5100) attached to transmission electron microscope (Hitachi H500). The X-ray energy spectra from the rod outer segments showed remarkable peaks for P and S, considerably higher peaks for Na, Cl and K and a poor peak for Ca. These remarkable peaks for P and S were considered to originate from protein and phospholipid composing membrane structures of rod outer segments. Elemental contents of the rod outer segments were estimated from the ratios of peaks to backgrounds and standard calibration curves of various kinds of elements. Results of rod outer segments from dark adapted retina were as follows; Na:65 ± 20, Cl:64 ± 30, K:41 ± 8, Ca:8 ± 5, P:120 ± 33, S:105 ± 21 mmol/Kg wet weight. On the other hand, results from light adapted retinas showed that Na was less but K was higher than those of dark adapted retinas. The X-ray energy spectrum of melanosome in a thin section showed a remarkable peak for Ca, which corresponded to concentration of about 60 mmol/Kg wet weight.

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ROLE OF CYCLIC-GMP IN PHOTOTRANSDUCTION MECHANISM

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It is well known that Ca^{2+} mimics light effects on photoreceptor potential. On this basis, Ca^{2+} has been postulated as a candidate for an intra-cellular transmitter mediating disk membranes and plasma membranes in rod photoreceptor outer segments. Biochemical experiments, instead, have suggested cyclic-GMP (c-GMP) being a mediator. So far this conflict has not been resolved yet.

The experiments presented in this paper gives clear evidence showing that Ca^{2+} effects on photoreceptor potential is through affecting the metabolic pathway of c-GMP. Ca^{2+} affects phosphodiesterase activity, an enzyme activated in the light and hydrolyzing c-GMP. PDE is activated by Ca^{2+} in both the dark and the light. Under low Ca^{2+} condition, PDE activity is almost constant in the light with ATP. The results suggest the possible involvement of Ca^{2+} in light adaptation mechanism in rod photoreceptor cells.

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THE PIII RESPONSE OF THE ISOLATED FROG RETINA IN THE PRESENCE OF Ba^{2+}.
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The PIII response of the isolated, aspartate-treated bullfrog retina consists of the fast and slow PIII responses. By the application of 0.5 mM Ba^{2+}, the slow PIII response was disappeared but the fast one was little affected in amplitude. The following results were obtained from the retina immersed in 0.5 mM Ba^{2+} solution. The log relative sensitivity of the PIII response was not linearly related to the rhodopsin content and the shape of curve was depicted as being sigmoidal. Reducing Ca^{2+} concentration in the extracellular fluid, the thermal decay rates of rhodopsin intermediates were accelerated and the recovery time of the PIII response amplitude after switching off the adapting light was decreased. The log of PIII response threshold was inversely proportional to the amount of 380 nm-absorbing rhodopsin intermediates in both the 1.0 mM and 0.01 mM Ca^{2+} solutions.

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FAST AND SLOW PIII RESPONSES IN THE ISOLATED FROG RETINA. MATSUURA, T. Dept. of Physiol., Sch. of Med., Kinki Univ. Osaka

PIII response is composed of the fast component which arises from the photoreceptors and the slow component which is produced by the Müller cells as they hyperpolarize in response to the light-induced potassium decrease around receptors. Separation of these two potentials from the aspartate-isolated PIII response was performed by inserting two micropipettes into the bullfrog retina and the effects of stimulus irradiance upon PIII, fast PIII and slow PIII responses were compared: As the mathematical model (Matsuura et al. 1978) predicted, the slow PIII response continued to increase its peak amplitude with increasing the light intensity after the amplitude growth of the fast PIII response was saturated. But, within the scotopic range, the amplitudes of the PIII and slow PIII responses were roughly proportional to that of the fast PIII response and, therefore, can be used as the measure of the photoreceptor sensitivity change within the scotopic range.
The contribution of the fast PIII response to the peak amplitude of the PIII response was also discussed.

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VOLTAGE DEPENDENT MEMBRANE RESISTANCE OF THE ARTHROPOD'S PHOTORECEPTOR. SUZUKI,H.and TASAKI,K. Dept. of Physiol., Tohoku University School of Medicine, Sendai

Light induced currents were recorded under voltage clamp using double-barreled electrodes placed in photoreceptors of mantis shrimp to study the effects of membrane potential on the single-channel conductance. A second electrode was penetrated 150 um away from the double-barreled electrode to confirm that the space clamp condition was satisfied. The relation between the receptor potential and the light intensity (Log I) was sigmoid, but light induced current was in proportion to Log I. The reversal potential of light response was 30 mV above the reference potential. The spectral distribution of the fluctuation of light induced current was represented by a single Lorentzian. The corner frequency was 10.7 Hz, and the mean open time of the ion channel activated by light was 15 msec. The conductance of single channel was 37 pS. The corner frequency was independent of the membrane potential from -70 mV to -15 mV, but the light induced current and the power of fluctuation at f=0 were in direct proportion to and in squared proportion to the membrane potential, respectively. These results suggest that the single-channel conductance is independent of the membrane potential. Therefore, the non-linear relation between light induced current and the photoreceptor potential is caused by the voltage dependence of the load resistance to the light induced current.

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CHARACTERISTICS OF THE SPONTANEOUS NP-WAVE OBSERVED IN THE ISOLATED CHICKEN RETINA. T. SATO, N. MATSUMOTO, K. TAIRA AND T.A. SUZUKI. Dept. of Oral Physiol., Sch. of Dent., Iwate Med. Univ., Morioka, Iwate.

Eighty-six eyeballs isolated from 55 chickens were used. Activity of the retinal preparation punched out from the posterior part of the eyeball was maintained by the previously reported methods (Sato et al., 1981). At 15 minutes later from beginning of the experiment, standing potential (SP) of the isolated retina leached to 9.8 $\pm$ 3.5 mV. The ERG was also recorded. During the experiment for 1 hour, spontaneous negative-positive deflections (NP-wave) were occasionally observed on the record of SP. At the phase from negative peak to positive peak of the NP-wave, the a-wave and the b-wave of ERG were augmented in amplitude to 152 $\pm$ 42% and 221 $\pm$ 87%, respectively. Five minutes later, however, their amplitude decreased to the level lower than that of control in majority cases, and did not recover to the original level throughout the experiment.

Intensity versus amplitude curves of the b-wave were analyzed under varying potassium concentration of vitreous side ($[K]_V$) from 2.3 to 15 mM/L. As the $[K]_V$ increased, the maximum value of the each curve increased but satulated between 4.5 and 9 mM/L, whereas the intensity of the stimulus light which produced the maximum response decreased.

From these results, the augmentation of the b-wave in the NP-process seemed due to increase of excitability caused by potassium accumulation within the isolated retina.

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AN ANALYSIS OF DARK NOISE RECORDED FROM SINGLE RODS.
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Intracellular potentials in dark and during illumination were recorded from single rods in isolated retinas of the bullfrog. Variance and frequency spectrum of each record were calculated.

The measurements revealed that in darkness the potential fluctuated spontaneously. The magnitude of the random noise was several hundred μV and varied from cell to cell. Average variances of the noise in a dark and during even illumination (1.5×10^6 Rh* / flash /rod) were about 0.024 and 0.011 mV^2 respectively. A major component of the noise was similar to the response to a very weak flash (1 Rh* /flash /rod) of light in the time course. The fluctuations lessened during and just after bright illumination, but were independent upon the membrane potential to a certain extent. From the results of the frequency spectrum analysis, decreasing effect of illumination on the noise appeared in a low frequency range (less than 0.3 Hz).

It is thought that the 'discrete' events appeared in each rod spread to the neighboring cells through the rod-rod network and are averaged. Existance of the dark events might decrease S-N ratio in the signal transmission, but this effect of dark events could be reduced by rod-rod network.

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OSCILLATORY PHOTORESPONSES OF RODS IN LIGHT-ADAPTED BULLFROG RETINA. YAMADA,M., TAUCHI,M.* and SHIGEMATSU,Y. Applied Optics Sect., Electrotechnical Lab., Tsukuba Sci. City, Ibaraki and *Dept. of Information Physiol., National Inst. Physiol. Sci., Okazaki, Aichi.

It was observed that oscillatory responses were able to be evoked in rods simply by photic stimulation in light-adapted bullfrog (Rana catesiana) retinae. This study was prompted by our observation that (1) the unit impulse responses of rods to photostimuli which were intensity-modulated in a white-noise manner consist of a monophasic peak followed by oscillations at a frequency of approx. 2 Hz, and that (2) the power spectrum of rod responses to the photostimuli resonated also at about 2 Hz. Consistent with these results, we recorded oscillations (ca. 2 Hz) in the membrane potentials of rods in response to light flashes delivered in the presence of a maintained background light. They persisted transiently after the offset of the background. Either way, the emergence of the membrane potential oscillations invariably accompanied a conspicuous acceleration of the decay of the flash-evoked rod response. The appearance of oscillations could be explained by a phase lag between counteracting membrane currents. That is, light adaption may depress a hyperpolarizing effect which normally slows the repolarization of rods, enhance an ionic mechanism which depolarizes rods following the initial light-evoked hyperpolarization, or both.

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CONNECTIONS BETWEEN RECEPTOR CELLS AND HORIZONTAL CELLS IN THE RETINA OF JAPANESE DACE, Tribolodon hakonensis.
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The cone cells of Japanese dace retina show row mosaic pattern. Their elements were mostly unequal double cones, long single cones and short single cones without a myoid. In the electron microscopic examination, the mosaic pattern was most demonstrable in the DAB treated retina, because of their differences in electron density. Two different types of cone terminals can be distinguished, one was small in size and possessed only one synaptic ribbon (s-pedicle), and the other was large in size and possessed many synaptic ribbons (l-pedicle). The l-pedicles were located more proximally than the s-pedicles. Other members of receptors of this retina were rods and miniature cones. The rod terminals were small in size but complicated in shape and sometimes more dense than cone terminals. They were located between the l- and s-pedicles.

The L-type horizontal cells contact mostly the l-pedicles, and its fine processes extend more than 100 um. The RB-type horizontal cells made contact with both the l- and s-pedicles and its ratio was 1:9. The RGB-type horizontal cells were connected with only the s-pedicles.

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A DOUBLE-INPUT MODEL OF R/G TYPE HORIZONTAL CELL IN THE CARP RETINA.
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Some R/G cells, though in a minority, were found to show that the positive peak of the E-response to the maximum current stimulus did not reach the maximum depolarizing photoresponse to deep red light on which the E-response was superimposed. This suggests that the membrane potential of green-sensitive cones is little affected directly or indirectly by deep red illumination and that there are two independent inputs to R/G cell; one responsible for the positive E-response driven by green-sensitive cones and the other for the inverted photo- as well as E-response from L-cell but without passing through the green-sensitive cones. An equivalent circuit of the subsynaptic membrane model of R/G cell was presented for discussion. Replacement of Ca^{++} by Ba^{++} or by Sr^{++} depolarized the dark membrane potential of R/G cells and enhanced the photoresponses to green light but suppressed those to red one resulting in a reversal of their polarity. Since L-cell's photoresponses to green and red lights are almost equally suppressed by Ba^{++}, the depolarizing red S-potential of R/G cells appears to be derived from L-cell. The reversal of photoresponse to 710nm by Ba^{++} suggests that involvement of green-sensitive cones might be excluded in generation of the red response of R/G cells.

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INTERCONNECTION BETWEEN PHOTORECEPTORS AND LUMINOSITY TYPE HORIZONTAL CELLS IN THE RETINA OF THE TURTLE (CHINEMYS REEVESII).
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Synaptic contacts between photoreceptors and luminosity type horizontal cells (LHCs) were studied by light and electron microscopy, after horizontal cells were injected intracellularly with horseradish peroxidase (HRP). The types of photoreceptors were determined from the color of their oil droplets in reference to our previous study. An LHC is made of a soma (CB) and an expanded axon terminal (AT), and these structures (>100 μm apart) are connected by a fine (<1μm dia.) process. Both CB and AT were seen to send out fine processes (dendrites) towards photoreceptor terminals. Dendritic fields of CB and of AT never overlapped.

Electron microscopic observation revealed that CB sent HRP-positive processes to all type of cones, but not to rods. At cone terminals, processes from CB occupied most of the lateral elements of the synaptic complex. Processes from ATs (n=2) were found both in rod terminals and in terminals of chief member of double (red-sensitive) cones, but in only a few of about 10 synaptic complexes of these photoreceptors. The present observations suggest that LHC receives mixed inputs from cones and rods at AT, while CB receives inputs from cones only.

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ISOLATION OF ROD-ORIGINATING COMPONENTS IN THE RESPONSE OF LUMINOSITY TYPE HORIZONTAL CELLS IN THE RETINA OF THE TURTLE (CHINEMYS REEVESII).

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Light and electron microscopic observations of luminosity type horizontal cells (LHCs) revealed that processes from the cell body (CB) have connections with all types of cones, but not with rods, while those from the axon terminal (AT) connected with both rods and cones. Rod-originating components in the response of LHC were studied by making intracellular recording in dark-adapted eye-cup preparations. LHC and their sites of recording were identified by intracellular injection of horseradish peroxidase. Of sixty cells, 13 cells were recorded at CB, and 47 at AT.

Responses of LHC to brief (20 ms), dim flash of white light (<100 $nW/cm^2/sec$) consisted of fast and slow hyperpolarizations. Responses recorded at CB had only the fast component. Responses from AT had both. The origin of the fast component was identified as cones, and that of the slow component as rods: (1) the fast component was maximally sensitive at red region of the spectrum, while the slow component was maximally sensitive at blue-green region of the spectrum. (2) the slow component was suppressed in the presence of moderate background. These results indicate that rod inputs to AT can be detected at a lower mesopic level, toghether with the cone component.

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PROPERTIES OF COUPLING BETWEEN HORIZONTAL CELLS IN CARP RETINA.

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The large distant spread of light response through the horizontal cell (HC) layer has been attributed to the presence of gap-junctional contacts. However, the direct measurement of the space constant by electrical current injection gave much smaller value (ca. 50 μm) which could not account for the large spread of light response. For possible explanation of this discrepancy, we expected that the light stimulation might facilitate the coupling. Thus, the effect of light on the coupling ratio in the external HCs was studied in the isolated carp retina by comparing the spread of the current injected into HC under the dark and light conditions. The results showed that the coupling was facilitated increasingly with background intensity, suggesting that a light-induced increase of the subsynaptic membrane resistance resulted in the increase of current flowing through the coupling site. Further, hyperpolarizing command voltages yielded larger ratios than depolarizing voltages, showing rectification of lateral spread. Nevertheless, the maximum increase of coupling ratio was only 20%, which was still smaller than expected for light response. It was suggested that the connection between HCs may not only be electrical.

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RESPONSE PROPERTIES AND STRUCTURAL ARRANGEMENT OF CHROMATIC HORIZONTAL CELLS IN THE MUGIL RETINA.

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It was previously reported by Svaetichin et al. (1961, 65) and Tamura (1967) that S-potentials from mugil retina showed exceptional chromatic (C) responses (i.e., G/R, Y/B and Y/RB) which originate from the second layer horizontal cells (HCs). The present study was done to reconfirm them and identify their locations with micropipettes filled with Procion Yellow dye. All C types except for RB/Y were encountered. As the results, both G/R and B/Y were found to locate in the second layer HCs, and usual C types were the third layer HCs. The first layer, external HCs, gave rise to exclusively the photopic L types reported in other species, but they were divided into two spectrally different types, one having a spectral peak at 520 nm and the other at 560 nm. As another discriminating property, the responses of both C and photopic L cells showed a distinct antagonism between red and green inputs. The fourth layer HCs delivered typical scotopic L type, showing that HCs in this fish are arranged into four layers as described by Parthe (1972). The present results proved that the inverted C types also originated from HCs and mugil retina have four basic color-opponent systems already in HCs.

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EFFECTS OF PROLONGED DARK ADAPTATION ON THE SENSITIVITY OF L-TYPE EXTERNAL HORIZONTAL CELLS.

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Effects of prolonged dark adaptation were examined in L-type external horizontal cells (LEHCs) in live immobilized goldfish. When the fish was kept in the dark after 3 min exposure to $2.34 \times 10^{-2} \mu W/cm^2$ of white light, the sensitivity (defined as a reciprocal of the light intensity giving rise to a criterion response amplitude) increased gradually in the initial period of about 20 min. The change was smooth, and no inflection was seen. With further dark adaptation, the sensitivity then declined with time, and at about 70 min, it became 1.0 log unit less than the maximum. The lowered sensitivity could be raised by giving a weak ($2.34 \times 10^{-7} \mu W/cm^2$) background illumination, indicating that deterioration of preparation or of cells is an unlikely interpretation of the lowered sensitivity. Since the sensitivity to green (502 nm) and red (706 nm) flashes showed similar changes, this seems to be a common feature of dark adaptation of inputs from both green- and red-sensitive cones. Lowered gain in the synaptic transmission from cones to LEHCs could be responsible, at least partly, for this phenomenon.

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INPUTS FROM RED-SENSITIVE AND GREEN-SENSITIVE CONES TO L-TYPE EXTERNAL HORIZONTAL CELLS IDENTIFIED BY CHROMATIC ADAPTATION

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L-type external horizontal cells (LEHCs) were recorded in live, immobilized goldfish (Carassius auratus), under selective adaptation, in order to identify the types of cones which send input to LEHC. Assuming that inputs from different types of cones differ in time course, spectral sensitivities were measured at the initial phase (60 ms after the flash onset), at the peak (85 ms), and at the plateau phase of the response (flash duration 400 ms). In the presence of a strong blue (451 nm) adapting light, spectral sensitivity curves agreed with that of red-sensitive (R) cones at all the timings tested. In contrast, when a strong red background (655 nm) was presented, spectral sensitivities measured at the initial phase and at the peak agreed well with that of green-sensitive (G) cones, while the sensitivity measured at the plateau peaked between those of R and G cones. Surprisingly, under the strong red background, weak green flashes evoked a prominent depolarizing response in luminosity type cells, so named for monophasic spectral responses. Spectral characteristics of the depolarizing component suggest that it originated from G cones. Since the depolarizing component appeared with prominent delay, it seems to be the result of synaptic interaction.

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SPATIOTEMPORAL MECHANISM OF L-TYPE HORIZONTAL CELL RESPONSE IN THE CARP RETINA

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The area and intensity effects of a brief light stimulus on the L-type horizontal cell response were recorded and analyzed from their waveforms dynamically. We discovered clear nonlinearities in the behavior of the response which could not be explained by the simple summative model presently accepted. Proposed here is a new model, "the discrete nonlinear spatial reduction mechanism", which elucidates more precisely the mechanism of their intercellular connections. A basic difference between the classical model and our new model is that the former is additive, i.e., the actual response is given by the sum of the individual horizontal cells' response. Our new model, however, is reductive, i.e., the pure (=maximum) hyperpolarizing response is given by large field stimulation, and decreasing spot diameter causes the response amplitude to be nonlinearly reduced through the current spread from more depolarizing neighboring cells.

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SIGNAL TRANSMISSION AMONG CATFISH RETINAL NEURONS, 2

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Responses from interneurons in the retina of channel catfish, Ictalurus punctatus, were evoked either by white-noise modulated light input or white-noise modulated current injected into the horizontal cells. White-noise inputs had a flat power spectrum from near DC to 50 Hz. Responses were crosscorrelated with the input to obtain Wiener kernels. Linear, second and third order components in the evoked responses were segregated by convoluting the input signals with the kernels of similar order. Light-evoked horizontal-cell responses were quasi-linear with a low-pass characteristic. Signal transmission from horizontal to bipolar cells was quasi-linear. White-noise modulated field of light produced a low-pass or band-pass response from the bipolar cells. The responses from type N cells evoked either by current or light had a remarkable nonlinearity which produced the oscillatory response from the cells. In the type C cells the second order component produced the major share of their response. These observations reinforce our conclusions that signal transmission in the outer retinal layer is essentially linear and that nonlinearities appear when the (linear) signal is transmitted to the inner layer.

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POTENTIATION OF HORIZONTAL CELL RESPONSES TO L-GLUTAMATE BY D-ASPARTATE.

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Depolarizations of type H1 horizontal cells in goldfish retinas were measured by intracellular recording, in response to L-glutamate ("L-glu") and L-aspartate ("L-asp"), with each L-amino acid bath-applied alone or in the presence of D-asp or D-glu. Following exposures of retinas to 3.2 mM D-asp, the dose-response curve resulting from L-glu (but not L-asp) applications shifted laterally with respect to that measured in the normal retina to a significantly lower range of effective doses. Under these conditions, e.g., L-glu produced depolarizations (8-11 mV) at 100 uM. 3.2 mM D-asp can also enhance the endogenous depolarizing input to H1 cells. Since the latter is thought to be provided by photoreceptors, L-glu may be released by photoreceptors as a neurotransmitter.

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CIRCUMFERENTIAL ADDITION OF RETINAL MONOAMINE-ACCUMULATING CELLS IN GROWING GOLDFISH.

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The retinas of juvenile goldfish were processed for fluorescence-microscopic studies with flat-mounts. The fish were reared in an aquarium for one to 3 months (from June to September) after either one class of dopaminergic (DA-) or indoleamine-accumulating (IA-) cells had been destroyed by intravitreal injection of 6-hydroxydopamine (6-OHDA; 2.5 μg) or 5,7-dihydroxytryptamine (5,7-DHT; 2.5 μg), respectively. The fish grew from 10 to 12 cm in body length for 3 months. The retinas were isolated at various survival times after pretreatment with 6-OHDA or 5,7-DHT. To improve detection of the cells, a mixture of noradrenaline (NA; 0.5 μg) and 5,6-DHT (0.5 μg) was intravitreally injected into the eyes 2 hr prior to enucleation. Retinal growth occurred by cell proliferation at the circumferential margin. DA- and IA-cells were added in concentric rings, with a rate of cell accretion of approximately 12 cells per day, estimated from the mean cell density in the growth zone (about 252 μm width) over a 3-month period. To compensate for loss of dopaminergic innervation induced by pretreatment with 6-OHDA, the proliferation rate appeared to be fascilitated significantly as compared to that in normal retinas, and long and centrally extending processes were found to emerge from the DA-cells formed earliest after intravitreal injection of 6-OHDA.

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REGIONAL DIFFERENCE IN DENSITY OF MONOAMINE-ACCUMULATING CELLS OF CARP AND CATFISH RETINAS. NEGISHI, K., TERANISHI, T., HIDAKA, S.*, HIDA, E.* AND NAKA, K.-I.* Dept. of Neurophysiol., Neuroinformation Res. Inst., Sch. Med., Univ. Kanazawa, Kanazawa and *Dept. of Sensory Physiol., Natl. Inst. Basic Biology, Okazaki

By means of a histofluorescence technique, a comparative study was carried out on the regional density of dopaminergic (DA-) cells and indoleamine-accumulating (IA-) cells in the carp (Cyprinus carpio) and catfish (Ictalurus punctatus) retinas. DA- and IA-cells were counted systematically in space on flat-mounts. In general, both classes of cell were found to be distributed similarly in the two species of fish. However, differences in the distribution of the cells between carp and catfish were as follows: (i) the cell density is higher over all the retinal field in carp (the mean density ± S.D., 34 ± 16 cells/mm^2 for DA-cells) than in catfish (13 ± 7 cells/mm^2); (ii) the region where the density is slightly higher than in the surround is restricted immediatedly dorsolateral to the optic disc in carp, while it is relatively broadly placed dorsal to the optic discs, forming a horizontal band in catfish; (iii) the density ratio of DA-cells to IA-cells is 1:1 in carp but 1:2 in catfish; (iv) catfish DA-cells seem to be more irregular than carp DA-cells in shape, size, dendritic arborization, uptake preference for the monoamines intravitreally injected, and also in depth location seen in radial cryo-sections.

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PHYSIOLOGICAL AND MORPHOLOGICAL IDENTIFICATION OF BIPOLAR CELLS IN THE CARP RETINA. Saito, T. and Kujiraoka, T. Dept. Physiol. St. Marianna Univ. Sch. Med.

Bipolar cells in the dark-adapted retina were studied by means of intracellular recording and Lucifer-yellow dye injection. Type I on-center bipolar cells responded to a light spot with a transient depolarization followed by a plateau. They were subdivided into two types (Ia and Ib). Type Ia cells had a positive reversal potential only, while type Ib cells had two reversal potentials of opposite polarity. In spite of different electrical properties, There were no consistent morphological differences between them, and all the type I cells were characterized by a big swelling of axon terminals. Type II on-center bipolar cells responded to the light with an approximately rectangular depolarization. They were subdivided into two types (IIa and IIb). Type IIa had a negative reversal potential only, while type IIb had either a positive reversal, or two reversal potentials of opposite polarity. All the type II cells were characterized by highly branched axon terminals. Off-center bipolar cells had a positive reversal potential. However, some of them showed a strong membrane rectification, which sometimes made their responses complicated as the results of the sum of electrical properties of synaptic and non-synaptic membrane. Such cells had always the cell bodies located in the proximal half of the inner nuclear layer.

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CONNECTIONS BETWEEN PHOTORECEPTORS AND BIPOLAR CELLS IN THE CARP RETINA. Saito, T., Kujiraoka, T. and Yonaha, T. Dept. of Physiol. St. Marianna Univ. Sch. of Med.

HRP-injected bipolar cells in the carp retina have been examined by serial section electron microscopy. The results have shown that there are three types of bipolar cells (type I and II on-center cells and off-center cells) on the basis of their response waveforms, morphological properties and receptor connectivities. The fine dendritic terminals of all of these bipolar cells invaginated into rod spherules and occupied at least a position opposite a synaptic ridge. The dendritic terminals of these bipolar cells also invaginated into cone pedicles, but in quite distinct patterns. Type I on-center bipolar cells did not send processes to the synaptic ribbons. In contrast, type II on-center bipolar and off-center bipolar cells sent their processes to the synaptic ribbons. The dendritic terminals of off-center bipolar cells occupied a position opposite the synaptic ridges. Although type II on-center bipolar dendrites approached to the synaptic ribbons very closely, there was a single process from a off-center bipolar cell interposed between them.

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EFFECTS OF SOME CHEMICALS ON THE INTERACTION OF SECOND-ORDER NEURONS IN THE RETINA. TOYODA, J. AND FUJIMOTO, M. Dept. of Physiol., St. Marianna Univ. Sch. of Med., Kawasaki

Hyperpolarization of L-type horizontal cells (H1) elicits a depolarizing response in RG-type horizontal cells (H2), and hyperpolarization of H2 cells elicits a depolarizing response in RGB-type horizontal cells (H3). Effects of some putative transmitters and their antagonists were tested in the perfused carp retina to elucidate the possible route of such interaction. Picrotoxin, the GABA antagonist, depolarized H2 but not H3 cells and selectively abolished the red component of H2 and H3 cells without affecting other components. Application of GABA resulted in a hyperpolarization of H2 cells, but after suppression of synaptic transmission by cobalt ions, it did not mimic the effect of the native transmitter. The results suggest that the interaction from H1 to H2 cells is mediated by GABA but probably through an indirect route such as a feedback pathway to cones. Interaction from H2 to H3 cells is not affected by GABA nor by several other transmitter candidates tested in the present experiments except for glycine which occasionally suppressed the green component of H3 cells.

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SYNAPTIC ORGANIZATION OF AMACRINE CELLS IN THE CATFISH RETINA

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Electron microscopic observations were made on HRP-injected amacrine cells(physiologically identified transient-type(n=2) and sustained-type(n=2)amacrines). Serial tracing and reconstructions were made on the principal dendrites and cell bodies along the 500 μm distance.

The cell bodies of both types of amacrines were post-synaptic to amacrine cells and to processes which probably originated efferent mylinated fibers. Dendrites of both types of amacrine cells made reciprocal synapses with bipolar cells. They also made contacts with other amacrine cells via chemical synapses and gap junctions. Dendrites of sustained-type amacrines formed spine-like appendage, where the bipolar cell ribbon synapses contacted. Dendrites of transient-type amacrine cells, which bistratified in the inner plexiform layer, did not show distinct differences in their synaptic organization between the distal and proximal parts.

Synapses were made on both types of amacrine cell dendrites in such a way that presynaptic and post-synaptic sites were intermingled to form a synaptic zone(5-10μm length of 1.5μm diameter dendrites). These synaptic zones were seperated from the adjacent ones at 60-120μm intervals, suggesting that they may function as local circuits.

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AXONAL CONDUCTION VELOCITIES OF RETINAL GANGLION CELLS PROJECTING TO THE LATERAL GENICULATE NUCLEUS (LGN) OF THE EASTERN CHIPMUNK. WAKAKUWA, K., WASHIDA. A., FUKUDA, Y. AND IWAMA, K. Dept. of Neurophysiol., Inst. Higher Nerv. Activ ., Med. Sch. Osaka Univ., Osaka 530

Single unit activities of retinal ganglion cells were recorded extracellularly in immobilized eastern chipmunks (Tamias sibiricus asiaticus). From latencies of antidromic responses to stimulation of the optic chiasm and the LGN, axonal conduction velocities were estimated. They ranged from 2 to 32 m/sec, forming three distinct groups; the fast (F), intermediate (I) and slow (S) groups. In the ganglion cell sample from the central retina the I and S axons predominated, while the F and S axons were characteristic of the sample from the temporal periphery. Ganglion cell axons of the nasal retina were exclusively of I type. This physiological finding is consistent with the histological data for the soma size of ganglion cells at different retinal locations. A considerable fraction of the ganglion cell sample was fired not only from the LGN but also from the superior colliculus. Ratios of such doubly projecting cells were about 0.5 for the central retina and about 0.25 for the temporal and nasal peripheries.

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AN INTRACELLULAR ANALYSIS ON THE RESPONSE PATTERNS OF X- AND Y-CELLS IN THE EXCISED AND PERFUSED EYE-CUP PREPARATIONS OF THE CAT.

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Intracellular recordings of on- and off-center X- and Y-type retinal ganglion cells (X_{on}, X_{off}, Y_{on} and Y_{off}) were made in the excised and perfused eye-cups of the cat.

X_{on} cells responded to a light spot centered at the receptive field with a sustained depolarization and X_{off} cells responded with a sustained hyperpolarization. An annulus antagonized the center response in a sustained manner. On the other hand, the responses of Y-cells were characterized by transient polarizations. Y_{on} cells were transiently depolarized by an onset of the spot and a cessation of the annulus. They were transiently hyperpolarized by the cessation of the spot and the onset of the annulus. The polarities of the responses of Y_{off} cells were just the opposite of that of Y_{on} cells.

Effects of strychnine and bicuculline on these intracellularly recorded potentials were next studied. The effects were different between X_{on} and Y_{on} cells: In X_{on} cells, strychnine markedly diminished the hyperpolarizing responses elicited either by the annulus or by the cessation of the spot, but bicuculline did not. In Y_{on} cells, these hyperpolarizing responses were diminished by bicuculline. In the case of the off-center cells, bicuculline reduced all of the photic responses regardless of X- or Y-type.

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RESPONSES OF THE CARP OPTIC TECTUM IN Cl-DEFICIENT MEDIUM IN VITRO

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Anatomical studies have suggested the existence of reverberating circuit in the optic tectum. To detect electrophysiological evidence of reverberating circuit, field potential and intracellular responses to optic nerve stimulation were examined in the standard and Cl-deficient medium. In Cl-deficient medium, amplitude of the field potential was augmented and the time course was prolonged. Near the border of SFGS and SGC, Oscillatory wavelets were superimposed on the negative going potential. The minimum interval of oscillation was 2.8 ms. Following these early components, large negative potential which lasted for more than several seconds appeared. The peak of the late component occurred in SGC.

Intarcellular recordings from presumed pyramidal and fusiform neurons in Cl-deficient medium presented DS(deporalizing shift) or seizure discharges as observed in mammalian hippocampus.

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ELECTROPHYSIOLOGICAL STUDY ON PROJECTION FROM SUPERIOR COLLICULUS TO LATERAL POSTERIOR NUCLEUS IN THE CAT.

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The projection from the superior colliculus (SC) to the interjacent part of the lateral posterior nucleus (LPi) or the tectorecipient zone of the LP was studied in chloralose-anesthetized cats. In most of LPi neurons electrical stimulation of optic chiasm (OX), optic tract (OT) and SC ipsilateral to LPi studied produced EPSPs on which a spike discharge was superimposed followed by a long-lasting IPSP with latencies ranging from 4 to 7 msec, from 5 to 11 msec and from 2 to 6 msec, respectively. LPi neurons stained intracellularly with procion yellow were medium-sized multipolar cells. Stimulation of LPi activated antidromically SC neurons located in the superficial layers which were driven orthodromically by stimulation of OX and OT. Respective latencies of these responses ranged from 0.5 to 4 msec, from 3 to 12 msec and from 4 to 14 msec. Conduction velocities estimated from OX-OT latency differences of input afferent fibers to SC neurons were found to be inversely correlated with antidromic latencies. These results clearly show that LPi neurons receive input from superficial collicular neurons which in turn receive Y and W retinotectal fibers.

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SYNAPTIC DELAY IN THE LATERAL GENICULATE NUCLEUS (LGN) FOR UNCROSSED OPTIC NERVE FIBERS IN NEONATALLY ONE-EYE-ENUCLEATED RATS. SHIROKAWA, T., FUKUDA, Y. AND IWAMA, K. Dept. of Neurophysiol., Inst. Higher Nerv. Activ., Med. Sch. Osaka Univ.

By stimulating the optic nerve (ON) electrically in rats with one eye taken out neonatally, single unit discharges were recorded from LGN rely cells, both ipsi- and contra-lateral to the remaining eye. The response latencies were not significantly different between the two sides of LGN, though in the ipsilateral side some units were fired at unusually long latencies. The crossed and uncrossed ON fibers were not significantly different in the conduction velocities that were calculated from response latencies to stimulation of two separate sites along the ON. From the values of conduction velocities thus determined and with assumption that the conduction velocity does not change up to the LGN level, it is concluded that synaptic delays for activating LGN relay cells were elongated for the uncrossed fibers.

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INFORMATION PROCESSING OF SIMPLE CELLS IN SPATIAL FREQUENCY DOMAIN
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Neural interaction of spatial frequency components were studied in the simple cells recorded from the striate cortex of anesthetized and immobilized cat. The stimuli: 1. regular gratings of given unit widths, 2. one-dimensional random gratings, 3. two-dimensional random patterns consisted from unit rectangles in given rate of length and width, were projected from the opposit side of a translucent screen. Following determination of the properties of the receptive field using stationary and moving slit, spatial tuning curve was obtained using regular gratings. Ideal responses to 1-D random gratings were calculated and compared with the real responses to the same stimuli. Cells were more responsive to 1-D random gratings of narrow unit width than expected from calculated values. To 2-D random patterns which have randomness along length dimension additionaly to 1-D random gratings, cell responded only weakly although the unit width was effective enough for 1-D random gratings. Since 2-D random patterns contain the spatial frequency components in all directions, the results suggest that the frequency components of non optimal directions altogether interacted inhibitory on the response to the component in optimal direction.

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RESPONSE SELECTIVITY OF CLARE-BISHOP CELLS TO STIMULUS MOVEMENTS PRESENTED TO BOTH EYES WITH OR WITHOUT DISPARITIES. KEISUKE TOYAMA, TOHRU KOZASA* AND KOZI MAEDA* Dept. Physiol. Kyoto Pref. Univ. of Med. and Dept. Physiol. Fac. of Med. Univ. of Tokyo*.

Seventy-seven Clare-Bishop (CB) cells were studied under N_2O anaesthesia, using a 3-dimensional visual stimulator which presents a light spot to both eyes with variable disparities. They were classified into 3 response types. Approaching cells (29) were activated optimally, when movement was presented to both eyes in a medio-lateral direction so that movement disparity occurred as if the stimulus was approaching the animal. These cells responded slightly, when movement was presented either eye in the medio-lateral direction, indicating that the response to movement disparity was a sum of the responses to movement presented to each eye. Similarly responses of departing cells (23), activated by movement with departing disparity, and those of distance cells (25), activated when movement was presented with no disparity as if the stimulus was moved along the fronto-parallel plane, were a sum of the responses to movement presented to each eye. These observations indicate that response selectivity of CB cells for movements with approaching or departing disparity and those with no disparity was determined by combinations of directional selectivities of responses evoked through each eye.

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RESPONSES OF MONKEY PRESTRIATE NEURONS DURING FIXATION TASK.
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Single neuron activity was recorded in foveal OC (striate , A17) , OB (prestriate, A18) , OA (prestriate, A19) and IT (inferior temporal) cortex of rhesus monkeys trained to fixate the eyes to a tiny spot (0.05° in visual angle). Many neurons showed relatively high maintained activity (about 7 to 30 spikes/sec) while the animal kept fixation. Electrode penetration track was identified histologically , and these neuron showing fixation-activity were almost exclusively located in foveal OB (27 / 58 recorded , 46.6 %) and OC (22 / 49 , 44.9 %) , and were distributed through layer II - V . On the other hand , very few such cells were found in OA and IT area . Some neurons in prestriate cortex showed various type of task related response ; discharge at lever-on (start of trial) , discharge after each task end , and inhibition during fixation .

These results suggest that there may be cells in foveal OB and OC which participate in visual fixation , and cells receiving behavioral modulation.

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CONTROL OF THE LOCUS COERULEUS (LC) OVER ACTIVITY OF VISUAL CELLS IN THE RAT SUPERIOR COLLICULUS (SC).
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In urethane-anesthetized rats effects of repetitive electrical stimulation of LC upon neurons of SC were studied in comparison with those of iontophoretically applied noradrenaline (NA). Unit discharges of SC, both spontaneous and evoked by photic stimulation, were either facilitated or inhibited. Some neurons of the visual layer suffered modification of the response pattern to photic stimulation as well. While all the neurons of the upper half of the visual layer were inhibited, about 20% of the neurons were facilitated and 60% inhibited in the deeper half of the same layer. In the non-visual layer, which lies beneath the visual layer, incidence of the facilitatory effect was greater than in the visual layer; more than 30% were facilitated and less than 60% inhibited.

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MORPHOLOGICAL ANALYSIS OF POSTNATAL DEVELOPMENT OF VISUAL CORTICAL NEURONS PROJECTING TO THE SUPERIOR COLLICULUS IN KITTENS.
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Postnatal development of striate cortical neurons projecting to the superior colliculus (SC) was studied in kittens, ranging in age from the 1st day to the 14th week, by injection of horseradish perioxidase (HRP) into the SC. At the 1st day postnatally, HRP-positive cells were widely distributed throughout the cortex between the splenial and suprasylvian sulci. In terms of laminar distribution, however, they were confined to layer V of the cortex, as it is the case in the adult. The packing density of the HRP-positive cells was very high at the 1st day and reduced slightly during the 3rd-5th days. A rapid, marked reduction in the cell density occurred at the 8th day. Thereafter, the density was again gradually reduced and attained to the adult value at the 8th week. Quantitative measurement of the cross-sectional areas of the cells also indicated that a rapid increase in size of cells took place at the 8th day and became adultlike at the 8th week. Since the eyes of kittens open around the 8th day of age, the results presented above suggest that drastic developmental changes in the cortico-collicular projection may take place at the time when afferent visual inputs arrive at the cortex and SC.

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THE NEURAL MECHANISM OF PUPILLARY CONSTRICTION TO LIGHT AND NEAR VISION INOUE, T.
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The present experiments were designed to study the neural mechanism of pupillary constriction produced by light stimulation and that produced by electrical stimulation of the cerebral cortex. So-called "ON" and "OFF" units are present in the ciliary nerve of cervical sympathectomized rabbits (Inoue, Brain Research, vol 186, pp43-53, 1980). Both types of units were evoked by electrical stimulation of the pretectum, and the latency of the evoked spike ranged from 17 to 35 msec. Therefore, it appears that both types of units concern the control of the pupil. On the other hand, pupillary constriction was also evoked by electrical stimulation of the visual cortex, which is not the pathway of the pupillary light reflex. The pupillo-constrictor area was corresponding to the striate cortex (Area 17 of Brodmann).

Further, "ON" units described above were elicited by electrical stimulation of the cerebral pupillo-constrictor area, and the latency to cortical stimulation was 5 to 10 msec longer than that to pretectal stimulation in individual units. From these observations, it is thought that "ON" units in the ciliary nerve concerning the pupillary light reflex participate also in pupillary constriction to near vision.

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DIFFERENCE OF COLOR VISION BETWEEN BILATERAL EYES DEPENDING ON DIRECTION OF ROTATION TSUKEDA, K. Dept. of Physiol., Faculty of Med., Univ. of Tokyo, Tokyo

Natanson's law holds good in color vision also. As reported previously, in both eyes alike, red light is perceived as a left-rotating circular polarized light and blue light as a right-rotating. The difference of color vision between the bilateral eyes was studied by observing after-image. Red stimulus light was given through a small hole in a rotating disc and the after-images in the right and left eyes in right- and left-rotation were compared. The brightest and longest after-image was resulted when a left rotation was viewed by the left eye. Similarly, with blue stimulus light, the after-image was brightest and longest when a right rotation was viewed by the right eye. It is considered that the maximum excitation is presented when the direction of the circular polarized light, the direction of the stimulus, and the direction of the conduction of excitation were all the same.

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SPATIAL ORGANIZATION OF L-TYPE HORIZONTAL CELLS IN JAPANESE DACE RETINA. HASHIMOTO, Y.*, HIDA, E., YASUI, S. AND NAKA, K.-I. Dept. of Physiol., Tokyo Wom. Med. Coll., Shinjuku-ku, Tokyo 162*; Nat'l. Inst. Basci Biol., Okazaki, 444.

TV snow noise and one-dimensional random-grating were applied to reveal the spatial and temporal organization of the receptive fields of L-type horizontal cells in Japanese dace, Tribolodon hakonensis. Main results are: 1) Horizontal cell soma and axon formed the monotonic receptive fields whose half-width were about 250 μm and 500 μm, respectively. Spatial slop of the receptive field periphery of the axon was more gradual than that of the soma. The response amplitude and wave form to both stimuli showed different characteristics in each. The ratio of the response amplitude of the soma and axon was about 1.5:1.0, and the response wave form was more steeper in the soma. Such spatial and response characterisitcs were detected by both TV snow noise and one-dimensional random-grating. 2) Temporal organization of the receptive fields measured by TV snow noise was similar to the time course of the first order Wiener kernel by white noise modulated light.

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THE EFFECTS OF X-RAY IRRADIATION ON THE HEARING IN GUINEA PIGS
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We studied the effects of single doses of X-ray irradiation on the inner ears of guinea pigs by continuously measuring cochlear microphonics (CM) and auditory brainstem responses (ABR) with permanent electrodes implanted on the round window and on the skull. Sensorineural hearing loss was induced by more than 8,000 rad. With 10,000 rad, both CM and ABR, by air and bone conduction stimuli, disappeared simultaneously about 6 hours after irradiation. The amplitudes of responses were usually enhanced by 20-50% above the pre-irradiation levels for the second hour after irradiation. Hearing impairment began earlier with each increase in irradiation: about 10 hours at 8,000 rad, about 6 hours at 10,000 rad and about 2 and a half hours at 12,000 rad. Responses to high frequency sounds generally disappeared a little earlier than those to low frequencies. Changes were also observed in endocochlear potential (EP) and in electronmicroscopy (TEM and SEM).

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SYNTHESIZED FORMANTS BY A TERMINAL ANALOG METHOD FOR A STUDY OF AUDITORY CORTEX UNITS.
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We found formant detector neurons which responded exclusively to synthesized formants by a modulation method (MDSF) in the auditory cortex (AI) of cats. Some of the formant detectors had more than one effective range for the formant frequency. It is interesting to investigate the responses of these neurons to combined formants or synthesized voices. But when two MDSFs are combined, it is almost impossible to keep constant periodicity, because pitch frequencies of two MDSFs are adjusted independently. So we made the synthesized formant by a terminal analog method (TASF). The TASF is the output of the resonant circuit to which periodic pulses are fed. The spectrum of the TASF consists of the components of which frequencies are integer multiples of the fundamental frequency. The spectrum envelope is dependent on the tuning character of resonant circuit. Therefore, each parameter of the formant, such as formant frequency, formant bandwidth, amplitude and pitch frequency can be chosen independently to others. Combined formants were made by parallel connection of resonant circuits. We found a unit which responded exclusively to a special combination of TASFs. We synthesized human vowels and some of cat cries by this method.

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CHANGES IN SOUND TRANSMISSION CHARACTERISTICS IN THE MIDDLE EAR OF RATS DUE TO THE ACOUSTIC REFLEX. MURATA, K., HORIKAWA, J. and ITO, S., Dept. of Neurophysiol. Medical Research Institute, Tokyo Med. & Dent. Univ., Tokyo

Albino rats(Rattus norvegicus) were anaesthetized with chloralose and ultrasonic condenser earphone was inserted into the respective external auditory meati with a probe tube for sound pressure calibration. Contraction of the middle-ear muscles resulted from the reflex was recorded in three ways; change in EMG from the muscles, in CM amplitude and in sound pressure and phase of sound at the eardrum. The frequency-threshold curve of the reflex in response to a pure tone was shaped like an U-form with the lowest frequency at 3-6 kHz. Any tone with a frequency higher than 30 kHz did not elicit the reflex at least up to 110 dB SPL. The closed loop transfer-function for the reflex contraction had a cut-off frequency of around 20 Hz and the phase lag appeared to be more than 180 degree. Though both of the tensor muscle and the stapes muscle more or less contributed to change in acoustic impedance at the eardrum, the proportion of their contribution differed from animal to animal. The reflex suppressed the middle-ear transmission of sounds most efficiently at lower frequencies than 1 kHz and less efficiently at the higher. At above 30 kHz the transmission loss could not be observed.

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SPONTANEOUS ACTIVITIES AND RESPONSES TO STIMULUS TONE OF THE GOLDFISH'S AUDITORY FIBERS. KAWASE, I., KUNO, MIYUKI and MATSUURA, S. Dept. of Physiol. Osaka City Univ. Med. Sch., Abeno-ku, Osaka

Small auditory fibers of the goldfish showed spontaneous firings of 30 to more than 300 Hz. Their firing patterns could be classified into burst and other types. Fibers of the burst type had various numbers of impulses (maximum; less than 8) in each burst. In many fibers, frequencies of bursts were about one thirds of firing frequencies of impulses at room temperature (18-20°C). Intervals between two successive bursts tend to increase in accordance with the increase in number of impulses in the preceeding burst. Many fibers showed adaptation more or less in the firings of impulses to stimulus tone. However, some showed no apparent adaptation, initiating impulses to almost every waves of the tone (100-400 Hz) at room temperature. All fibers intensified adaptive rundown of firings to the tone when temperature was lowered. Frequencies of bursts in spontaneous activities and number of impulses in each burst also decreased with cooling. Duration of spontaneous and sound-induced spike potentials increased with cooling. Latency to the initiation of impulses to the tone also increased when temperature was lowered.

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EFFECTS OF HYPOXIA ON EXCITATORY SYNAPTIC TRANSMISSION BETWEEN HAIR CELLS AND AFFERENT AUDITORY FIBERS IN GOLDFISH. Wu,G.-B., Suzue, T. & Furukawa, T. Dept. of Physiol. Tokyo Med. & Dent. Univ. Sch. of Med., Bunkyo-ku, Tokyo 113

Effects of hypoxia on goldfish's ear were studied by recording the excitatory postsynaptic potentials (e.p.s.p.s) intracellulary from single auditory fibers. When hypoxia was introduced, e.g., by suspending water flow to the gill, sound-evoked e.p.s.p.s were suppressed reversibly within several minutes after the onset of hypoxia. Since any marked change was observed neither in the microphonic potentials, nor in resting and action potentials of the auditory fibers, the hypoxic effects were attributed to a primary disturbance in the mechanism related to the release of transmitter from hair cells. Statistical analyses revealed that the reduction in the amount of release during hypoxia was related to a reduction in the value of parameter n (pertaining to the amount of readily available store), while the value of p (release probability) and γ (amplitude of unit e.p.s.p.s) stayed largely unchanged.

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TWO TONE SUPPRESSION AND TWO TONE FACILITATION OBSERVED IN THE AMPHIBIAN PAPILLA NERVE OF THE TOAD. OOYAMA, H., SUGAI, T., YANO, J. and CHUJO, T. Dept. of Physiol., Kanazawa Med. Univ., Uchinada, Ishikawa

Single unit responses to two tone stimuli were studied in the amphibian papilla nerve of the Japanese toad anesthetized with urethane. Upon a test tone (f1) of the characteristic frequency (CF), a high frequency tone (f2) was superposed, the latter, if strong enough, completely suppressed the response to the f1 (two tone suppression, TTS). Two tone facilitation (TTF), an increase of the firing rate above that to the f1 alone, occurred, when the f2 was twice in frequency and 0-20 dB in intensity above those of the f1. The timing of spikes was well related to the sound wave form obtained by an algebraic summation of the f1 and f2. When the frequency tuning curve of each unit was taken in consideration, the result seems somewhat perplexing, since a simple application of the place theory to the tuning curve leads to a vibration by the f2, 1/10-1/100 in amplitude of that by the f1 at the place corresponding to the CF. Most recently, application of d-tubocurarine was found to suppress the TTF but not the TTS. This differential effect might be utilized for a further analysis of both the TTS and TTF.

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TONOTOPIC ORGANIZATION IN FROG AMPHIBIAN PAPILLAE STUDIED BY HORSERADISH PEROZIDASE INJECTED WITHIN THE EIGHTH NERVE GANGLION. YANO, J., OOYAMA, H., SUGAI, T. and CHUJO, T. Dept. of Physiol., Kanazawa Med. Univ., Uchinada, Ishikawa

In frog amphibian papillae (AP), evidences suggest a tonotopic organization in which neurons with lower and higher characteristic frequencies (CF's) innervate anterior and posterior macula, respectively. In order to elucidate this point, single unit discharges were recorded in the 8th nerve ganglion by micropipette electrode filled with 0.6 M KCl and 3-5 % horseradish peroxidase (HRP) solution. After identification of the CF of the unit, HRP was iontophoretically ejected at the site. In four preparations, transported HRP was observed in the peripheral nerve fibers within the AP. In two preparations, HRP was injected near each neuron with the CF of 400 and 550 Hz, and labeled fibers were found in the middle portion of the posterior S-shaped macula. In other two preparations, HRP was injected near neurons with low CF (150 and 160 Hz), and labeled fibers were observed in the nerve fibers innervating the anterior macula. Although the HRP applications were all extracellular in this study, results are consistent with the prior suggestion.

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HYSTERESIS LOOP OF CM INPUT-OUTPUT FUNCTION. UCHIYAMA, H. Dept. of Neurophysiol. Medical Research Institute, Tokyo Med. and Dent. Univ. Tokyo

Study was made on the shapes of hysteresis loops of CM input-output function as obtained to stepwise cyclic load. CMs to 13 kHz or 300-1000 Hz tone were recorded through a band pass filter from the 1st or the 3rd turn of the guinea pig cochlea. The curves were obtained from the pictures on two oscilloscopes, one for amplitude and the other for phase(photographed with a 16 mm movie camera). If the turn load level was set before the secondary rise,the returning curves were rather simple, lower in amplitude and lagger in phase. However in case of turn after occurrence of the secondary rise, the two curves crossed, the phase during the return being not always lagger. For instance at 13 kHz and 3 sec/step, the minimum CM level appeared at ca 20 dB for load level above the maximum. After turning at 4dB more rise(ca 6 dB rise for CM level) the curve still rised gradually and the crossing occurred between 12 and 16 dB below the turn level. At the second successive trial, higher level loop as well as the minimum disappeared and the lower level loop area diminished. The formation of the higher level loop was explained to be due to phase nonequilibrium, and the effct of the second load on the loops due to stiffening, in a large deformation of an elastomer of the cochlear partition.

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FORMANT DETECTORS IN THE CAT'S AUDITORY CORTEX.
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About 7% of the auditory cortex (AI) units were formant detectors which responded exclusively to synthesized formants in unanesthetized cats. The effective range of formant frequency of each unit was extremely restricted. Some of formant detectors had more than one effective range. In the present experiments, we studied the responses of formant detectors to synthesized formants generated by a terminal analog method (TASF) used in speech synthesis and those generated by modulation methods (MDSF). In most cases the best formant frequency was a little different between TASF and MDSF, but the effective range was almost the same. In one of the units, two local maxima for formant frequency were defined in terms of driven discharge. The response to combined formants, where respective formant frequencies were at the local maxima, was 1.5 times larger than the sum of the responses to the individual formants. One unit responded to combined formants, though individual synthesized formants inhibited the spontaneous discharges. As the formant frequency of either of the combined formants was shifted, evoked discharges decreased, and spontaneous discharges were inhibited by further formant frequency shifts.

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SOME BINAURAL INTERACTIONS IN THE INFERIOR COLLICULUS AS DEMONSTRATED BY ^{14}C-2-DEOXY-GLUCOSE METHOD: HORIKAWA, J., ITO, S., MURATA, K., Dept. of Neurophysiol., Medical Research Institute, Tokyo Med. & Dent. Univ., Tokyo.

A white noise and a pure tone of 20 kHz were presented to each ear of rats separately through polyethylene tubes connected to ultrasonic speakers. The mean level of the noise to one ear being kept constant at 75 dB SPL, the intensity of the pure tone to the other ear was changed to 65, 75 and 85 dB SPL. For controls one cochlea was destroyed and the noise was presented. Autoradiographs of the brains were prepared after Sokoloff et al. A stripe of reduced labeling of 2DG surrounded by heavy and diffuse labeling area was seen in the inferior colliculus(IC) contralateral to the ear stimulated by the noise, while a stripe of labeling corresponding to the pure tone was seen in the other IC. This reduced labeling was located in the position corresponding to the stripe of labeling of the opposite IC. The width of the reduced labeling stripe was increased with the increase of pure tone intensity. In control rats labeling of 2DG in IC was diffuse on the side contralateral to the stimulated ear, and little on the ipsilateral. The stripe of low optical density in IC might result from inhibitions of the auditory pathways ipsilateral to the side of the pure tone stimulation.

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FACILITATION AND INHIBITION OF AUDITORY EVOKED POTENTIALS IN THE PIGEON
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Auditory evoked potentials were recorded from the Field L of neostriatum in the pigeon in response to clicks and pure tone bursts. The auditory evoked potentials to clicks were classified into four types according to the effect of continuous pure tone background stimulation. The first type remained unchanged under the application of continuous pure tones. The second showed facilitation of the auditory evoked potentials to clicks. The third showed inhibition. The fourth had both facilitation and inhibition. A 1 to 2 kHz continuous pure tone which caused the facilitation of the auditory evoked potentials to clicks was independent of the frequency that produced the largest auditory response to the pure tone burst. Frequency of a continuous pure tone which caused the inhibition of auditory evoked potentials to clicks was related to the frequency that produced the maximum response to its pure tone burst, although their frequencies were not completely identical. It was observed in the nucleus angularis that the inhibition of auditory evoked potentials to clicks occurred by presentation of a continuous pure tone at a suitable frequency and the facilitation did not occurred. Therefore, the facilitation was attributed to the neural mechanisms between nucleus angularis and Field L.

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TONOTOPIC ORGANIZATION ON THE HUMAN SCALP
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The left ear advantage for the 1 kHz pure tone changed to the right ear advantage under emotional stimulation such as olfactory, sex and autonomic nervous system arousals. The change is characteristically observed in the Japanese people. In the present study employing the shift of the dominance for pure tones by Tsunoda Method, we gave thermal stimulation on many points of the left and right sides of the head specified by the International EEG Society alternately and found that points located over the auditory cortex and the posterior speech area in the linguistically dominant hemisphere were more deeply related to the all frequencies of test sounds including 4 kHz and 8 kHz pure tones, whereas shift occurred for only low tones under the thermal stimulation on the frontal and peripheral parts of the left half of the head. Since similar reversal is observed in Japanese by the thermal stimulation of points close to the cranio-sacral localization of parasympathetic nerves, it is believed that stimulation of the autonomic nervous system is responsible for such reversal.

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RIGHT-LEFT ASYMMETRY WAS NOT FOUND WITH DICHOTIC FUSION.

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Right-left asymmetry of ears was found with interaural switching of CV syllables (*J. Physiol. Soc. Jpn. 39:*351[1977]). We interpreted this asymmetry as another case of right ear advantage(REA) due to cerebral *speech* function asymmetry. However,this could result from cerebral *auditory* function asymmetry if we assume left hemisphere to be more specific for high frequency processing. This question could be settled through the technique introduced by Broadbent(1955) : high and low frequency region of speech signal was fed separately to two ears, and subjects were asked about fusion of these dichotically applied signals. I repeated this, but used Fourier and invert-Fourier transform for filtering high and low. No asymmetry of ears was found in fusion of dichotically applied speech signals, hence no auditory specialization needs to be assumed.

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IONIC MECHANISM OF THE SPINDLE POTENTIAL IN THE FROG MUSCLE SPINDLE.
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The spindle potential in response to stretch was recorded across an air-gap which was made on the first myelinated segment immediately outside the capsule in isolated muscle spindles of the frog. The static component of the spindle potential was reversed during application of depolarizing currents from 0.5 to 9 nA in normal Ringer solution and also in Na-free Ringer solution. Application of the almost same intensity of the current caused rhythmic hyperpolarizations, which have been known to be due to a Ca-activated K conductance increase. The amplitude of the static component of the spindle potential was modified markedly by the treatment of K and/or Ca blockers or 0.1 mM quinine. This suggests that the reversal of the spindle potential is a part of the rhythmic changes. The hypothesis was supported by the different deflections of the spindle potential elicited in each phase of the rhythmic changes. It is concluded that the static component of the spindle potential is due to Ca - activated K conductance in the intracapsular nodal membrane.

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EFFECTS OF CA^{++} AND BA^{++} ON AFFERENT DISCHARGES OF SNAKE MUSCLE SPINDLES
ICHIKI, M. Dept. of Physiol. Mie Univ. Sch. Med. Tsu, Mie 514

In Ca^{++}-free solution, the two types of snake muscke spindles respond in quite different manners, that is, the phasic type of the spindles increases the afferent discharges while the tonic tends to decrease. These changes of afferent discharges are inhibited in K^{+}-free solution in the both types. Ba^{++} seems not to substitute for ca^{++}. Quinine of 100uM inhibites afferent discharges from the both types of muscle spindles. Possible existence of Ca^{++}-activated K^{+} channel is suggested. It is also reported that 4-Aminopyridine below 100uM concentration enhances afferent discharges in frog and snake muscle spindles, although the underlying mechanism is left to be solved.

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RE-EVALUATION OF SPINDLE POTENTIAL IN THE FROG MUSCLE SPINDLE.
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The thresholds for eliciting afferent impulse by electrical stimulation applied antidromically across an air-gap on which the first myelinated segment outside the capsule was bridged were measured in isolated frog muscle spindles. The stimulation was derived with a delay of 10 msec following individual impulse, within which the excitability was recovered from a relative refractoriness to the normal level. The threshold was decreased from 4 nA at the in situ length to 2.1 nA at the end of dynamic stretch, and then gradually restored to 3 - 3.6 nA during the static stretch. These intensity changes reflect the threshold changes at the branching node in the capsule which is changed in almost parallel with the course of the spindle potential. The threshold of 36 nA was measured with intracellular microelectrode inserted into the terminal node. The amount of the spindle current at the terminal node during the static stretch was calculated of approximately 0.9 nA which was far low to attain the threshold by itself.

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FLUORESCENT LABELING OF THE SWEET TASTE RECEPTOR PROTEIN ON TASTE PAPILLAE WITH THIOL REAGENTS IMOTO, T., YAMADA, H. AND HIJI, Y. Dept. of Physiology, Tottori Univ. School of Medicine, Yonago 683

In the histological study of the tongue epithelium of rat and pig treated with fluorescent thiol reagents such as N-(7-dimethylamino-4-methylcoumarinyl)maleimide and S-mercuric N-dansylcysteine(DNS-Cys-SHg^+), strong fluorescence was observed on the tip of the taste bud in the fungiform papillae as well as on the keratinized layer of the epithelium. Furthermore, the responses to sucrose and saccharine recorded from the chorda tympani of the rat were suppressed by the treatment of the tongue with DNS-Cys-SHg^+ without producing any significant change on the responses to the other three basic taste stimuli, indicating that the specific binding of sweet molecule with the sweet taste receptor protein on taste cell membrane was inhibited by the modification of thiol groups of the protein with the reagent. Therefore, the fluorescent chemical modifiers tested in the present experiment would be of great use for isolating the receptor protein from the whole homogenate of tongue epithelium tissue with monitoring the fluorescence as a marker.

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ATPase IN RABBIT FOLIATE PAPILLA. HIRAKAWA, Y. AND NOMURA, H. Dept. of Oral Physiol., Matsumoto Dental Coll., Shiojiri

Adenosin triphosphatase (ATPase) activity stimulated by either Ca^{2+} alone or Mg^{2+} alone was studied in the foliate papilla of rabbit. The highest specific activity was obtained in microsomal fractions. Optimal ATP hydrolysis was obtained at approx. 5 mM of either Ca^{2+} or Mg^{2+}. Mn^{2+}, Cd^{2+} and Co^{2+} also activated ATP hydrolysis but Zn^{2+} and Sr^{2+} did not show clear activation. Potency of the divalent cations as cofactor was in the order $Ca^{2+} > Mg^{2+} > Mn^{2+} > Cd^{2+} > Co^{2+}$. At 5 mM-$Ca^{2+}$, an addition of varying concentrations of Mg^{2+} did not show additive effects but inhibited ATP hydrolysis. Other divalent cations also depressed Ca^{2+} activation but monovalent cations, Na^+ and K^+ did not. When the rate of ATP hydrolysis was measured in the presence of 5 mM-Ca^{2+} or 5 mM-Mg^{2+} at different ATP concentrations, similar substrate dependence curves that had high substrate concentration inhibitions were obtained. These inhibitions suggest that true substrate is the complex of divalent cation and ATP. Apparent Km values for Ca^{2+}- and Mg^{2+}-ATP hydrolysis were similar: approx. 1 mM. The ATPase activity displayed pH maxima around 7.0 in the presence of 5 mM-Ca^{2+} or 5 mM-Mg^{2+}. These results indicate that this enzyme is different from mitochondrial and myosin ATPase.

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ACTION OF COBALT IONS ON FISH TASTE BUDS. YAMASHITA, S., KIYOHARA, S. AND KITOH, J.* Inst. of Biol., Coll. of Lib. Arts, Kagoshima Univ., Kagoshima 890 *Dept. of Anat., Nagoya Univ. Sch. of Med., Nagoya 466

The fish taste bud contains two types of cells reaching the taste pore, i.e. t-cell having a thick rod-shaped process which protrudes toward the taste pore and tortuously tangled tubules in the supranuclear region, and f-cell having microvilli and bundles of filaments. The tubules in the t-cell occasionally open directly to the taste pore at the base of the process, suggesting that the stimulus molecules or ions may enter into the t-cell through the tubules.

To examine the permeation of ions into the cell the cobalt iontophoresis technique was applied to the barbel of carp. By treating with $CoCl_2$ and H_2S-alcohol solution the cells constructing taste buds in the berbel were selectively stained black. It was shown light-microscopically that cobalt-permeated cells may be the t-cell rather than the f-cell. If the barbel was fixed prior to the treatment with $CoCl_2$, however, f-cells rather than t-cells appeared to be selectively stained, suggesting that the permeation of cobalt ions into the cell membrane may vary largely by fixing. Although cobalt-permeation was examined to confirm with X-ray microanalyses under a transmission electron microscope, it was impossible to identify the cell type of permeated cells, since their cytoplasm was considerably damaged.

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TASTE RESPONSES TO AMINO ACIDS IN THE RAINBOW TROUT PALATE. MARUI, T. AND HARA, T.J.* Dept. Oral Physiol.,Sch. Dent., Kagoshima Univ., Kagoshima-890, * Freshwater Institute, University Crescent, Winnipeg, Canada R3T 2N6.

The importance of amino acids as olfactory and taste stimuli for fishes has generally been recognized. However, the relative significance of their effectiveness between two systems has not been fully understood. This is primarily due to the lack of systematic investigations into both chemoreceptor functions in the same species under controlled experimental conditions. In the present study, the stimulating effects of amino acids and related chemicals on the trout taste receptors were studied by recording electrical responses from the palatine nerve. Among chemicals tested at 1 mM or lower, only L-Pro, L-Hyp, L-Ala, L-Leu, L-α-amino-β-guanidinopropionic acid, L-argininic acid, betaine and some of their derivatives were effective at neutral pHs (7.5-7.7). The threshold concentration for L-Pro, the most effective amino acid tested, ranged between 0.01 and 0.1 μM. L-Arg, one of the most stimulatory amino acids previously reported, and related compounds including D-Arg were effective only at basic pHs (> 8.5). The dose-response relation for most of the stimulatory chemicals, plotted semi-logarithmically, were of sigmoid nature, saturating at 1 mM or higher. These data demonstrate that the palatal taste receptor of trout might be highly specific, responding to a more narrowly-tuned spectrum of chemicals than does the olfactory system.

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RECEPTOR POTENTIAL OF FROG TASTE CELL IN RESPONSE TO ACID STIMULI. OKADA, Y. and SATO, T. Dept. of Physiol., Nagasaki Univ. Sch. of Dent., Nagasaki, 852

The receptor potential of a frog taste cell was recorded with a glass capillary microelectrode, and the input resistance of the taste cell was determined by applying hyperpolarizing constant current pulses of 100 ms at 1 Hz. Acetic, hydrochloric, oxalic, and tartaric acids dissolved in 0.1 M NaCl were used for taste stimuli. Upon acid stimulation of a taste cell, sustained depolarization usually appeared. The stimulus concentration curve of taste cells was very similar to that of glossopharyngeal nerves. The input resistance of a taste cell decreased, on the average, by 12% with a 20 mV depolarization in response to acids. The amplitudes of receptor potentials produced by acids scarcely changed in spite of a wide range of change in the membrane potential. The receptor potential amplitudes for 0.001 M acetic and hydrochloric acids scarcely changed even when Na^+ and Ca^{2+} ions in the intercellular fluid sorrounding taste cells were removed. This result suggests that the generation of taste receptor potential in response to acids may be related to the phenomena occurring at the taste receptor membrane.

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THE TASTE CELL RESPONSES IN THE MOUSE AS STUDIED BY INTRACELLULAR RECORDING. TONOSAKI, K. AND FUNAKOSHI, M. Dept. of Oral Physiol., Gifu College of Dentistry, Gifu 501-02

The taste cell intracellular responses and the accompanying membrane resistance changes were studied in the mouse taste bud. The responses were selected for this experiment, only if they responded to 1.0M sucrose with depolarization response. After recording cell responses, procion dye was injected. Injection of dye into a cell after recording served as a reliable indicater that the electrode tip was actually inside a cell and allowed location of the cell histologically. Tongue was adapted to constantly flowing of distilled water. Taste solutions (1.0M sucrose,0.1M NaCl,0.01M HCl & 0.02M quinine) replaced the adapted water without interrupting fluid flow. Twenty intracellular recordings from taste cells were obtained. The membrane potentials ranged from -30--65 mV(mean -38.6mV). Taste cells responded with membrane potential and resistance changes to a variety of stimuli representing the four taste qualities. NaCl, HCl and quinine evoked the complexity of the potential changes, and sucrose and quinine increased the membrane resistance.

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EFFECTS OF IONIC CHANGE IN ADAPTING SOLUTION ON NaCl- OR WATER-INDUCED TONGUE SURFACE POTENTIAL

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As previously revealed, application of NaCl or water to the dorsal surface can produce a slow potential change on the tongue of bullfrogs after adaptation to Ringer's solution. Potential change was also produced at the small areas of tongue included single taste discs or not, and its amplitude was larger in the former than that in the latter, statistically. Decrease or increase of NaCl in adapting Ringer augmented or depressed NaCl- and water-induced potentials. After adaptation to NaCl-free sucrose solution, NaCl-induced potential was augmented, but small water-induced potential was remained. Addition of sucrose in adapting Ringer depressed both potentials. Effect of Ca in adapting solution on water-induced potential was maximum in 1.8 mM and reduced in either lower or higher Ca than 1.8 mM, while NaCl-induced potential could not be influenced by the change in Ca. Both potentials were augmented by low K and depressed by high K. The results indicate that the potential change may contribute to the taste sensation because of its similarity to the receptor potential in taste cells or the activity of taste fibers.

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GUSTATORY NERVE RESPONSES IN MICE

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Responses to lingual stimulation by various chemicals were recorded from the chorda tympani in mice. Good responses were demonstrated to the four basic taste stimuli. Sucrose, quinine and saccharin elicited bigger responses than those in rats. Among cations Ca, Mg and Sr were most effective, and NH_4 and K had effectiveness nearly equal to that of Na. Replacement of Cl by other anions decreased the response magnitude in proportion to the relative hydrated size of anions. Also amino acids produced good gustatory responses compared with those in rats. The phasic response magnitudes for 0.2 M solutions are in the order of L-HIS HCl > L-ARG HCl > L-LYS HCl > L-ORN HCl > L-MSA > L-MSG > L-SER > GLY > L-CYS > L-GLN > ß-ALA > L-ALA > D-ALA > L-ASN > homoserine > L-MET > L-THR > L-VAL > L-ILE > L-PRO > L-LEU (0.1 M) > taurine > L-PHE (0.1 M) > betaine. Taste preferences in mice for NaCl, sucrose and saccharin were also studied by employing the two-bottle preference technique. Mice showed marked preferences for 3 mM - 1 M sucrose and 1 - 30 mM saccharin, but a weak preference for 0.1 M NaCl. These results indicate a difference in taste sensitivity between mice and rats and a high sensitivity to amino acids in mice.

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COMPARISON OF LINGUO-HYPOGLOSSAL AND LINGUO-TRIGEMINAL REFLEXES IN THE FROG.

NOMURA, H. AND KUMAI, T. Dept. of Oral Physiol., Matsumoto Dental Coll., Shiojiri.

Reflex discharges in the hypoglossal and trigeminal nerves elicited by electrical stimulation of the glossopharyngeal nerve were studied in respect to the reflex time and other characteristics of the reflex in the frog, Rana nigromaculata. Reflex discharges were always greater ipsilaterally than contralaterally in both the hypoglossal and trigeminal nerves, but ipsilateral predominancy was prominent in the hypoglossal nerve than in the trigeminal nerve. Facilitation and post-tetanic potentiation were remarkable in the reflex discharges of the trigeminal nerve, but not in those of the hypoglossal nerve. The mean minimum reflex time is shorter ipsilaterally than contralaterally in both the nerves, and were shorter in the hypoglossal nerve than in the trigeminal nerve. These results suggest that the linguo-trigeminal reflex is more complicate than the linguo-hypoglossal reflex in the frog. The complexity of the linguo-trigeminal reflex arc may be needed in forming a tonic discharge in the trigeminal nerve bilaterally that plays an essential role in keeping the nostril closed.

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CEREBELLAR PROJECTION OF THE GLOSSOPHARYNGEAL NERVE AFFERENTS IN FROGS. HANAMORI, T. AND ISHIKO, N. Dept. of Physiol., Miyazaki Med. Coll., Miyazaki

Cerebellar potentials evoked by electric stimulation of the glossopharyngeal(IXth) nerve in the frog immobilized with succinylcholine chloride were studied. When a glasscapillary electrode filled with 0.5 M sodium acetate solution containing 2 % pontamine sky blue was penetrated dorso-ventral direction, a slow negative potential with the latency of about 34 msec was recorded bilaterally. It reached maximum magnitude at 1 mm in depth, 0.6 mm lateral from the midline in ipsilateral hemisphere and 0.5 mm caudal from rostral end of the cerebellum. Histological examination revealed that the position corresponded to the molecular layer near the Purkinje cells. When the IXth nerve was stimulated by paired pulses, the second negative wave was almost completely depressed at the interval of 50 msec. Recovery from the depression took about 30 sec. Single unit responses resulting from the nerve stimulation consisted of simple and complex spikes or both, characteristic of the Purkinje cell discharges. That the electrode was in the Purkinje cell layer had been confirmed histologically.

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FLUCTUATION OF PAROTID SALIVA SECRETION AND ITS pH. INOMATA,K. ITO,K., TAMAGAWA,K., NAKAMURA,H*. AND KURAHASHI,M*., Dept. of Physiol. and Oral Physiol*., Higashi-Nippon-Gakuen Univ. Sch. of Dent., Ishikari-Tobetsu, Hokkaido, 061-02

The parotid saliva secretion volume, secretion velocity and saliva pH were recorded simultaneously, and the relations in these phenomena were studied. In this experiment, 3% tartaric acid(ca. 0.5ml) was used as gustatory stimulus. Small pH and reference electrodes which diameter was 1.6 and 2 mm respectively were used to measure saliva pH, and both electrodes were set in narrow tube(1 mm diameter) through which the saliva was collected. Ten to twenty seconds after stimulation, maximum secretion velocity which 0.1 to 0.2 ml per 5 seconds was shown, but about 1 minute after maximum response, secretion velocity decreased to about 0.01 ml per 5 seconds. On the other hand, saliva pH was 7.4 to 7.8 one to three minutes after stimulation, and this value was maintained for 7 to 15 minutes, but 10 to 20 minutes after, the pH was gradually decreased to about 5.3. However, the ultimate saliva pH did not decrease below 5.2. The fluctuation of saliva secretion velocity did not parallel with that of saliva pH.

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ELECTROMYOGRAM OF JAW AND TONGUE MUSCLES PRODUCED REFLEXLY BY MECHANICAL AND CHEMICAL STIMULATION OF THE ORAL MUCOSA AND THE TONGUE IN THE FROG. KUMAI, T. AND NOMURA, H. Dept. of Oral Physiol., Matsumoto Dental Coll., Shiojiri

Electrical activities of jaw and tongue muscles produced reflexly by mechanical and chemical stimulations of the oral mucosa and the tongue were studied by recording EMG in the frog, Rana nigromaculata, using biporlar iron electrodes. The activity of the temporal muscle occured mainly when mechanical stimuli were applied ipsilaterally to the palatal list of the upperlip, the fold of the lowerlip, the root of the tongue, the ventral surface of the lower jaw and the oesophagus. The contralateral stimulations also produced moderate activities. The masseter muscle produced the potential in response to the ipsilateral stimulation of the palatal list and the rostral region of the fold of the lowerlip. No activity of the mandibular depressor muscle was obtained whatever portions of the mouth were stimulated. The activity of the submaxillary muscle was observed mainly when the area around the oesophagus was stimulated mechanically. This muscle also produced the potential synchronously with the respiration, which was suppressed by the mechanical stimulation of the oral mucosa. The hyoglossal and the intrinsic tongue muscles generated the potentials in response not only to mechanical but also to chemical stimulation of the tongue. The intrinsic tongue muscle was also activated by the mechanical stimulation of the oesophagus.

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SENSORY ORGAN OF ORAL FLOOR MUCOSA IN XENOPUS LAEVIS

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The response characteristics of sensory organ on the oral floor mucosa in Xenopus laevis to mechanical and chemical stimuli were investigated.

The gustatory receptors on the oral floor mucosa of this toad did not elicit an obvious response to pouring water. These sensory organs were sensitive to 4 basic taste substances. The thresholds for NaCl, sucrose, acetic acid and quinine-HCl were 1/4096 M, 1/8 M, 1/4096 M and 1/16384 M respectively. The stimulating effectiveness of various salt solutions to these sensory organs were compared. The order of effectiveness of monovalent salts was $KCl > NaCl > NH_4Cl > LiCl$ and that of divalent salts was $CaCl_2 > MgCl_2$. Responses of 64 single taste fibers to 4 basic taste substances were investigated and classified into 4 types according to their response patterns.

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EFFECTS OF GUANETHIDINE AND VERAPAMIL ON THE LATERAL-LINE ORGAN.

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Phosphatidylinositol (PI) is known to have an extraordinary high affinity for Ca^{2+} and guanethidine is known to have a strong inhibitory effect on Ca^{2+} uptake by PI. Verapamil is known to reduce Ca^{2+} entry in a variety of cells. Using *Necturus maculosus* effects of these drugs on the mechano-sensitivity of the lateral-line organ were studied to demonstrate the action site of Ca^{2+} on the hair cell. The mechano-sensitivity was expressed as an increment of the synchrony between mechanical vibrations and afferent nerve discharges. The synchronization was remarkably improved by Ca^{2+} and it was strongly suppressed by application of guanethidine (100 μM) and verapamil (50 μM). These effects were reversible. The synchronization was also suppressed by La^{3+}, Mn^{2+}, Co^{2+} and Mg^{2+}. The order of effectiveness was La^{3+} > verapamil > guanethidine > Mn^{2+} > Co^{2+} > Mg^{2+}. Suppressive effects were decreased with increasing concentration of Ca^{2+} in the medium, and double reciprocal plots indicated competitive interaction between these substances and Ca^{2+} for a specific site on the lateral-line organ. These results suggest that the action site of Ca^{2+} may be related to PI in the membrane.

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OLFACTORY INPUT TO DEEP AMYGDALOID NUCLEI.

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Extracellular unit responses of deep amygdaloid nuclei-lateral(La), basolateral(BL), basomedial(BM) and central(Ce) amygdaloid nuclei-following stimulation of the prepyriform(PPF) or the lateral olfactory tract(LOT) were recorded in the cat anesthetized with pentobarbital sodium. The location of electrode tips was marked with the fast green FCF.

Stimulation of both PPF and LOT produced responses showing the play of latency and with long recovery time. In addition cells of those nuclei frequently fired repetitively to a single stimulus. Latencies of these orthodromic, postsynaptic responses were 4-30 msec(mean; 11.7 msec in La, 10.9 msec in BL and BM, and 10.2 msec in Ce). PPF stimulation also elicited antidromic spikes. Latencies of these responses were 3-30 msec(mean; 10.2 msec in La, 12.7 msec in BL and BM, and 8.8 msec in Ce). LOT stimulation could not elicit an antidromic spike. Double shocks at an interval of 5 msec evoked the third, orthodromic responses after two antidromic spikes to each shocks. This indicate the existense of a reverbrating circuit between PPF and the deep amygdaloid nuclei. The distribution of response types was different in recording sites: in La(n=95, orthodromic 17 %, antidromic 31 % and unresponsive 52 %); in BL and BM(n= 114, 40 %, 23 % and 37 %); in Ce(n=37, 27 %, 11 % and 62 %).

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REGULARITY OF BACKGROUND DISCHARGE OF INFRARED RECEPTORS

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We recorded background discharges from infrared receptors(warm fibers in snake pit organs) at various temperatures and calculated their mean intervals ($\bar{X}$), standard deviations (S), and coefficients of variation (C). In four units $\bar{X}$s and Ss were lowest between 24 and 29°C. Cs were lowest between 24 and 28°C in three units. We plotted spike interval histograms for 17 units at 61 separate temperature conditions and calculated the goodness of fit (P) to the corresponding normal distribution curves with the chi-square method. The null hypothesis that the histograms fit the normal distribution curve was not rejected in 25 cases of the 61 at the significance level of 1%. This indicates that in some cases the receptors were firing at certain regular intervals, for unknown reasons. We also checked the same values for central units, but their P values were all below the 1% level. We conclude that the regular discharge in the peripheral units of this system is not used in central information processing.

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CHANGES OF SKIN TEMPERATURE IN RESPONSE TO WARMING AND COOLING OF VARIOUS SKIN AREA.

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Cutaneous vasomotor responses to warming and cooling of face, arm and leg were examined mainly by the use of a thermograph in various thermal conditions. The response was generally confined to hands and feet and its pattern depended largely on environmental and skin temperatures. The response consisted of immediate and transient response (fast response, FR) and delayed and more sustained one (slow response, SR). Arterial occlusion of the stimulated area abolished SR but not FR, indicating that FR was elicited reflexly by dynamic response of cutaneous thermoreceptors. In thermal neutrality with the temperature of the test area around 30°C, the response was most remarkable and yet was often masked by spontaneous vasomotor oscillations. The lower the skin temperature was, the weaker was FR and the later was the appearance of SR. In a warmer environment with a relatively high skin temperature, FR as well as SR was attenuated. Changes in skin temperature was initiated at the tips of fingers and toes, suggesting participation of arteriovenous anastomoses. Some dermatomal influence of the stimulated area was noted upon the response area. Stimulation of the face or the arm generally induced a stronger response than that of the leg, and further, that of the forearm was more effective than that of the hand.

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HEATING EFFECTS ON CHEMICAL RESPONSES OF POLYMODAL RECEPTORS.

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Low reproducibility of responses is one of the characteristics of polymodal receptors. This might reflect that the activity of this receptor is modulated by environmental changes at the receptive area. Whether subthreshold heating of receptive area modulates chemical responses of this receptor was studied on testicular polymodal receptor units in vitro. The testis and epididymis with spermatic cord were excised from the dog. Single unit discharges were recorded from the superior spermatic nerve in the oil pool, while the testis and epididymis exposed at the tunica vaginalis visceralis were immersed in Krebs-Henseleit solution equilibrated with 5% CO_2 and 95% O_2. Mean discharge rate(I) during application of 4.5% NaCl solution monotonously increased, when the temperature of the solution(T) was raised by 3°C step from 34°C to 43°C. The relation between I and T was expressed as $I = 1.651 \times 10^{-7} T^{4.859}$ (r:0.985). Mean discharge rates responding to NaCl increased with increasing the concentration of the solution from 1.8% to 4.5% measured both at 34°C and at 40°C. The slope of the regression-line of the concentration-response curve obtained at 40°C was significantly steeper than that at 34°C. A similar tendency was also observed in the responses to bradykinin. After "heat sensitization", responses to NaCl were enhanced, but those to bradykinin were supressed.

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INDUCEMENT OF FLIGHT IN CRICKETS, Teleogryllus taiwanemma. N.YAMAGUCHI, N.AI. Dept. of Biol., Tokyo Gakugei Univ., Koganei-shi, Tokyo

Induced flight composed of forewing-flapping and flapping-flight in adult cricket is appeared by air puff or wind stimulation. These two patterns of flight are investigated easily when air flow stimulations are given to the cerci of cricket. Several kinds of mechanosensory hairs are located on the surface of the cerci and these hairs are bent by air flow to originate input signals. When the stimulations are given to the cerci, muscle action potentials are appeared in M127(direct flight muscle) and M112(indirect f. m.),which are innervated from N1 dorsal root of metathoracic ganglion. So input signals originated in the region of cercal sensory hairs are able to conduct to the metathoracic ganglion and induce the flight bilaterally or sometimes ipsilaterally by efferent signals from metathoracic ganglion. To confirm the process of the flight electrophysiologically, 5th abdominal ganglion is stimulated electrically by intracellular electrode and action potentials for wing movements are recorded ipsilaterally, not bilaterally.

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RESPONSES OF SLOWLY ADAPTING MECHANORECEPTOR AFFERENT UNITS INNERVATING THE FROG FOOT SKIN DURING ARTIFICIAL PERFUSION OF THE LEG. OGAWA, H., YAMASHITA, Y., NOMURA, T., AND TANIGUCHI, K. Dept. of Physiol., Kumamoto Univ. Med. Sch., Kumamoto.

Changes in the response characteristics were studied of the slowly adapting mechanoreceptor afferent (SA) units innervating the frog foot skin, while perfusing the leg with various artificial solutions. After identifying the SA units, the leg were perfused with a normal Ringer solution containing heparin using a roller pump at a rate of 40/min and mean pressure of 20 cmH_2O for 1 hr or more. Then various perfusates without heparin, including a normal Ringer soln., were used, and the response to a ramp stimulation was recorded. Discharge rates of the SA units usually increased after the onset of perfusion with a normal Ringer soln.. Increased osmotic pressure in a perfusate decreased the discharge rates. An addition of Mg ions to the Ringer solution increased discharge rates in a SA type II unit, but decreased the rate in most of the SA type I units studied. Differences in the contribution of Ca ions to the stimulus-reception process were suggested between the two types of the SA units in the frog.

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TWO KINDS OF ANALGESIA DIFFERENTIATED BY DEXAMETHASONE MURAI, M. FUJISHITA, Y. HISAMITSU, M. AND TAKESHIGE, T. Dept. of physiol. Showa Univ. Sch. of Med., Shinagawa-ku, Tokyo

The skin impedance of rat was measured at 86 different point on the body. Low impedance point were mostly found in lower extremities and were not found in the thigh and body trunk. Low frequency muscle stimulation of lower extremities, showing low impedance, cause analgesia which was measured by the tail-flick test. While stimulation of thigh and abdominal muscles showed no analgesia effect. After treatment with 250mg/kg D-phenylalanine, analgesia was produced by thigh muscle or abdominal muscle stimulation. Analgesia caused by stimulation of the lower extremity muscles was not antagonized by dexamethasone, while analgesia caused by thigh and abdominal muscles stimulation after D-phenylalanine was antagonized by dexamethasone. These data indicate that analgesia caused by stimulation of thigh muscle is caused by different endogenouse morphine-like releasing mechanisms by stimulation of lower extremity muscles.

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THE EFFECT OF GASTROINTESTINAL AND PANCREATIC (GEP) HORMONES AND SOME FATTY ACIDS ON THE DISCHARGE RATE OF VAGAL HEPATIC AFFERENTS.

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Afferent discharges were recorded from filaments dissected from the hepatic branch of the vagus nerve in the rat. Experiments were conducted in vivo or in isolated and perfused liver preparations with vagus nerves. Injections of insulin, 3-deoxytetronic acid into the portal vein increased the discharge rate, while glucagon, CCK-PZ, 2-deoxytetronic acid decreased it. The results suggest that GEP hormones and tested fatty acids which appear in the blood after starvation may modulate appetite and body functions through the hepatic vagal afferents.

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RESPONSES OF RAPHE NEURONS TO PERIPHERAL STIMULATION AND CROSS-CORRELATION BETWEEN RAPHE NEURONS AND DORSAL HORN NEURONS.

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The purpose of the present experiment is to study the functional influences of raphe neurons responsive to noxious or innoxious inputs on the spinal dorsal horn neurons.

8 cats were anaesthetized with α-chloralose and urethane (i.v.). Extra-cellular recordings were obtained from 56 raphe neurons and 18 spinal dorsal horn neurons. Some raphe neurons showed to be excited by cutaneous noxious stimulus. But, electrical stimulation of hindlimb nerves and stretch or pressure applied to the muscle were particularly effective in evoking raphe neuronal responses. We, also, applied a cross-correlation analysis to 56 pairs of simultaneously recorded dorsal horn and raphe neurons. Cross-correlograms were flat in 52 cell pairs. One pair had a cross-correlogram with broad trough suggesting that the raphe neuron had inhibitory effect on the dorsal horn neuron in this case. Another pair had a cross-correlogram with a peak around t=+40 ms suggesting that the raphe neuron had excitatory effect on the dorsal horn neuron. The other two pairs had cross-correlograms with peaks around t=0 ms, suggestive of causal relationship between them. These observations suggest that the organization of these areas and their involvement in analgesic effects are much more complicated than previously described.

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RESPONSE PROPERTIES OF BARREL NEURONS IN THE RAT SOMATOSENSORY CORTEX.

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Response properties of layer IV neurons in the rat somatosensory cortex was studied by measuring the threshold amplitude of whisker deflection with exponential ramp onset at different time constants. Recording loci were identified in relation to barrels that are specific to layer IV. The "trade-off" between the threshold amplitude and the time constant was the measure of the extent to which a neuron was oriented for detection of velocity component of mechanical stimuli; a velocity-sensitive neuron would require less amplitude with shortening the risetime whereas an amplitude-sensitive neuron would require a more or less constant amplitude regardless of whether the onset was rapid or slow. It was found that these neurons tended to signal the amplitude component and showed sustained firing during whisker deflection. This was in a sharp contrast with layer V neurons that had previously been shown to be more oriented for velocity detection.

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SELECTIVE BLOCKING EFFECT OF P-CHLOROMERCURIBENZOATE ON THE RESPONSES TO ODORS AS STUDIED IN THE BULLFROG OLFACTORY BULB.

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The responses elicited by application of 0.1% n-amyl acetate(AA), 0.2% ethyl n-butyrate(ENB), 0.5% ethyl ether(EE) and 0.5% n-buthyl alcohol(BA) on the olfactory epithelium were recorded extracellarly from single olfactory bulb neurons of the bullfrog. Responses to the four odors were compared before and after the epithelium was treated with 0.5 to 1mM p-chloromercuribenzoate(PCMB). After PCMB was applied, responses to AA, ENB and EE were blocked, but the response to BA was not. Then, the olfactory epithelium was treated with a mixed solution of PCMB and AA. Responses to the other odors were affected, while response to AA was not. Thus, it was clear that the receptive site for AA can be protected from the blocking effect of PCMB, but the sites for the other odors are not.

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GUSTATORY RESPONSES OF CHORDA TYMPANI FIBERS IN THE MOUSE.

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Gustatory responses from the mouse chorda tympani fibers were tested with the four basic taste stimuli and other various chemicals. Many of single chorda tympani fibers responded to one or two of the four basic taste stimuli, although relative responsiveness of these fibers to the four taste stimuli considerably varied with the stimulus concentration. Entropy measures of the breadth of responsiveness showed that chorda tympani fibers in the mouse were much more narrowly tuned than those in the hamster and the rat. Taste sensitivities of the mouse chorda tympani fibers were also characterized by high magnitudes of mean responses to HCl and divalent chloride salts, such as $CaCl_2$ and $MgCl_2$. Forty-five single fibers were classified into the following 5 types according to their responsiveness to taste stimuli; 12 fibers were specifically sensitive to sugars and saccharin Na (sweet-type), 8 fibers were to NaCl (Na-type), and 11 fibers strongly responded to $CaCl_2$ and/or $MgCl_2$ (Ca-Mg-type), and 10 fibers did to HCl and/or tartaric acid (acid-type), and 4 fibers did to quinine-HCl (quinine-type).

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DISCRIMINATION OF THE TOOTH POSITION AFTER MECHANICAL TAPPING:EFFECT OF FEEDBACK CONTROL.

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In the present study, quantitative analysis of discrimination of the tooth position and the effect of bio-feedback control, which was designed by notifying the correct answer at each trial, were done in the dental students with normal occulusion. In non-feedback group, the degree of correct answer for discrimination of tooth position was about 30% smaller than that in the feedback group. In the incisor tooth, the subjects answered more correctly than in the premolar or molar tooth. There were no significant differences between the degree of correct answer for discrimination in both sides of the jaws and in the upper and lower jaws. The recognition time of the discrimination of the tooth position in the non-feedback group was longer than in the feedback one. The mean recognition time was 4.6 sec in the non-feedback and 2.1 sec in the feedback group.

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THE ROLE OF CATIONS IN THE OLFACTORY RECEPTION

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The effects of changed ionic environments on the olfactory responses in the carp, the rainbow trout, and the bullfrog were examined by recording the stimulant-induced waves from the olfactory bulb. a) Application of stimulants(various species of amino acids and β-ionone) dissolved in deionized water to the EDTA-treated olfactory epithelium of the carp did not induce any response. The addition of various species of salts to the stimulating solution recovered reversibly the response. b)The responses of the carp to L-alanine in the presence of $MgCl_2$ and $MgSO_4$ and those in the presence of KCl and $K_4Fe(CN)_6$ were illustrated with a single curve as a function of concentration of the cations, respectively, suggesting that the cations support the olfactory reception. c) All the inorganic cations examined(Li^+, NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Co^{2+}, Mn^{2+}, Cd^{2+}) and organic cations(Tris$^+$, choline$^+$, Bis-Tris Propnae^{2+}) were effective to support the response of the carp, whereas TEA$^+$, TBA$^+$, triethanolamine$^+$, and Bis-Tris$^+$ were ineffective. d) The olfactory responses of the rainbow trout and the bullfrog were also reduced by removal of ions from the surface of the epithelia and recovered by addition of ions. e) It is discussed that the cations do not act as current carrier across the apical olfactory cell membrane for the generation of the receptor potential but the binding of the cations on the receptor membrane plays an essential role in the reception.

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MUSCLE CONTRACTION UNDER HIGH HYDROSTATIC PRESSURE.(III). A ELECTRONMICROSCOPIC STUDY.
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When skeletal muscle of frog is immersed in liquid paraffin and applied to high hydrostatic pressure, there is marked increas in muscle tention durung period of compression. Muscle exposed to 600 Kg/cm^2 appeared to have an unreversible contracture. Isolated satrius muscle was fixed under compression (from 300 Kg/cm^2 to 600 Kg/cm^2 at 20°C for duration of 10 min.) by specially designed apparatus. Ultrastructural change in the pressurized muscle was compared with that both in tetanus muscle with electric stimulation and nonpressurized muscle.

Pressurized muscle length (300 Kg/cm^2 - 500 Kg/cm^2) was contracted down to about 69% of resting length regardless of its magnitude of pressure. This value corresponded to length of thick filaments. Length of tetanic muscle with electric stimulation was contracted down to 83% of its resting length, which approximately corresponded to length of thin filaments. Under compression, electron dense bands appeared on both sides of pseudo H-zone, and the I-bands disappeared and the A-bands came into contact with the ajacent Z-line. Ultrastructural deorganization, however, was imperceptible below 500 Kg/cm^2, and destruction of Z-line and irregularity of striation pattern were observed in muscle exposed to 600 Kg/cm^2.

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DIFFRACTION INTENSITY CHANGE AND TENSION DEVELOPMENT AS A FUNCTION OF SARCOMERE LENGTH IN FROG SKELETAL MUSCLE.
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Diffraction intensity (I) changes of the first order line and tension development (T) was simultaneously observed as a function of sarcomere length (SL) in frog semitendinosus. In single fibers, I decrease upon tetanus at 2-4°C proceeded more rapidly than T between 2.23 and 2.76 μm of SL, although the onset of I change was simultaneous with the onset of T. The half-maximum of I decrease was reached 8.8 ± 1.3 msec (N=11) before the half-maximum of T. At 3.12 μm, the rate of I change was completely synchronized with that of T. The same observation was obtained in skinned fibers being activated by Ca. Therefore, the time lag between I decrease and T observed around the slack length of SL may be explained by greater myofibril misalignmant. The relationship between I decrease and Ca conct is also studied and compared with the isometric T-pCa curve. Both curves showed a similar steep S-shape, although the I-pCa curve shifted slightly to the left. This suggests that Ca conct is monitored by I measurement more sensitively than T.

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STUDIES ON THE UNIFORMITY OF SARCOMERE LENGTH CHANGES ALONG THE LENGTH OF SINGLE MUSCLE FIBERS DURING THEIR DYNAMIC MECHANICAL RESPONSES.
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To examine the uniformity of sarcomere length changes along the length of single frog muscle fibers during their dynamic mechanical responses, an apparatus was constructed with which the sarcomere length changes of less than 10Å could be detected by use of diffraction with He-Ne laser light. Data were obtained only with fibers exhibiting perfect sinusoidal sarcomere length signals in response to sinusoidal length changes applied to one fiber end during tetanus. When a quick release (complete in 0.3msec) was applied to one end of a tetanized fiber, the beginning of the resulting fall in tension tended to precede the beginning of the sarcomere shortening if the sarcomere length was measured near the fixed fiber end. It was always observed irrespective of the location of laser beam that the quick tension recovery took place while the sarcomere shortening in response to a quick release was still in progress. These results indicate that the tension changes following a quick release may not give correct information about the cross-bridge properties. An appropriate viscoelastic sarcomere model is being constructed to account for the present results.

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EFFECT OF MUSCLE LENGTH ON MAINTENANCE HEAT PRODUCTION IN FROG SKELETAL MUSCLE
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The maintenance heat production in 3 s tetanic contractions was measured on frog semitendinosus muscles at 0°, 5° and 10°C, and at various sarcomere lengths between 2.0 and 3.7 μm as determined by laser diffraction. The maintenance heat was divited on the basis of its time course into the two parts, labile and stable, which were defined by Aubert (1956). The stable heat, as well as active tension developed, declined linearly when sarcomere length was increased. These relationships were unaffected by 1 mM caffeine. When no active tension was developed at l_{max}, about 20% (3 mJ g^{-1} s^{-1}) of the stable heat at l_o remained at 0°C, whereas this was about 40% (20 mJ g^{-1} s^{-1}) at 10°C. The labile heat also declined linearly as sarcomere length was increased. However in 1mM caffeine the labile heat became independent on sarcomere length at 5°C, and the labile heat became much less dependent on sarcomere length at 10°C. The "recovery" of the labile heat in the second tetanic contraction was delayed in 1 mM caffeine.

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SARCOMERE LENGTH-FORCE RELATION IN VERTEBRATE GLYCERINATED MUSCLE MYOFIBRILS.
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The sarcomere length-force relation in glycerinated rabbit psoas muscle myofibrils during Ca-activation was studied in an auxotonic condition in which the fibrils had to shorten actively by 10-20% against a glass needle of appropriate compliance before isometric force generation. The sarcomere length along the entire length of the preparation was continuously recorded with a TV camera mounted on a microscope. The extent of the auxitonic shortening differed from part to part within the same preparation, and when the steady isometric force was reached, the local sarcomere length changes were no longer observable, indicating that the forces are balanced between the sarcomeres. By comparing the final isometric force developed and the shortest sarcomere length during the isometric force generation, it was found that, in Ca-activated myofibrils, the maximum isometric force (about 3 kg/cm^2) could still be produced even when the sarcomeres shortened to about 1 μm, indication the virtual absence of the ascending limb of sarcomere length-force relation obtained from intact muscle fibers. These results also suggest that the double overlap between the thin filaments or the collision of the thick filaments against the Z-line do not appreciably impair the force generating capacity within a single sarcomere.

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THE MgATP CONCENTRATION DEPENDENCE OF CONTRACTION CHARACTERISTICS IN GLYCERINATED HORSESHOE CRAB MUSCLE FIBERS.
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The MgATP concentration dependence of isometric tension, ATPase activity during isometric contraction and unloaded shortening velocity was studied on glycerinated horseshoe crab skeletal muscle fibers. Isometric tension and ATPase activity were monophasic function of MgATP concentration. They reached their maxima at 10^{-5} M MgATP and 10^{-4} M MgATP respectively. The unloaded shortening velocity continued to increase with increasing MgATP concentration up to 3 x 10^{-3} M. The values of both the ATPase activity and the unloaded shortening velocity in horseshoe crab fibers were smaller than the corresponding values in rabbit psoas fibers. These results obtained on horseshoe crab muscle fibers can at present be explained by assuming that the rate constant for breaking cross links is smaller in horseshoe crab muscle fibers than in rabbit psoas fibers.

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TRANSIENT A-BAND LENGTHENING DURING CONTRACTION IN HORSESHOE CRAB STRIATED MUSCLE.
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Glycerinated myofibril bundles of horseshoe crab striated muscle were maximally activated by Ca ions, and the resulting sarcomere shortening was recorded by a 16mm cine-camera to explore the possibility of thick filament shortening during contraction suggested by Dewey and his coworkers. In relaxed myofibrils, the A-band width (about 4μm) did not increase appreciably when the sarcomeres were stretched from 10 to 15μm. If the myofibrils were activated by Ca ions at the initial sarcomere length of 12-15μm, the sarcomeres shortened almost linearly with time, while the A-band width first increased by about 50% and then returned to the initial value during the course of the sarcomere shortening. The transient A-band lengthening during contraction can be accounted for by the transient increase in misalignment of thick filaments with a constant length at the early phase of contraction; the initial thick filament misalignment is followed by their realignment as contraction proceeds. Since such a misalignment causes a marked imbalance in the number of cross-links between the two halves of a thick filament, the subsequent thick filament realignment implies that the force exerted by a single cross-bridge is not constant but differs according to its mechanical conditions.

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TIME-RESOLVED X-RAY DIFFRACTION DURING LENGTHENING OF SKELETAL MUSCLE.
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During a 3 sec isometric tetanus in frog skeletal muscle, a load greater than P_o was applied to induce lengthening of the muscle. The effect of lengthening of the muscle on two equatorial reflections, I(1,0) and I(1,1) was studied with the time-resolved X-ray diffraction technique. The application of a load (1.0-1.3 P_o) induced lengthening of the muscle of about 5% of its initial length, but intensities of both I(1,0) and I(1,1) during lengthening of the muscle were nearly equal to the intensities before lengthening. The reciprocal changes in I(1,0) and I(1,1) have been explained as a transfer of mass of cross-bridges from the vicinity of thick filaments to that of thin filaments. However, in some cases, I(1,1) once increased on activation of the muscle decreased during contraction while I(1,0) was kept to be low. This result can be explained as a result of a change of the ordering of thin filaments within a hexagonal lattice as a primary effect.

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BEHAVIOUR OF CROSS-BRIDGES DURING SLOW LENGTH CHANGES OF ACTIVE MUSCLE.
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Cross-bridge movements in frog sartorius muscle was studied at a time resolution of 200 msec using a fast X-ray diffraction technique. When a tetanized muscle was stretched or released at a constant speed(7% muscle length in 2.4 sec) the intensity of the myosin meridional reflection at 14.3nm decreased indicating a change in the axial distribution of cross-bridges. The intensity decrease was greater during stretch than release suggesting that the cross-bridge alignment was more disordered during stretch. The intensity decrease was accompanied by an increase in tension during stretch and a decrease during release. The tension deviation from the isometric level was proportional to the intensity decrease both during stretch and release. However, the proportionality constants were different; for a given amount of the intensity decrease, the tension change was greater during stretch than release. This suggests that the elastic modulus of cross-bridges is greater during stretch.

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LATERAL SHRINKAGE OF THE MAYOFILAMENT LATTICE IN "SKINNED" MUSCLES DURING CONTRACTION. MATSUBARA, I., UMAZUME, Y. AND YAGI, N. Dept. of Pharmacol., Univ. of Tohoku Sch. of Med., Sendai

A toe muscle of the mouse was skinned chemically with saponin and held isometrically at the sarcomere length of 2.5 m. At the resting state (17-21 °C) the 1,0 spacing of the hexagonal lattice was 40.7 nm at the ionic strength of 0.20. When the muscle was maximally activated at pCa =4.2, a steady tension of 1.3 kg/cm² was produced and the spacing decreased to 38.4nm. The magnitude of this decrease was almost the same as that observed on going into rigor (although the rigor tension was only 0.1 kg/cm²). This indicates that the side force responsible for the shrinkage is not proportional to the contractile force. During the maximal activation a considerable number of myosin heads were transferred radially to the thin filaments; i.e. 90% of the rigor transfer. When the muscle was activated at a lower Ca concentration, the lattice shrinkage was smaller and the radial transfer was also smaller. These findings suggest that the side force may be proportional to the number of myosin heads attached to actin.

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INFLUENCE OF OSMOTIC COMPRESSION ON LOAD-VELOCITY RELATION IN SKINNED MUSCLE FIBERS OF FROG.. TSUCHIYA, T. AND SUGI, H. Dept. Physiol., Sch. Med., Teikyo Univ.

Several mechanical properties of osmotically compressed frog muscle fibers by PVP (polyvinylpyrrolidone) were investigated. The isotonic shortening and lengthening were appreciably and almost inhibited at 4% and 12% PVP respectively. The redevelopment of tension after a quick release to zero tension was slower as the concentration of PVP was increased. The stiffness of the fiber in activating solution, measured by small stretch, increased with the concentration of PVP. The fiber did not give when the tension rose above three times as high as isometric tension at 8% PVP by constant velocity stretch, indicating the increased difficulty of detachment of cross-bridge from thin filament. The above results seem to be explained by the decrease of the rate of detachment of cross-bridge.

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RADIAL STIFFNESS IN RELAXED AND RIGOR SKINNED MUSCLE FIBERS. UMAZUME,Y. AND KASUGA,N. Dept. of Physiol., Jikei Univ. Sch. of Med., Minatoku Tokyo

Lateral stiffness may reflect the cross-bridge attachment in skinned fibers. Adding PVP K-30 (polyvinylpyrrolidone M.W. av. 40,000) in incubating medium, the width (D) of the fiber was measured. Below 6% PVP, fibers acted as complete elastic body. The compression ratio at $3X10^{4}$ Pa was proportional to the sarcomere length in rigor condition. From width-pressure relations, the bulk moduli were calculated. The bulk moduli changed in various manner by ATP,PPi and calcium ion.

bulk modulus at 92% of D at 0 Pa ($Pa\ X10^{-4}$, mean±S.D.,n)

A	0.50 3	
B	0.86±0.27, 5	A: KCl ghost in relaxing solution
C	2.37±0.44,12	B: 4mM Mg-ATP, Ca(-)
D	3.61±0.98, 6	C and D: 4mM PPi, Ca(-) and Ca(+)
E	4.37±1.06,12	E and F: no ATP or PPi, Ca(-) and Ca(+)
F	5.97±2.16, 6	

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CALCIUM NON-SENSITIVE CONTRACTION OF GLYCERINATED MUSCLE FIBER

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The normal glycerinated skeletal muscle fiber possesses Ca sensitivity in its contraction (S fiber). When S fiber was treated with trypsin, its Ca sensitivity was lost (Ca non sensitive fiber : NS fiber). NS fiber was also found in some glycerinated muscle which was standing in glycerol medium at room temperature for few days (Tem-NS fiber). NS fiber contracted without Ca, and particularly, distinguished from S fiber in the rate of rise in the tension development. However, Tem-NS fiber was different from trypsinated NS fiber (Try-NS fiber) in the SDS-polyacrylamide gel electrophoretic pattern of Tn-TM bands, respectively. Moreover, S fiber was incubated at 25°C in a Ca free buffer solution to examine the effect of intrinsic protease, but did not lose its Ca sensitivity, and changed to NS fiber by addition of Ca.

These facts suggest that S fiber altered to NS fiber by digestion of Tn-TM system with some intrinsic protease, such as CANP.

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Ca^{2+} RELEASE FROM SR VESICLES DERIVED FROM LONGITUDINAL RETICULUM AND TERMINAL CISTERNAE OF SKELETAL MUSCLE.

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Fragmented sarcoplasmic reticulum from bullfrog skeletal muscle was fractionated into longitudinal reticulum and terminal cisternae fractions. Ca^{2+} preloaded in the both fractions was released by changing the medium from K-gluconate to KCl. This KCl-induced Ca^{2+} release was inhibited by sucrose and glucose without affecting potential change, but not inhibited by dantrolene sodium (DAN). Anion exchange from gluconate$^-$ to Cl^-, or exchange of both ions from K^+ and gluconate$^-$ to choline$^+$ and Cl^- caused large potential changes negative inside, but did not induce Ca^{2+} release from the both fractions. Similar results were obtained using SR vesicles which took up Ca^{2+} in the presence of DAN. KCl-induced Ca^{2+} release was not inhibited by urea and glycerol, which did not have osmotic action because of their high permeability to SR membrane. These results indicate that KCl-induced Ca^{2+} release from SR vesicles is due to an osmotic effect rather than "depolarization" of SR membrane.

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ORIGIN OF ACTIVATOR CALCIUM IN DOG CORONARY ARTERY SMOOTH MUSCLE

SUZUKI, S., FUJIEDA, N. AND SUGI, H. Dept. Pysiol., Sch. Med., Teikyo Univ., Tokyo

Physiological and cytochemical studies were carried out to obtain information about the origin of activator Ca for contraction in dog coronary artery smooth muscle. The tension development of K-induced contracture was dependent on the external Ca concentration, and suppressed by Ca-free solution, 20 mM Mn, 20 mM La, 1 mM verapamil and low pH. Since these factors are known to inhibit the Ca-influx across the muscle membrane, these results indicate the external origin of activator Ca. Cytochemical study using K-pyroantimonate also indicated that, in the resting muscle fibers, prominent pyroantimonate precipitate was localized in the caveolar lumen, but not in any intracellular structures except for the nucleus. In the fibers fixed during mechanical activity, the pyroantimonate precipitate was diffusely distributed in the myoplasm, and disappeared in the caveolar lumen. The existence of Ca in the pyroantimonate precipitate was revealed by means of electron probe X-ray microanalysis. Meanwhile, the development of contracture tension in Na-free solution indicated the presence of Na-Ca exchange mechanism. It is concluded that the mechanical activity in dog coronary artery smooth muscle is regulated by the Ca-influx from the external medium.

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BIPHASIC TIME COURSE OF POTASSIUM CONTRACTURES IN FROG SINGLE TWITCH MUSCLE FIBERS UNDER VARIOUS CONDITIONS.

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We previously reported that the time course of potassium contractures is biphasic, consisting of the initial component and the secondary component under optimal conditions; at 70-80 mM K^+, 120 mM Cl^-, using single fibers with 75-100 μm of diameters (TAKAUJI et al., Jpn. J. Physiol., 30: 205-218, 1980). In the present report, we observed that even in the potassium contractures with 190 mM K^+ the time course becomes biphasic after pretreatment of the fibers with high Ca^{++} (5 and 10 mM) or Mn^{++} (3, 5 and 10 mM) for 30 sec and after conditioning depolarization with 25 mM K^+ in the presence of Mn^{++} or Ni^{++}. We further examined the effects of divalent cations (Mg^{++}, Ni^{++}, Mn^{++} and Ca^{++}) on the initial component and the secondary component which are modified, respectively, by reduction of external Ca^{++} for 30 sec. These cations substituted for extracellular Ca^{++} to restore the tension of both components. The former results indicate that in the usually obtained monophasic contractures with high concentrations of K^+ (100-190 mM), the initial component and the secondary component are fused with each other and the latter results suggest that extracellular Ca^{++} has not direct effect not only on the initial component but also on the secondary component.

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EFFECTS OF NO_3^- ON Ca-TRANSIENT IN ARSENAZO III-LOADED TWITCH MUSCLE FIBERS.

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Intracellular changes in free Ca^{2+} concentration during contraction (Ca-transients) were recorded from twitch muscle fibers of the frog, using the metallochromic indicator dye arsenazo III. The effects of NO_3^- (twitch potentiating agent) on Ca-transients were also examined. Arsenazo III was iontophoretically injected into muscle fibers with pipette negative pulse currents of 30-50 nA over a period of 15 min. Changes in transparency at the wave lengths of 534.5 nm and 604.5 nm were detected by a pair of photomultipliers, whose output current was amplified through a cutoff filter of 80-1000 Hz, to reduce the optical noise. Usually, arsenazo signals of 32 twitches were averaged. As movement artefacts during contraction of the muscle interfere seriously with the optical recording, the muscle was obliged to be highly stretched to minimize contraction. When the muscle was stimulated at a temperature of 27°C, the arsenazo signals began to rise after 4 msec latency and reached a maximum after a further 12 msec, and then declined in nearly exponential manner. Replacing 110 mM Cl^- in normal Ringer solution with equimolar NO_3^-, both latency and time to maximum response were shortened to 2 msec and 9 msec, respectively, and maximum amplitude was increased. It is concluded that NO_3^- increases Ca^{2+} release from sarcoplasmic reticulum at an early phase of contraction.

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EXCITATION-CONTRACTION (E-C) UNCOUPLING DUE TO ACROLEIN-DTE COMPLEX IN SINGLE XENOPUS MUSCLE FIBERS, AND CHARACTERISTICS OF E-C SITES.

FUJINO, M., SATO, Y., ARIMA, T., AND TAKAI, H. (1. Dept. Physiol., Natl. Def. Med. Coll., Tokorozawa-359)

In the course of studies on possibility of chemically inhibiting E-C coupling, we found a potent inhibitory action of acrolein on E-C coupling, with additional interesting results as follows: 1. Dithiols (e.g., dithioerythritol (DTE)) form complexes with acrolein (acrotols), which have aldehyde-radicals but no SH-groups. 2. Acrotols, e.g., acrolein-DTE complex, show a complete reversible inhibiting action on E-C coupling. 3. In this case, action and resting potentials are almost completely intact, and only change in static electrical properties consists in an increase of Rm twice the normal. 4. "Creep" is removed by the complex, in presence of which a remarkable negative staircase appears. 5. Inactivation of depolarization-contraction is accelerated and activation is inhibited by the complex, which inhibits E-C coupling of tonic fibers. 6. The complex does not inhibit caffeine-RCC.

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EFFECTS OF HYDRAZINE-DERIVATIVES OBTAINED FROM NIALAMIDE ON CONTRACTILE BEHAVIORS IN AMPHIBIAN SKELETAL MUSCLE, AND EXCITATION-CONTRACTION (E-C) COUPLING.

FUJINO, M., SATO, T., ARIMA, T., AND TAKAI, H. (1. Dept. Physiol., Natl. Def. Med. Coll., Tokorozawa-359)

1. 5 mM nialamide (a hydrazine-derivative) evoke a twitch potentiation, which is accompanied by a slight change in the form of action potentials. 2. 0.1 - 1N NaOH-treated nialamide (nialamide') show an additional action, a remarkable prolongation of K-contracture, 60 min or more. 3. The prolongation is not removed by Ca-deprivation or by removing nialamide'. 4. Experiments on single fibers exhibit similar results on whole muscles. 5. Not only K-depolarization but also a train of action potentials, occasionally even one action potential, evokes a prolonged contracture. 6. A series of experiments to isolate effective principles responsible for the effects of nialamide' shows that: a. the principles isolated by means of adsorption and gel permeation chromatographic procedures are at present accompanied by contaminants; b. the principles seem to consist at least of three or four effective substances, the one prolongs K-contracture, the second has a depolarizing action, the third evokes a contracture, and/or the fourth has a twitch-potentiation action.

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ON THE CONTRACTION OF SINGLE SKELETAL MUSCLE FIBRES OF FROG DURING EXCHANGE OF THE FLUID OF TRANSVERSE TUBULES.

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Factors with respect to the process of ionic diffusion in the fluid within the transverse tubule of frog single twitch fibres during exchange of the external fluid was discussed. The discussion was focused on a possibility of flow of fluid within the transverse tubule during contraction.

An experiment was undertaken to find out any experimental evidence suggesting this possibility. This experiment needs a single fibre whose excitability is nearly the same along the whole length of fibre. Several procedures was tried to obtain such a suitable single fibre. Our experimental results suggesting this possibility have not yet been obtained.

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^{1}H-NUCLEAR MAGNETIC RESONANCE STUDY ON LACTATE AND INTRACELLULAR pH IN MUSCLE.

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High resolution proton magnetic resonance spectra of intact frog muscle can be obtained by the selective saturation of water signal with 15 min accumulation. The spectra consisted of the broad component and high resolution portion. The high resolution portion showed well-resolved signals from creatine phosphate, creatine, carnosine, and lactate. This technique was used to monitor the intracellular pH from the chemical shift of carnosine and the change in lactate content in muscle. Simultaneous spectra of ^{31}P-NMR gave the ratio of creatine phosphate to total creatine. Resting muscle under anaerobic conditions showed gradual increase in lactate content accompanied with intracellular acidification for about 5 hr and thereafter the production of lactate and the corresponding intracellular acidification were accelerated. When anaerobic muscle was electrically stimulated for 5 sec, the lactate production and the acidification were transiently accelerated. The acceleration lasted only for 15 min.

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DIAMETER DISTRIBUTION OF CENTRALLY-NUCLEATED MUSCLE FIBERS IN DYSTROPHIC MICE

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The fore and hindlimb muscles in normal and dystrophic mice (57BL/6J dy/dy) were examined histologically. The muscle specimens were 7 μm-thick cross sections of paraffin-embedded m. triceps brachii and m. biceps femoris stained with hematoxylin and eosin. Microscopic observation revealed increase in fiber-splitting, fiber necrosis, central and clustered nucleation, atrophic fiber, connective tissue, and fiber-diameter variation in the fore and hindlimb muscles of dystrophic mice. The present study focussed fiber-diameter variation and central nucleation in the muscle. The mean diameter of dystrophic muscle fibers was much smaller than that of normal ones. In a noticeable number of dystrophic fibers, central nuclei were observed, whereas they rarely observed in normal muscle fibers.

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GROWTH IMBALANCE BETWEEN BONES AND MUSCLES IN DYSTROPHIC MICE.

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Growth of bones and muscles in normal and dystrophic (C57BL/6J dy/dy) mice were investigated.

Muscle weight per leg in both the fore- and hindlegs of dystrophic mice increased up to about 4 weeks of age but thereafter stopped to increase. On the contrary, muscle weight per leg in both the fore- and hindlegs of normal mice continued to increase even after 4 weeks of age, up to about 10 weeks.

The length and breadth of bones (ulna, humerus, tibia and femur) were measured through the photomicrographs. The bones were found to grow almost normally in dystrophic mice.

The present findings clearly demonstrate that bone-muscle imbalance increases with age in dystrophic mice. Recently, we reported that a major dystrophic symptom (dragging of hindlegs) is latent in genotypically dystrophic-dwarf mice. Thus, the age-related increase in bone-muscle imbalance may play an important role in the pathogenesis of murine muscular dystrophy.

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TIME COURSES OF CHANGES IN EVOKED MUSCULAR POTENTIALS IN DYSTROPHIC, DWARF AND THEIR CROSSBRED MICE.

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Time courses of changes in muscular potentials to prolonged, sciatic stimulation of 5 per sec were studied in dystrophic (strain: C57BL/6J-dy), dwarf (strain: DW/J) and their crossbred mice of various ages. Muscular potentials, whose peak-to-peak amplitudes were measured, before 5 Hz-stimulation were taken as a control and those at various times were expressed in a percentage of the control level.

After the onset of 5 Hz-stimulation, muscular potentials were reduced to a notable extent in the strain C57BL/6J-dy normal mice and more severe depression was observed in the strain DW/J mice, while a slight decrease or even an increase in them was characteristic of dystrophic mice (2-3 months of age). Less reduction was obtained in younger mice. Muscular potentials in crossbred (non-dystrophic and dwarf or non-dwarf) mice were also rapidly and severely depressed. In contrast, the ratio in dystrophic and non-dwarf mice (2-3 months) was 95.2±4.7 % (n=11). Only two mice were probably dystrophic and dwarf, showing little reduction (24 days) and a progressive decline to 83.5 % after 5 Hz-stimulation for 10 min (98 days).

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FINE IRREGULAR MECHANICAL ACTIVITIES OF THE ISOLATED ABDOMINAL ARTERIES OF HUMANS IN VITRO.: MASUDA,Y., OKUI,K., and FUKUDA,Y.*, Dept. of Surg., *Dept. of Physiol., Sch. of Med., Chiba Univ., Inohana, Chiba.

Fine irregular mechanical activities(FMA) of the vascular wall were recorded from the ringed preparation of the isolated abdominal arteries of humans which was obtained during surgical operation. FMA could be registered without any chemical stimuli. With increment in passive tension, the amplitude and frequency of FMA were augmented. Cooling suppressed FMA and relaxed the artery. Cooled artery showed no augmentation in FMA when passive stretch was loaded. FMA and tension of the artery were recovered by rewarming. Application of norepinephrine or high potassium augmented FMA and constricted the artery. Removal of external calcium from the perfusion fluid or administration of Ca-antagonist(verapamil or cinnarizine) had no obvious effects on FMA and tension of the artery. Also, alpha blockade(phentolamine) had no clear influences on FMA.

These results suggested that FMA relates to the intracellular calcium metabolism and plays an important role to maintain the vascular tone.

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THE EFFECTS OF TETRAETHYLAMMOMIUM ON THE ELECTRICAL AND MECHANICAL RESPONSES IN THE SMOOTH MUSCLE OF GUINEA-PIG VAS DEFERENS.
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Tetraethylammonium ions(TEA) increased the rate of rise and the height of spike discharges caused by depolarizing current application both in Ca saline solution and in Sr one in the smooth muscle of guinea-pig vas deferens. The magnitude of evoked contraction was also enhanced by application of TEA into both solutions. These findings mean that TEA increases the membrane permeability of Ca and Sr ions. Meanwhile the curves of contraction in TEA contained solution showed similar figures to that in Ca solution, but the falling phase was steeper in TEA contained Sr solution than control.

The results obtained in this experiment suggest that there is a linear relation between internal Ca concentration and the amount of bound Ca on the contractile units and also the ability of pumping out system of Ca ions, but the ratio of bound ions on the contractile units to internal ion concentration may be getting down as elevation of internal value in case of Sr ions.

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SYNCHRONOUS ELECTRICAL ACTIVITY OF LONGITUDINAL AND CIRCULAR MUSCLE DURING PERISTALSIS IN THE GUINEA-PIG SMALL INTESTINE. NORTH, R. A. AND YOKOYAMA, S. Dept. of Pharmacol., Loyola Univ. Med. Center. Chicago. U.S.A.

Peristaltic reflex could be evoked by raising the intraluminal pressure. At low intraluminal pressure, intervals between spike bursts of the circular muscle were longer than those of the longitudinal muscle. The higher the intraluminal pressure, the shorter became the intervals between spike bursts in the circular muscle, until both muscle layers showed synchronous discharge of action potentials. Atropine (100 nM) reduced the excitatory effects of raising pressure on both muscles but did not abolish them. The atropine-resistant excitation of the circular but not the longitudinal muscle was reversibly blocked by exposure to substance P (100-500 nM). Chymotrypsin (200 μg/ml) reversibly abolished the atropine-resistant excitation of the circular muscle.

It was concluded that during peristalsis both longitudinal and circular muscle layers are activated synchronously: Circular muscle activation during peristalsis is not entirely cholinergic but may involve in addition a substance P-like peptide.

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KINETIC ANALYSIS OF CONTRACTION IN UTERINE SMOOTH MUSCLE.
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The isometric tension changes during tension development and relaxation were kinetically analysed on electrically evoked contractions in longitudinal muscle of estrogen-treated rat uterus. The tension development and relaxation were represented by single and dual exponential curves, respectively. These rate constants depended on temperature, but not on preparation thickness and resting load. The rate constant of tension development was independent of the change in ionic composition (Na, K, Ca and Mg) of the perfused solution. Low Na and high K decreased the relaxation rate, on the other hand, high Mg decreased it. These changes were ascribed to the change in ratio of two exponential components, but not to the change in rate constants. The results were explained by two-compartments model in which it was assumed that the rate-limiting step during tension change was Ca movements through sarcolemma and intracellular Ca store.

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CALCIUM MOVEMENTS IN THE ISOLATED SINGLE SMOOTH MUSCLE CELLS. OBARA, K. AND YABU, H. Dept. of Physiol., Sapporo Med. College, Sapporo 060.

Single smooth muscle cells were dispersed from the guinea-pig taenia coli by the digestion with 0.3% collagenase for 30 min at 35°C. Contraction of single muscle cells was induced by 4mM caffeine. The extent of contraction in Ca^{2+}-free medium was about 50% of that in 0.18mM Ca^{2+} containing medium. 1mM La^{3+}-treated single cells in 0.18mM Ca^{2+} medium contracted about 70% of that in 0.18mM Ca^{2+} medium. The movements of intracellular Ca^{2+} were monitored by chlorotetracycline(CTC) fluorescent probe. The fluorescence of the Ca^{2+}-CTC-cell system was decreased by an addition of caffeine. The single cells loaded with ^{45}Ca were washed with medium containing 10mM EGTA and then were perfused continuously on Millipore filter. ^{45}Ca in the perfusate was counted on a liquid scintillation spectrometer. The efflux of ^{45}Ca from single cells in Ca^{2+}-free medium consisted of three components. Half time of the first, second and third component was about 0.9, 4.6 and 45 min, respectively. Amount of the efflux at the third component was clearly increased by caffeine. These results suggest that Ca^{2+} which contributes to the contraction is released by caffeine from the intracellular site (especially third component) and that the decrease of the intensity of the fluorescence by caffeine may be considered to monitor the release of Ca^{2+} from the third component.

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EFFECTS OF TEA ON THE ELECTRICAL AND MECHANICAL ACTIVITIES OF THE SMOOTH MUSCLE OF PROSTATE GLAND.
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The resting membrane potential of the smooth muscle obtained from the guinea-pig prostate gland was about -50 - 60mV. The majority of cells was electrically quiescent but some cells generated action potentials spontaneously. The repetitive stimulation evoked the e.j.p.s and an action potential. The repetitive stimulation produced the phasic contraction and the response was frequency-dependent. Ad and Norad exhibited an excitatory effect on the membrane activity. The frequency and the duration of the action potential were increased of these drugs. The excitatory action of Ad on the mechanical activity was antagonized by phentolamine. TEA potentiated the mechanical response and the frequency of action potential. The action of TEA was blocked by phentolamine but not by propranolol.

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THE CONTRIBUTION OF THE NA-CA EXCHANGE TO SMOOTH MUSCLE DEPOLARIZATION INDUCED BY NA-REMOVAL. SAKAMOTO, Y. AND NASU, Y. Dept. of Physiol. Sch. of Med., Fukuoka Univ., Nishi-ku, Fukuoka

When external Na ions were completely replaced with choline, the circular muscle membrane of guinea-pig stomach was depolarized by about 20 mV and contracture developed. High concentration of external Ca- and Mg-ions inhibited the contracture, but did not affect on the membrane potential. Although the contracture was not observed in Na, Ca-free solution, the membrane depolarization was still observed. This depolarization and Na-removal depolarization were not blocked by Ca-antagonists, such as verapamil, D-600, Mn and high Ca, but the contracture was inhibited by them. The Na-removal depolarization was inhibited by reintroduction of small amount of Na ion to the Na-free solution containing high Ca ion or Ca-antagonists and first component of the slow wave was restored but not in the presence of Mn. Nitroprusside, which was an antihypertensive agent, almost inhibited the membrane depolarization and the contracture. These evidence supported the possibility that initial depolarizing phase of the slow wave may be produced by Na-Ca exchange process.

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Membrane mechanism concerning Sr^{2+}-induced activity of rat portal vein Y. Yamamoto and K. Hotta Dept. Physiol., Nagoya City Univ. Med. Sch., Mizuho-ku, Nagoya 467

The membrane activity of the smooth muscle of rat portal vein was investigated with a microelectrode in a solution in which all Ca^{2+} was substituted with Sr^{2+}. The Sr^{2+}-induced activity consisted of four components; gradual depolarization at the resting phase, initial depolarization prior to spikes, train of spikes and long-lasting plateau. The peak potential of the spike changed by 15mV for each decade change of $[Sr^{2+}]_o$, and this value was much smaller than that of a pure Sr^{2+} spike. The plateau was abolished by Na^+ deficiency or Mn^{2+}(1-2mM). 20mM-TEA increased the amplitude of the plateau. When $[Sr^{2+}]_o$ was increased, the plateau was prolonged. The initial depolarization persisted in a solution with 2mM-Mn^{2+}, and ouabain (10^{-4}M) failed to stop the rhythmical activity. These results indicate that both Na^+ and K^+ conductances are increased during the plateau and that the Na^+ current may enter through "so-called Ca^{2+} channel". And this membrane seems to have Sr^{2+}-activated K^+ conductance which concerns the falling phase of the spike.

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THE INHIBITORY ACTIONS OF CALCIUM IONS ON THE MEMBRANE CURRENTS CARRIED BY BARIUM IONS IN THE INTESTINAL SMOOTH MUSCLE. INOMATA, H. AND MIMATA, T. Dept. of Applied Physiol., Sch. Med., Tohoku Univ. Sendai-980

The interactions between Ca ions and Ba ions on the smooth muscle of guinea-pig taenia coli were studied under current-and voltage-clamp conditions, Under current-clamped condition,when Ba^{2+} concentrations were changed in range from 2.5 to 68 mM,the amplitude of the spike was increased, and the duration prolonged, proportionally to their concentrations. Ca ions at concentration of 1 or 3.5 mM added to Ba^{2+}media shortened the plateau phase of Ba-action potential and retarded its rate of rise without affecting its amplitude. Under voltage-clamped condition, Ba ions carried the early current. Inward current by Ba ions was composed of two components; one was an early fast inactivation phase, another slower inactivation one.The time constants of both phases tended to increase with increasing Ba^{2+} concentrations. In all concentrations, Ba ions reduced the late current of K ion. The degree of reduction was greater in higher concentrations of Ba ions. Adding Ca ions decreased the maximum Ba^{2+} inward current and its conductance without affecting the chord conductance for K ion, while reversal potential for Ba ions remained fixed. The degree of its inhibitory action was more prominent in lower concenrations of Ba ions. They suggest that Ca ions might suppress Ba^{2+}inward current by competiting for the same channel or binding sites or both with Ba ions.

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EFFECTS OF Cl REMOVAL ON THE MECHANICAL ACTIVITY OF RAT MYOMETRIUM.

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The effect of replacement of the external Cl with some other anions on the spontaneous activity and on the 0 Na contracture of the myometrium were studied in non-pregnant and pregnant rats. The spontaneous activity was markedly potentiated when Cl was replaced with a small anion such as NO_3, but replacement with large anions such as isethionate or sulphate suppressed the spontaneous activity. In some pregnant myometrial strips, Cl replacement with the large anions produced a sustained increase in tension. The 0 Na contracture developed slowly with some delay and the maximum tention was suppressed in the presence of the large anions. This suppression was stronger in the pregnant than in the non-pregnant myometrium. Simultaneous removal of Na and Cl (large anion replacement) potentiated the initial phase of 0 Na contracture, while after pretreatment with Cl deficient solution for 20 min, removal of Na and readmission of Cl caused the suppression of the initial phase of 0 Na contracture.

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EFFECTS OF SODIUM VANADATE ON SMOOTH MUSCLES

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Effects of sodium vanadate ($NaVO_3$) on the vas deferens, ureter, taenia coli and portal vein of the guinea-pig were studied. In the vas deferens, $NaVO_3$ of the concentration higher than $5x10^{-6}$ M caused the tension development. No obvious change in membrane potential and resis tance was observed by the drug of the concentration up to $5x10^{-4}$ M, but higher concentration of the drug often caused slight depolarization and spontaneous spike discharges. The drug potentiated the phasic and tonic contraction of K-contracture. The former was caused by the change in membrane excitability. When the drug was applied in the course of the tonic contraction of K-contracture, the elevation of the tension was observed. The tension development was sensitive to Ca but about half of the tension remained even after prolonged treatment with Ca-free solution or in the presence of verapamil. Similar effects were observed in taenia coli, ureter and portal vein. Cooling-induced tension development of depolarized vas deferens and ureter was also potentiated by the drug and cooling-induced relaxation of taenia coli was reversed into contraction. In the polarized preparations or in the absence of Ca, cooling-treatment caused relaxation in all types of smooth muscles tested. Thus, the potentiating effects of $NaVO_3$ may be explained partly by the changes in membrane excitability and by the inhibition of Ca extrusion, and mainly by intracellular Ca release.

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EFFECTS OF PGD_2 AND PGF_{2a} ON THE SMOOTH MUSCLES OF GUINEA-PIG COLON.

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The effects of PGD_2 and PGF_{2a} on longitudinal and circular muscles(10mm long,1mm wide) dissected from the proximal colon of guinea-pig were investigated. PGD_2,PGF_{2a} (1 nM-10 μM) produced concentration-dependent contractions in both muscles. When the contraction induced by acetylcholine(10 μM) was taken as 100 %, PGD_2 and PGF_{2a} (10 μM) induced contractions were 78 ± 7.7 %,67.5 ± 6.1 % in circular muscle, and 50.1 ± 5.1 %,66.9 ± 4.9 % in longitudinal muscle, respectively. PGD_2 and PGF_{2a} induced contractions in both muscles were unaffected by atropine(1 μM), but were slightly inhibited by tetrodotoxin(1 μM) in longitudinal muscle, indicating that these PGs may mainly act on both muscle cells and partly act through noncholinergic intrinsic nerves in longitudinal muscle. A Ca-antagonist, verapamil(10 μM),almost completely inhibited PGD_2 and PGF_{2a} induced contractions in longitudinal muscle, and slightly inhibited these contractions in circular muscle. These results suggest that in longitudinal muscle PGD_2 and PGF_{2a} induced contractions may depend on an increase in influx of Ca ions, but in circular muscle these PGs may largely depend on a verapamil insensitive utilization of Ca ions.

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METABOLIC FACTORS IN THE SPONTANEOUS ACTIVITY IN THE STOMACH MUSCLE.
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The circular muscle of the guinea-pig stomach has a regular(4-6/min) spontaneous activity.Mitochondrial inhibitors,such as carbonil cyanide m-chlorophenylhydrazone or rotenone reduced the tension development, but they did not significantly affect the frequency of the activity. The suppression of the mechanical activity was stronger than that of the electrical activity (the slow wave). Fluoroacetate decreased the frequency with some suppression of the tension and this effect remained after washout.Removal of glucose slightly reduced the frequency after 3-5 hours and fluoroacetate became very effective in reducing the frequency in the absence of glucose. Pyruvate or 3-hydroxybutyrate increased the tension, but not the frequency in glucose free solution, while glucose tended to produce the recovery both in tension and frequency. It is tentatively suggested that the TCA cycle mainly determines the tension development through ATP production and some step in anaerobic glycolysis controls the frequqncy of rhythm through interaction with the plasma memberane.

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EFFECTS OF THYROTROPIN-RELEASING HORMONE (TRH) ON GUINEA-PIG STOMACH MUSCLE.
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The antrum strips were used for experiments. In longitudinal muscle TRH mainly increased the muscle tonus dose dependently (10^{-9}-10^{-6}M), and in circular muscle it enhanced the amplitude of spontaneous contraction dose dependently (10^{-8}-10^{-6}M). These effects of TRH were not affected C_6 (10^{-4}M), enhanced by physostigmine (10^{-7}M), but abolished by atropine (10^{-7}M) and tetrodotoxin (3×10^{-7}M) in both preparations. The longitudinal muscle is more sensitive to TRH and to acetylcholine than the circular muscle. After treatment of atropine (10^{-7}M), TRH decreased tonus and suppressed the contractions in longitudinal muscle, but not in circular muscle. These effects were not affected by guanethidine (10^{-6}M), phentolamine (10^{-6}M), propranolol (10^{-6}M) and C_6 (10^{-4}M), but abolished by TTX (3×10^{-7}M).

It could be concluded that TRH enhanced the acetylcholine release from cholinergic neurones on both preparations, and stimulated the non-adrenergic inhibitory neurones to decrease tonus and suppress contractions in the longitudinal muscle.

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THE RELATION BETWEEN PRESSURE AND VOLUME IN THE RABBIT LEFT VENTRICLE. OKUYAMA,H., OKADA,T. AND MASHIMA,H. Dept. of Physiol., Sch. of Med., Juntendo Univ., Tokyo

The dynamic properties of rabbit left ventricle were examined and compared with the corresponding properties of isolated cardiac muscle. Maintaining the coronary circulation with oxygenated Krebs solution at 30-32°C, the heart was isolated and stimulated electrically at 120/min. A balloon was inserted into the ventricle and the volume of it was controlled with a shaker. (1) Pressure-volume relation of the left ventricle was obtained, measuring the systolic and diastolic pressures at various volumes. The developed pressure increased with increasing volume and reached the peak at about 2.5 ml, then decreased very gradually with further increase in volume. (2) When the volume change was made at a given pressure during systole, the pressure curve became horizontal if the velocity of volume change was appropriate. The relation between these pressure and velocity thus obtained was described with a hyperbolic equation. (3) Pressure-stiffness relation of the left ventricle was also determined, measuring the pressure change to a sudden small volume change at various pressures. This relation was almost linear. All these relations are almost similar to those of isolated cardiac muscle. Therefore, the dynamic properties of left ventricle could be explained from the corresponding properties of cardiac muscle.

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REST POTENTIATION AND REST DECAY AT VARIOUS LENGTHS IN THE CAT PAPILLARY MUSCLE
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If a cardiac muscle is regularly stimulated and then a rest is applied, the developed tension of the first beat after the rest is either greater (rest potentiation) or smaller (rest decay) than those of regular beats. Positive inotropic interventions such as high calcium concentration and high-frequency regular stimulation augment rest potentiation and retard rest decay. Recently cardiac muslce length has been suggested to affect muscle contractility. We therefore examined effects of muscle length on the rest potentiation and rest decay of isolated cat papillary muscles (n=12). Rest potentiation was augmented and rest decay was delayed at Lmax as compared to 90%Lmax. The delayed rest decay at the longer muscle length may be explained by the shift of the length-tension curve associated with a calcium concentration change. However, to account for the augmented rest potentiation, other factors such as delayed relaxation and increased calcium release should be considered. The similarlity of the augmented rest potentiation and delayed rest decay between the length increase and the positive inotropic interventions suggests the contractility is higher at a longer muscle length than at a shorter length.

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STRESS-STRAIN RELATION OF LEFT VENTRICULAR MYOCARDIUM AND STRESSES IN LEFT VENTRICULAR WALL DETERMINED FROM ISOVOLUMIC VENTRICULAR PRESSURE-VOLUME RELATION. NAKAMURA, T., OKINO, H. and ABE*,H. Dept. of Physiol., Sch. of Med., Tokai Univ., Isehara, Dept. of Mech. Eng*, Tohoku Univ., Sendai.

The stress-strain relation of systolic cardiac muscles which constitute the left ventricular wall of canine hearts was derived only from pressure-time relationships at different ventricular volumes of isovolumic beat by using a largely deformable thick spherical shell model as a first approximation. The strain energy function being independent of the magnitude of forces generated by contraction was found to be uniquely determined by assuming those forces caused by an eigen stress. The result based on this assumption was verified by several pressure-volume relationships obtained experimentally. The distribution of radial and tangential stress through the left ventricular wall were calculated. The magnitude of tangential stress was very large at endocardial surface and decreased rapidly toward epicardial surface.

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RELATIONSHIPS BETWEEN SURFACE LAYER ATP AND CONTRACTION IN ISOLATED, SUPERFUSED CANINE VENTRICULAR PAPILLARY MUSCLE.

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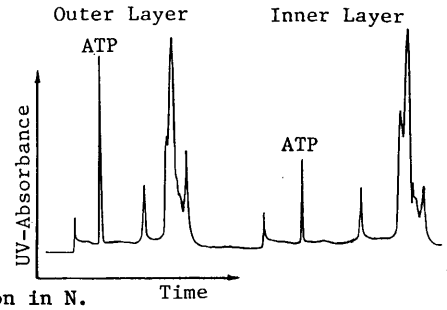
The relationships between contractile tension and concentration and content of ATP in canine papillary muscle were examined in normoxic (N)(PO_2=300mmHg) and hypoxic (H) (100mmHg) conditions, using isotachophoresis. After the muscles were exposed to either condition for 30 min, they were clashed to pieces in liquid N_2, followed by extraction in methanol-EDTA solution. H markedly depressed ATP content and concentration. In N there was a positive correlation between the tension and ATP concentration but not in H. Tension correlated with total ATP content positively in N, but negatively in H. ATP concentration tended to be inversely proportional to the muscle weight, suggesting the outer layer of the muscle contains more ATP than the inner layer. In fact this was the case, as shown in Figure. Results indicate that superficial ATP mostly plays a role in initiation of the contraction in N.



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EFFECTS OF CORONARY DILATORS ON THE CHARACTERISTICS OF MYOCARDIAL MECHANICS IN ACUTE ISCHEMIA AND REPERFUSION.

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The characteristics of myocardial contraction and relaxation in acute ischemia and reperfusion were studied using an isolated non-working heart model of canines.Continuous administration of nitroglycerin(NG) or prostacyclin(PG) prior to acute ischemia did not induce changes in peak tension,time to peak tension,max dF/dt,diastolic tension,time to $T_{1/2}$ and negative dF/dt.Verapamil(VER) decreased peak tension and negative dF/dt.In acute ischemia,both NG and PG didn't induce significant changes in the time course of peak tension,time to peak tension and max dF/dt compared with the control,though NG tended to increase negative dF/dt.VER accentuated the decreases of peak tension and negative dF/dt in acute ischemia.In reperfusion,PG and NG inhibited the prolongation of time to $T_{1/2}$.VER failed to restore peak tension and time to $T_{1/2}$.

These results indicate that (1) NG tends to accelerate the relaxation during ischemia and reperfusion, (2) PG does the relaxation during reperfusion and (3) VER attenuates the relaxation accompanied with decreased peak tension during reperfusion.

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INTRACELLULAR CA^{++} TRANSIENTS AT DIFFERENT MUSCLE LENGTHS IN MAMMALIAN CARDIAC MUSCLE.

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Length-tension relationship in skeletal muscle is explained by thick and thin filaments overlap. However, there is considerable evidence that activation factors have an important role in the length-tension relationship of cardiac muscle. In this study, intracellular free calcium concentration was measured using calcium-sensitive photoprotein aequorin at different length in rat and cat ventricular muscles. After an increase in muscle length, the developed tension increased immediately and then showed a slow rise over a period of minutes. The peak intracellular free calcium concentration was initially unchanged but then showed a slow increase with a time course similar to that of the slow tension change. Immediately after an increase in muscle length the calcium transient was abbreviated. These changes of Ca^{++} transient can be explained by the change of the binding constant of troponin for calcium.

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THE RATES OF CALCIUM RELEASE AND UPTAKE DURING CONTRACTION ESTIMATED BY THE CARDIAC MUSCLE MODEL. MASHIMA, H. AND KABASAWA, K.* Dept. of Physiol. and *Div. of Inf. Sci., Sch. of Med., Juntendo Univ., Bunkyo-ku Hongo, Tokyo

A mathematical model for the cardiac muscle contraction was developed, assumming the attachment and detachment cycle of the cross-bridge is activated by the internal Ca concentraction and the rate of the cycle depends on the sliding velocity of myofilaments. The inputs of the model are the rates of Ca release and uptake, and the output is the tension curve which reflects the number of activated cross-bridges. The variables are factored into a series of realizable functions and most parameters were determined from the dynamic constants of the force-velocity relation for the frog ventricular muscle at 20°C. Using this program, the rates of Ca release and uptake were calculated for a given experimental tension curve by a computer, PDP 11/60, varying the input parameters so as for the output tension curve to fit the experimental curve. When the tension was increased by increasing the initial muscle length, the rate of Ca release increased and that of uptake decreased. At higher external Ca concentrations the similar changes in the input paramerters were observed. In the presence of 5×10^{-6} g/ml adrenalin the duration of activation was markedly prolonged, while the rates of Ca release and uptake show little change.

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OPTICAL DEMONSTRATIONS OF EXCITATION SPREAD IN EARLY EMBRYONIC CHICK HEART USING A POTENTIAL SENSITIVE DYE. FUJII, S., HIROTA, A., SAKAI, T., KAMINO, K. Dept. of Physiol. Tokyo Med. and Dent. Univ. Sch. Med. Bunkyo-ku, Tokyo.

The conduction of spontaneous action potentials in the 7-9 somite embryonic chick hearts was monitored optically using a potential-sensitive merocyanine-rhodanine dye. Spontaneous optical action signals from 5-12 different regions of the primitive heart were recorded simultaneously. Usually, in the 7 somite to the early period of the 9 somite stage, (i) excitatory waves conducted radially over one side of the prebeating heart, at a uniform rate; (ii) the uniform radial propagation of excitation was distorted within the primordial fusion line at the midline of the heart; and (iii) once the fusion line is passed excitation progressively and radially propagated in the opposite side of the heart at a uniform rate. These observations suggest that electrical coupling among the cells within the primordial fusion line is poor during the 7-9 somite stage. The direction of conduction of excitation could be flexibly changed. Such a nature was tentatively recognized in experiments using preparations of which the site of the pacemaking area translocated from the left to the right pre-atrium or vice versa, or using micro-surgical division. Thus, we conclude that the specialized conduction system has not been differentiated in the embryonic heart at the early phases of cardiogenesis.

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IONIC BASIS OF SPONTANEOUS ACTION POTENTIAL IN EARLY EMBRYONIC CHICK HEART MONITORED BY OPTICAL POTENTIOMETRIC DYE. SAKAI, T., FUJII, S., HIROTA, A., KAMINO, K. Dept. of Physiol. Tokyo Med. and Dent. Univ. Sch. Med. Bunkyo-ku, Tokyo.

Using an optical method for monitoring membrane potential, spontaneous action potentials in the 7-9 somite embryonic precontractile chick hearts were measured. The optical action potential in the 7-9 somite embryonic heart was lacking 'phase 0' and 'phase 1' attributable to the fast Na^+-current. The embryonic precontractile heart continued to generate spontaneous action potentials in a Na^+-free solution or in the presence of tetrodotoxin. Such an action potential was blocked by adding Co^{2+}, Mn^{2+}, Ni^{2+}, La^{3+}, D-600 or GEDTA, and the frequency, the amplitude, and the rate of rise of the spontaneous action potentials were depended closely upon the external Ca^{2+}-concentration; reducing the external Ca^{2+}-concentration resulted in suppression of both the *excitability and spontaneity*. From the above results, we concluded that the spontaneous action potential in the early phases of cardiogenesis is characterized as a Ca^{2+}-dependent action potential. In addition, the rate of diastolic depolarization decreases with a reduction in the external Ca^{2+}-concentration. This suggests that the low Ca^{2+} induced decrease in the frequency of recurrence of the spontaneous action potential in the embryonic precontractile heart is closely related to the decrease in the rate of diastolic depolarization.

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OPTICAL INDICATION OF SPATIAL SPREAD OF FORMATION OF INITIAL E-C COUPLING IN EARLY EMBRYONIC CHICK HEART. HIROTA, A., SAKAI, S., FUJII, S., KAMINO, K. Dept. of Physiol. Tokyo Med. and Dent. Univ. Sch. Med. Bunkyo-ku, Tokyo.

The spatial spread of spontaneous action potentials and the coupled contraction in early embryonic chick heart, at the time of the initiation of beating, was measured optically using a potential-sensitive merocyanine-rhodanine dye. Voltage dependent light-absorption signals from eight different regions of the embryonic heart were recorded simultaneously. The absorption signals were well synchronized among the eight different regions. The contraction of the early embryonic heart was also monitored optically. Changes related to contraction could be distinguished easily from optical signals resulting from the membrane potential change. In a dyed preparation which have small contractions, the optical signal consists of two components: the first component (early phase) corresponds to absorption signal accompanying action potential, and the second component (later phase) due to the contraction. The spread of the initial contraction of the embryonic heart was detected during the middle and later periods of the 9 somite stage of development. The initial contractions were limited to the cono-ventricular part of the heart and entirely confined to its right side. These results display that subcellular and/or molecular system(s) related to E-C coupling process is completely formed first in the right cono-ventricular area at 9 somite stage of development.

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ELECTRICAL COUPLING IN PAIRED VENTRICULAR CELLS of GUINEA-PIG HEART KAMEYAMA, M. and IRISAWA, H. Natl. Inst. Physiol. Sci.

Electrical coupling between paired ventricular cells, dissociated from guinea-pig heart by collagenase treatment, was investigated with two microelectrodes. Coupling ratio, membrane input resistances and capacitances, and junctional resistance were calculated from deviation of potentials evoked by current injection during either of the cells was voltage-clamped. These analyses revealed that: (1), About 70% of the tested cells showed coupling ratios larger than 0.8, and coupling resistances between 0.3 - 3 MOhm. (2), Current-voltage relation of the junctional pathway was linear within tested voltage range (±10 mV), and its conductance was independent of the membrane potential. (3), Reduction of $[Na^+]o$ (to 40 - 10 %) or application of strophanthidin (10 μM), both of which was known to increase $[Ca^{2+}]i$, reduced the junctional conductance signifiantly. It was concluded that the paired ventricular cell was connected with low-resistance pathway, and its conductance was probably modulated by $[Ca^{2+}]i$.

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TRANSIENT DEPOLARIZATION AND SPONTANEOUS MINIATURE VOLTAGE FLUCTUATIONS IN SINGLE VENTRICULAR CELLS
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Under the influence of digitalis, single ventricular cells exhibit transient depolarization (TD) after a train of driven action potentials, or transient inward current (TI) after a depolarizing clamp pulse. TD or TI was abolished by intracellular injection of EGTA or by superfusion of 5mM caffeine. TD was elicited even in the control Tyrode solution by $CaCl_2$ injection or augmented by cAMP injection. Along with TD or TI, digitalis promoted spontaneous miniature fluctuations in membrane potential or current. Their power spectra showed peaks at frequencies ranging from 2 to 7 Hz, which coincided well with that of repetitive TD. The fluctuations were eliminated by EGTA and decreased in amplitude by 5mM caffeine with a shift toward higher frequencies. Depolarization of the membrane caused a shift of the spectrum peak toward higher frequencies. These results suggest that an oscillatory release of Ca from intracellular storage sites is the common basis underlying both the transient events (TD or TI) and spontaneous fluctuations in membrane potential or current.

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The reevaluation of the pacemaker i_{K2} current in cardiac Purkinje fibers.

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The nature of the pacemaker i_{K2} current was studied in sheep cardiac Purkinje fibers using conventional two microelectrode voltage clamp technique. Pacemaker i_{K2} current completely disappeared in the presence of 1 mM Cs, and the steady state current voltage relationship(CVR) shifted to the outward direction. The shift was roughly equal to the amplitude of the i_{K2} measured at each membrane potential in the pacemaker potential range. In the presence of 1 mM Ba, neither the reversal potential nor the inward going rectifier property could be detected. Lidocaine decreased the i_{K2} with little effects on its kinetics. In the presence of lidocaine(1.85×10^{-5} M), the steady state CVR shifted to the outward direction although instantaneous CVR did not show any change. The suppressive effect of lidocaine on i_{K2} was minimum around -60 mV, but it became maximum around -100 mV where $s_{\infty}=0$ according to the conventional interpretation. Thus the present results strongly suggest that the pacemaker i_{K2} current is not a pure outward K current but inward current which may be activated by hyperpolarization similar to i_f reported in the rabbit sino-atrial node.

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OPTICAL DEMONSTRATIONS OF ARRHYTHMIC EXCITATION USING VOLTAGE-SENSITIVE DYES.

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Ring shaped preparations of bullfrog atrium (size 2.0 x 1.5 cm) containing the physiological obstacle (sinus venosus opening) were used to study circus movement tachycardia (CMT) using voltage-sensitive dyes (Dye XVII and NK2761). The preparation stained with dyes was exposed to Ca^{2+} free solution for eliminating the artifacts of movement. Optical signals were recorded from eight different regions of the preparation to monitor spread of the activation wavefronts. A basic stimulus resulted in two activation wavefronts which colloided at the opposite side of a stimulating site. CMT was initiated by the application of an appropriately timed single premature stimulus having echo zone (mean 30 $\pm$ 15 msec) and also terminated by a properly timed stimulus or allowed to terminate spontaneously. The rate of tachycardias (from 136 to 480 beats/min) was closely related to the conduction velocity of circulating impulse (from 0.03 to 0.17 M/sec). The results show that a circus movement can easily initiated and monitored in a small preparation of bullfrog atrium containing an anatomical obstacle. Optical recording of action potentials in many different portions of the heart is useful technique to analyse arrhythmic excitation.

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SLOW INWARD CURRENT AND INTRACELLULAR Ca IONS IN A SINGLE VENTRICULAR CELL.

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Ca-current dependent inactivation of slow inward current (i_{si}) in guinea pig ventricular cell was studied. The experiment was done on a voltage-clamped, internally perfused, isolated ventricular cell. The inactivation of i_{si} was devided into two components(fast and slow). When $[Ca]_o$ was increased from 1.8 to 5.4 mM, the fast component became faster, whereas it became slower by the decrease of $[Ca]_o$ to 0.01 mM. The fast component also became slower by the increase of $[Ca]_i$. The amount of steady state inactivation, recorded by substituting $[Ca]_o$ by $[Sr]_o$, greatly reduced, comparing to that recorded in $[Ca]_o$. However, the amount of the steady state inactivation in $[Sr]_o$ was not significantly affected by the increase of $[Ca]_i$. These findings suggested that the Ca ion gradient between outside and inside of the membrane was a major factor to determine the amount of inactivation. Therefore, it was concluded that the amount of Ca ions passing through i_{si} channel might decide the degree of the inactivation of this channel.

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Na-Ca EXCHANGE TRANSPORT AFFECTS HOLDING CURRENT AS WELL AS INWARD CURRENT ACTIVATED BY HYPERPOLARIZATION IN MYOCARDIUM? AN EXAMINATION ON THE BULLFROG ATRIAL MUSCLE. GOTO, M., HYODO, T. AND URATA, M. Dept. of Physiol., Faculty of Medicine, Kyushu University.

The membrane currents related to Na-Ca exchange diffusion were studied on the atrial muscle bundle under voltage clamp with double sucrose-gap technique. Na-deficient Tris Ringer (10-30 mM Na) produced a distinct outward current, a decrease in membrane conductance, and a contracture. These effects were observed in the presence of TTX (1.5×10^{-6} M), Mn (4 mM), ouabain (10^{-6} M) and even in Ca-free EGTA (10-100 μM) media. The data strongly indicated that the outward current resulted from a decrease of the Na inward current which related to the electrogenic Na-Ca exchange. When long hyperpolarizing pulses (5 sec, 40-100 mV) were applied in the presence of Ba (0.2 mM), an inward current was activated being accompanied by an increase in conductance and relaxation of tension. The hyperpolarizing response was also observed in the presence of TTX, Mn, ouabain and in the absence of Ca, but disappeared in Na-free Ringer which concomitantly produced the outward shift of holding current. Thus, the inward current response was estimated to have some relation with the electrogenic Na-Ca exchange.

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RELATIONSHIP BETWEEN Na-Ca EXCHANGE AND DELAYED OUTWARD CURRENT IN MYOCARDIUM; AN ANALYSIS ON THE BULLFROG ATRIAL MUSCLE. GOTO, M., HYODO, T. AND FUJINO, T. Dept. of Physiol., Faculty of Medicine, Kyushu University.

Possible effect of Na-Ca exchange on the delayed outward current was investigated on the atrial muscle bundles under voltage clamp with double sucrose-gap technique. Instantaneous outward current (I_{K1}) decreased in Ca-free, while it increased in Na-deficient conditions. Activation of delayed outward current (I_x) became faster in Na-deficient media, but the amplitude of fully activated I_x diminished markedly (52 % in 10 mM Na). Contrarily, in Ca-free Ringer the activation was delayed, but the amplitude of fully activated I_x was almost unaltered. The current tail was composed of two exponentially declining components, and the slower (I_{xa}) of two components was suppressed in Ca-free, while the faster (I_{xs}) was diminished in Na-deficient media. Exponential tension decays after depolarizing pulses were analysed simultaneously in these conditions. The main relaxation phase was found to retard markedly in Na deficient media, and the retardation occurred concomitantly with the disappearance of I_{xs}. These observations strongly indicate that the Na-Ca exchange transport affects the instantaneous outward current as well as the delayed outward current.

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DEPENDENCE OF SLOW CHANNELS INACTIVATION ON Ca INFLUX THROUGH THE CHANNELS. OCHI, R., Dept. of Physiol., Sch. of Med., Juntendo Univ., Tokyo

In ventricular muscles the inactivation of the Ca-dependent slow inward current(Is) becomes slower with the increase in depolarization in the positive potential ranges, and it is responsible for the long plateau of action potentials. The membrane potential in guinea pig papillary muscle was controlled by a single sucrose gap voltage clamp method. Is was elicited by depolarization from a holding potential of -40mV and estimated by subtracting cobalt-resistant current. Inactivation produced by various conditioning pulses(P1) was examined by Is elicited by a test pulse(P2) to 0-10mV. 50msec P1 to 0-20mV preceding P2 by 100msec depressed test Is to about 50%. The depression was smaller after more positive P1 as well as after less positive P1. Thus the inactivation produced by P1 was roughly in parallel with the amplitude of Is elicited by P1. Although adrenaline increased and acetylcholine decreased Is amplitude , respectively, they did not affect the time course of Is decay and the efficacy of P1 to produce inactivation. 5 and 9 mM Ca increased the amplitude of Is , accelerated the rate of decay and inactivated test Is more markedly. It was suggested that the increase in the Ca concentration near the Is channels within the cell might produce the inactivation of the Is channels.

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Na^+-DEPENDENCE OF OSCILLATORY AFTERPOTENTIALS IN MUSCLE FIBERS

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Oscillatory afterpotentials (OAPs) and aftercontractions (ACs) were observed when ventricular muscle fibers were exposed to the K^+-free, high-Ca^{2+} solution. To examine the mechanism of OAPs, Na^+-dependence of these activities was studied using dog and guinea pig ventricular muscles. Both OAPs and ACs were easily and reversibly abolished by the application of K^+ or Rb^+. The threshold concentrations to achieve a complete abolition of them were almost equal with $(K^+)_o$ and $(Rb^+)_o$, and were 1.4 mM. $(Cs^+)_o$ and $(Li^+)_o$ were also effective to suppress them but the threshold concentrations were 7.2 mM with $(Cs^+)_o$ and 30 mM with $(Li^+)_o$. When preparations were pretreated with 10^{-5} M ouabain, the application of 50 mM-Li, which were effective enough to eliminate OAPs and ACs, did not cause any disappearance of them. When $(Na^+)_o$ were varied, the amplitude of OAPs increased after a transient deppression with the higher $(Na^+)_o$ and decreased with the lower $(Na^+)_o$ after a transient augmentation. The application of 1 mg/L tetrodotoxin caused suppression of both OAPs and ACs with rate-dependent manner. The current application during the development of OAPs revealed that the activities were voltage-dependent being manifest at potential levels between -90 mV and -70 mV. These results suggest that OAPs develop at conditions of the Na^+-pump blockade and they may be caused partially by an influx of Na^+ ions.

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OVERDRIVE INDUCED CHANGES OF MEMBRANE POTENTIAL AND RELATED CURRENT COMPONENTS IN BULLFROG ATRIAL MUSCLE.

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Effects of high rate stimulation on the membrane potential and current components of the atrial fibers were studied with double gap method. Postdrive hyperpolarization was markedly depressed in ouabain and K-free Ringer, and completely eliminated by Na-lack media. The time course of the basal membrane potential changes during and after overdrive was successfully analysed into four exponential components. To clarify their natures, voltage clamp experiments were done imposing depolarizing pulses (500 msec) on the atria at 1 Hz for ~2 min. The outward current (I_o) at the end of pulse gradually increased and the time course of the increase appeared in two exponential components. The basal current level immediately before the pulses shifted inward with a single exponential time course, reflecting the late component of I_o while the early component diminished. The fast inward current gradually decreased and approached the asymptote within 1 min. When the membrane potential was clamped at the original resting potential after overdriving, a slowly decaying outward current appeared following a single exponential time course. These results indicate that I_x-activation, K-accumulation, Na-K pump activation and deactivation are responsible for the appearance of four components of the membrane potential changes during and after overdrive.

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EFFECTS OF INTRACELLULAR pH ON THE ELECTRICAL ACTIVITY OF SINGLE VENTRICULAR CELL.

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In order to examine the effects of intracellular pH on the electrical activity of cardiac cells, acidic or alkaline solutions were injected into single isolated ventricular cells of guinea-pig heart. Acid injection shortened the action potential mainly through decreasing i_{si}, and sometimes caused oscillatory transient inward current. Alkali injection showed opposite effects on the action potential and i_{si}, and suppressed the oscillatory TI current which was evoked by 5.4 mM Ca-strophanthidin containing Tyrode's solution. The comparison of the effects of alteration in $[H]_i$ and those of $[Ca]_i$ on the action potential and the time course of i_{si} suggests that a combination of a direct effect of manupulation of $[H]_i$ and an effect due to the changes in $[Ca]_i$ secondary to proton injections causes the above electrical changes of single ventricular cells.

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EFFECTS OF CO_2 ON ELECTRICAL ACTIVITIES OF MAMMALIAN MYOCARDIUM (III).
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The isolated right ventricular papillary muscle of canine heart were used in this study. A depolarization of the membrane was induced as increase in PCO_2, such as 10.3mV at $CO_2$20%, 77.3mV (in average) 100%. It was weakened by $[Cl^-]_o$-free (replaced by isethionate), DNP and higher $[K^+]_o$. Input resistance (R_o) was raised initially, and decreased later as the depolarization was proceeded. A prolongation of the action potential duration (APD_{50}) induced as increase in PCO_2 showed maximum value about 1 min after a load of CO_2 and then restored gradually. It and its restoration was enhanced and delayed by $[Cl^-]_o$-free and DNP. It was reduced by higher $[K^+]_o$. Withdrawal effect of NH_4Cl lengthened APD_{50} in Tris-buffer solution. Reduction of $[HCO_3^-]_o$, titration with HCl and short lasting anoxia have no effects on the three parameters. Acetazolamide abolished the above effects of CO_2. These results suggest that such effects of CO_2 are due to changes of gK and gCl caused by a raise in $[H^+]_i$ and by the ensuing buffer action on the intracellular acidosis. $(Cl^-)_i$-$(HCO_3^-)_o$ exchange mechanism plays an important role in the buffer process.

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DUAL EFFECT OF HYPERTONIC SOLUTION ON THE ACTION POTENTIAL DURATION IN MAMMALIAN CARDIAC MUSCLE.
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When guinea-pig ventricular muscle is exposed to hypertonic Tyrode solution (HT), the action potential duration (APD) initially prolongs and then shortens progressively. The present study was undertaken to investigate the mechanism for the above phenomenon. The late shortening of APD developed only slightly in HT when the muscle was stimulated at low rates (e.g. 1/min) or when the fluid temperature was low (~26°C), whereas APD rapidly shortened in HT when $[Na]_o$ was reduced or when $[Ca]_o$ was raised. However, the resting potential and the maximum rate of rise of the action potential showed only a slight change even when APD was rapidly shortening in HT. The initial prolongation of APD appeared to be not directly influenced by the above changes in the experimental condition. In partially depolarized preparation, HT increased the duration of Ca-dependent slow action potentials without causing any parallel increase in their upstroke velocity. From these and other relevant results, it was postulated that the initial prolongation of APD was directly related to the development of cell dehydration, probably to the increase in $[K]_i$, and that the late shortening of APD was due to an internal accumulation of Ca ions that occurred secondarily to the developed cell dehydration. In these changes in APD, the slow Ca current seemed to play little or no role.

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CHANGES OF INTRACELLULAR CA^{++} TRANSIENT IN RAT VENTRICULAR MUSCLE INDUCED BY CAFFEINE.
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The action of caffeine on the skeletal and cardiac muscles has been widely accepted as a potentiator of contraction acting on the SR. In rat ventricular muscle, caffeine (0.5-10mM) could induce contracture but inhibited twitch response after initial slight increase of contraction. This inhibition was followed by the slight recovery over a period of minutes. In this study, inhibitory action of caffeine on the rat ventriculrar muscle was examined by measuring $[Ca^{++}]i$ using aequorin. Immediately after application of caffeine (0.1-1 mM), peak of Ca^{++} transient increased slightly and persisted. However, developed tension decreased gradually. At more than 1mM(up to 5 mM), peak of Ca^{++} transient decreased with prolongation of time to peak light and decay of light. Developed peak tension diminished, and duration of contraction and action potential was prolonged. Caffeine discharge Ca^{++} from SR and subsequently may deplete the intracellular Ca^{++} store.

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ROLE OF THE HYPOTHALAMUS IN THE TOXIC CARDIOVASCULAR ACTIONS OF DIGITALIS IN THE RAT.
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The role of the hypothalamus in the resistance to the arrhythmogenic effects of digitalis was investigated in the rat which is a digitalis-resistant species. 27 male Wistar rats, 9-19 weeks old (250-515g), were classified into 3 groups, the 1st was a group without transection in any levels of brain (n=10), the 2nd was a group transected below the hypothalamus (n=10), and the 3rd was a group transected above the hypothalamus (n=7). After fixing the rat's head in a stereotaxic frame under urethane anesthesia (1.0-1.2 g/kg, i.p.), the brain was exposed and transected at the level below or above the hypothalamus according to the atlas by Albe-Fessard et al. The following doses of ouabain were given i.p. at 30 min. intervals ; 1,3,5,10,15,20,25,30, 35 and 40 mg/kg. ECG records were obtained from lead II . In the 1st group, doses producing ventricular arrhythmias and lethal doses were 20-40 and 25-40 mg/kg, respectively. Transection below the hypothalamus decreased the arrhythmogenic and lethal doses to 1-5 mg/kg. On the other hand, transection above the hypothalamus showed no significant changes of the doses compared with the 1st group. These results suggest that the hypothalamus has a role in modulating the toxic cardiovascular actions of digitalis.

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EFFECT OF GRAYANOTOXIN (GTX) ON THE RABBIT SINO-ATRIAL (S-A) NODE CELLS.
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GTX has been known to increase the membrane permeability to sodium ion specifically. The administration of GTX (10μM) on the rabbit S-A node cells produced sinus arrest. Following application of tetrodotoxin (TTX) (1μM) on the top of GTX, these silent S-A node cells resumed spontaneous beating. The ionic mechanism of this sinus arrest was studied by two microelectrode voltage clamp technique on a small piece of S-A node preparation (250x250μm). In the presence of GTX, the maximum conductance of the slow inward current (Is) was decreased, while the outward current (Ik) and the inactivation process of Is did not change. When S-A node cells regained spontaneous activity by the administration of TTX, these effects persisted. Significant increase in holding current at -50 mV between control medium and GTX medium was observed. The increase in holding current disappeared under the action of TTX. The slope conductance at -50mV in GTX medium was estimated to be 1.7 times larger than that in control medium. After abolishing the commitment of Is to holding current by the application of D-600 ($1x10^{-7}$g/ml), GTX depolarized the membrane almost the same as that in quiescent S-A node cells. Subsequent application of TTX abolished the depolarizing effect of GTX in both cases. Therefore, these findings suggested that increase in holding current, presumably due to increase in background current carried by sodium ion, played a major role in sinus arrest.

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AFTER-POTENTIATION OF THE MYOCARDIAL CONTRACTILITY BY LANTHANUM AND ITS MODE OF ACTION
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La^{3+} (0.1-0.2 mM) showed a marked, long-lasting twitch potentiation during wash in both bullfrog's ventricle and atrial as well as papillary muscle of guinea-pig. The participation of endogeneous catecholamine release is unlikely since the positive inotropic effect was affected neither by alpha nor beta adrenergic blocker. The potentiation was not accompanied with any increase in the action potential plateau, suggesting the independence of the phenomenon on the slow Ca current. Effects of increasing or lowering the external sodium concentration were strongly depressed. In addition, Na-free contracture was augmented during both La^{3+} perfusion and wash. Electron microscopic study revealed that La-deposits were not completely removed from the myocardial cell surface during wash. These findings suggest the possibility that an elevation of the intracellular Ca level due to an inhibition of Na-Ca exchange mechanism is the principal cause for the twitch potentiation.

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A COMPARISON OF K-INDUCED HYPERPOLARIZATION WITH THAT OF Rb IN RABBIT SINOATRIAL NODE CELL.
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The effects of K and Rb on the electrogenic Na-pump of a rabbit sinoatrial node cell were studied, using conventional micro-electrode techniques. The K-induced hyperpolarizations after K-depletion depended linearly on a K-concentration ranging from 1.35 mM to 10.8 mM. Using 2 mM Rb as an activator, a subthreshold oscillation appeared and a potential in the Rb solution was larger than with the standard solution. As the concentration of Rb increased, Rb-activated hyperpolarization increased, but the potential amplitude in the Rb solution decreased. The hyperpolarization and recovery phenomenon of Rb as substitute for K in the sinoatrial node cell after K-depletion showed that the effect of a monovalent cation on the external site of the Na-pump was more delicate than with K. Since these activation effects were not obtained after the application of ouabain containing K-free solution, the hyperpolarizations were considered to be electrogenic. A small hyperpolarization of resting level correlated with the activation of external site of Na-pump seems to be necessary for the immediate recovery of automatism.

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EFFECT OF MAGNESIUM ON THE INACTIVATION SYSTEM OF THE FAST SODIUM CHANNELS IN MAMMALIAN VENTRICULAR MUSCLE.
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Späh and Fleckenstein (1979) claimed the existence of the "Mg channel" besides the fast Na and the slow Ca channel in guinea pig ventricular muscle. We reevaluated their hypothesis in comparable experimental conditions. The ventricular fibers depolarized in K-rich (19 mM) solution restored the electrical excitability when external magnesium concentration was increased to 14 mM as reported by Späh and Fleckenstein (1979).

However, this "Mg-induced action potential" was sensitive to TTX (3×10^{-6} M) and rapidly abolished following reduction in external Na concentrations (from 149 to 12 mM). These findings suggest that inward Na current plays a predominant role in the genesis of the "Mg-induced action potential". The steady state Na inactivation curve (h_∞ curve) was determined relating the maximum rate of rise of the action potential to the various resting membrane potentials which were attained by varying external K concentrations (from 2.7 to 19 mM). A Mg increase (from 1 to 14 mM) shifted the h_∞ curve by 6 mV in the depolarizing direction. Results indicate that the "Mg-induced action potential" is produced by the restoration of the fast Na current which could have been attained by increased external Mg concentrations.

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EFFECTS OF Ba^{2+} ON THE MECHANICAL PROPERTIES OF GLYCERINATED CAT HEART MUSCLE
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At a muscle length L_0 (just taut), isometric tension and tension transients in response to rapid step stretches in length (about 1% of L_0 within 2 msec) were studied at constant levels of Ba^{2+} activation of varying magnitude in glycerinated cat papillary muscles (2-3 mm in length and 150-200 μm in diameter). The preparations were fixed between a tension transducer (natural frequency = about 500 Hz) and displacement device with a fast setting glue. All experiments were carried out at room temperature (22-24°C) and at a pH of 6.8. The steady isometric tension increased with Ba^{2+} over the range slightly below 10^{-5} to slightly above 10^{-4}. This concentration range was roughly 100 times higher than that of Ca^{2+}-activated muscle. Maximum Ba^{2+}-activated tension was about 60% of that developed by Ca^{2+}. The tension transients of Ba^{2+}-activated muscle were characterized by at least three distinct phases; an immediate tension increase coincident with the stretch, a rapid exponential tension decrease (τ_1 = 15-25 msec) and a delayed exponential tension rise (τ_2 = 150-250 msec). The time course was quite similar to that seen in the Ca^{2+}-activated muscle. These results suggest that in Ba^{2+}-activated glycerinated heart muscle the cross-bridge turnover over and the rotation of the cross-bridge heads are taking place as in the Ca^{2+}-activated muscle.

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EFFECTS OF INTRACELLULAR INJECTION OF VARIOUS Na SALTS INTO THE ISOLATED A-V NODE CELL CLUSTER OF RABBIT HEART. IRISAWA, H., KURACHI, Y. AND NOMA, A. (NATIONAL INSTITUTE FOR PHYSIOLOGICAL SCIENCES, OKAZAKI)

In order to confirm the electrogenicity of the Na pump in A-V node cell, various Na salts were injected into the enzymatically isolated A-V node cell cluster of rabbit heart. Injection of Na glutamate caused hyperpolarization of the membrane and stopped the pacemaker activity of the cell. In 27 mM K Tyrode's solution, the Na glutamate-induced hyperpolarization reached -70 mV, which clearly exceeded E_K, and was inhibited by 10^{-5}M strophanthidin. Therefore, it is concluded that the Na pump in A-V node cell is electrogenic. Injection of Na acetate also hyperpolarized the membrane, while those of NaI and NaCl induced marked depolarization of the membrane. These results show that the net effect of the injection is determined by the balance between the outward-going Na pump current and the inward-going current carried by the injected anions.

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DEPENDENCE OF THE ACTION POTENTIAL ON INTRACELLULAR ATP IN GUINEA-PIG SINGLE VENTRICULAR CELLS.
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The change of action potential or Ca current was studied on injecting ATP into a single ventricular cell isolated from the guinea-pig heart. An ATP injection elevated the plateau level of action potential and augmented the Ca current, while superfusion of the glucose-free Tyrode containing 5.4 mM CN (CN-Tyrode) depressed the action potential. Such effects of ATP injection or superfusion of CN-Tyrode were mimicked by injecting creatine phosphate or creatine, which should increase or decrease the intracellular ATP level catalyzed by creatine kinase. These findings could not be explained solely by a possible change in intracellular free ionic concentration of Ca, because the sarcomere length remained constant in CN-Tyrode and an EGTA injection failed to recover the action potential in the CN-poisoned cell.

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THE INCREASE IN THE DELAYED OUTWARD CURRENT BY ISOPROTERENOL AND THEOPHYLLINE IN MAMMALIAN VENTRICULAR MUSCLE.
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The voltage- and time-dependent outward current (i_K) in guinea pig papillary muscle was studied under some conditions which raised the intracellular cyclic AMP levels, using the single sucrose-gap voltage-clamp method. As well as the slow inward current (i_S), i_K during depolarizations from a holding potential of around -40mV was markedly enhanced by 10^{-7}M isoproterenol. Correspondingly, the positive tail current of i_K on repolarization to the holding potential was increased with little change in its decaying time course. The amplitudes of the tail currents were increased by isoproterenol to the same extent on every preceding depolarizations of different magnitudes or of various durations, indicating that neither the voltage- nor the time-dependency of i_K activation was changed. The similar results were obtained with 2mM theophylline and 2mM dibutyryl cyclic AMP. The time-independent current was not affected by theophylline. The enhancing action of theophylline on i_K was observed in the presence of 2mM Co^{2+} which had abolished i_S. On the contrary, 9mM Ca^{2+} did not alter the amplitude of i_K although it clearly increased i_S. These results suggest that cyclic AMP produces the increase in the number of available i_K channels.

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DEVELOPMENT OF REPOLARIZATION PHASE ON TRANSMEMBRANE ACTION POTENTIAL AND ON ELECTRO-CARDIOGRAM FROM PRENATAL AND POSTNATAL RAT HEART. SATO,S. & MIYAZAWA,T. Dept. of Clin. Physiol., KANAGAWA Pref. College of Med. Tech., YOKOHAMA

The bipolar-electrocardiograms (ECG) of A-B leads and action potentials (APs) of the left ventricular muscle fibers were recorded from prenatal and postnatal rat heart. ECG and AP were displayed on a Nihonkoden VC-9 oscilloscope and photographed with kymographic camera. The APs, conventional microelectrode was used, were recorded both side of epicardium (EPI) and endocardium (END) of the left ventricules. The heart muscle driven by electrically at a frequency of 1, 3, and 6 Hz. The duration at 50% and 90% repolarization phase (APD50 and APD90) of action potential, were inversely related to heart rate, and were decreased with growing age. QT interval and QTc of the ECG correlated with APD50 and APD90. The distribution data from the APD50 and APD90 showed a wide variation on gestation stage, and a narrow variation on adult in many region of EPI and END.

In this study we suggest that QT interval and QTc of ECG are most related to change of the APD, most important factor is the repolarization phase, on rat heart.

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CENTRAL DISTRIBUTION OF PREGANGLIONIC NEURONS MEDIATING THE NON-CHOLINERGIC EXCITATORY TRANSMISSION IN AMPHIBIAN SYMPATHETIC GANGLIA. KAGIYAMA, N., HIJIRIKAWA, K., HIGASHI, H. AND NISHI, S. Dept. of Physiol., Kurume Univ. Sch. of Med., Kurume

The lumbar sympathetic ganglia of bullfrogs are endowed with a non-cholinergic transmission system that generates an extremely long-lasting synaptic potential, the late slow EPSP, which triggers a late afterdischarge (LAD) in postganglionic fibers. The transmitter for this EPSP has been suggested to be LHRH-like substance liberated exclusively from preganglionic C fibers. This investigation aimed to clarify the central location of the peptidergic neuron by 1) recording the antidromic activity of preganglionic C fibers in the CNS, 2) recording LAD from lumbar ganglia following stimulation of various level of CNS perfused with a low Ca/high Mg solution over 2 hrs, 3) recording the reflexly induced LAD of lumbar ganglia by sciatic nerve stimulation after transection of the CNS at various levels, and 4) measurement of ^{3}H-HRP in various parts of the CNS after a long (48 hrs) immersion of the lumber ganglia in a ^{3}H-HRP-containing solution. Results obtained from these experiments suggested that the peptidergic (LHRH-containing) C neurons that trigger the LAD in the lumbar sympathetic ganglia are located between the Vth and VIIIth cord segments ipsilateral to the innervating ganglia.

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EFFECTS OF BRADYKININ ON THE GUINEA-PIG SYMPATHETIC GANGLION CELLS IN VITRO. YONEMURA, K., MORIMOTO, K., MATSUOKA, Y. AND TANAKA, I. Dept. of Physiol., Kumamoto Univ. Med. Sch., Kumamoto

In the isolated superior cervical ganglia of guinea-pigs, the effects of bradykinin were studied by means of intracellular microelectrode techniques. The drug was dissolved in a physiological saline solution, and injected for test into the perfusion stream to the ganglion. At concentrations of 8-100 μg/ml, bradykinin produced dose-related depolarizations of 2-14 mV in some cells tested. The depolarization phenomena were observed with concomitant reduction in input resistance and action potential amplitudes of the cells. The threshold for initiation of action potentials in the ganglion cells either by indirect or by direct stimulation was also lowered after application of the drug. In a low Na (24 mM) solution, the amplitude of depolarization by bradykinin was reduced to 80% of control. Similar observations were made in a Ca-free solution or in a low Ca (0.5 mM) solution. It was also observed that a calcium antagonist, diltiazem (10^{-5} g/ml), depresses the depolarizing effect of bradykinin on the ganglion cells.

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EFFECTS OF PROPRANOLOL ON THE CHOLINERGIC RESPONSES IN THE SUPERIOR CERVICAL GANGLION (SCG) CELLS OF THE GUINEA-PIG. IMAWATARI, R., NAKAMURA, T., HIGASHI, H. AND NISHI, S. Dept. of Physiol., Kurume Univ. Sch. of Med., Kurume

Propranolol (1 ∿ 10 μM), a typical β adrenoceptor blocking agent, markedly depressed the nicotinic acetylcholine (ACh)-potential, but lowered the fast EPSP only slightly. Propranolol did not alter the quantal content of fast EPSPs elicited in a low Ca/high Mg medium while it decreased the quantal size slightly. The drug unaltered the degree of facilitation in fast EPSPs induced by double pulses. Contrary to propranolol, dTC depressed both the fast EPSP and the ACh-potential indiscriminately. The dose-response curve for the action of propranolol revealed that it non-competitively depresses the ACh-potential. Propranolol-induced depression of the ACh-potential was voltage dependent; higher the membrane potential, greater was the depression. Contrary, there was no recognizable potential dependency in depression of the fast EPSP by propranolol. Futhermore, EPSPs whose duration was markedly prolonged by a low temperature also showed no significant voltage-dependency of propranolol action. The results altogether suggest that there would be extrajunctional ACh receptors, in addition to ACh receptors in the subsynaptic membrane; both of which are activated by application of exogenous ACh. Propranolol may selectively depress the ionophore of the extrajunctional ACh receptors.

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EFFECTS OF Ca^{2+}-ANTAGONISTIC AGENTS ON GANGLION TRANSMISSIONS IN THE SUPERIOR CERVICAL GANGLION OF THE GUINEA PIG. ITO, H., OYAMA, Y. AND NISHI, K. Dept. of Pharmacol. Univ. Med. Sch., Honjo, Kumamoto, Japan.

Effects of propranolol (PROP), known to have a Ca^{2+}-antagonistic action, and diltiazem (DILT) on transmission in sympathetic ganglia were investigated with intracellular recording techniques in superior cervical ganglia of the guinea pig. Both PROP ($5x10^{-5}$ M) and DILT (10^{-5}M) decreased amplitude of EPSP, and finally blocked initiation of postsynaptic action potentials without changing the membrane resting potential. Lower concentration of these drug (10^{-6}M) induced a transmission failure in the ganglion when preganglionic nerves were stimulated at high frequency (30 Hz), but did not affect action potentials in the soma membrane induced by direct stimulation, nor acetylcholine-potentials by iontophoretically applied acetylcholine. Result indicate that both PROP and DILT, acting on the preganglionic nerve terminals, inhibit Ca^{2+} influx in a frequency dependent manner. Result suggests organic Ca^{2+}-antagonists may supress the excess sympathetic activity without much affecting normal ganglionic transmission in the sympathetic ganglion.

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CORRELATION BETWEEN THE ACTIVITIES OF THE "CARDIOVASCULAR" NEURONS AND THE PERIPHERAL SYMPATHETIC FIBERS KANAI, K. AND MIYAKAWA, K. 2nd Dept. of Physiol. Sch. Med.,Shinshu Univ. Matsumoto

Unit discharges of the neurons in the dorsal nucleus of the medullary reticular formation were recorded with floating microelectrode during systemic arterial pressure oscillation elicited by side pressure exertion procedure. By simple comparison between the simultaneously recorded discharge patterns of the reticular units and the renal nerve fibers, 22% of these units have been already identified as "cardiovascular" neurons. The rhythmicity of the both discharges, further confirmation of the correlation between the both discharges and the detection of the common periodicity shared by the both were subjected to quantitative analysis with DEC11/60 computer and a signal processor (Sanei 7T07A). Highly significant correlation was obtained between the activities of the renal nerve fibers and the unit discharges of the medullary neurons already identified as "cardiovascular". On an average, the activity of type II neurons indicated higher correlation with that of the renal fibers than that of the type I neurons. The fluctuation of the type II neuron activity occurred in the same phase of that of the renal nerve fibers but that of the type I neuron activity in the reversed phase. In conclusion it can be said that the computer analysis gave us a very useful quantitative information on the correlation between the central and peripheral unit activities.

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EFFECTS OF SOMATIC AND VISCERAL AFFERENT STIMULATION ON SYMPATHO-ADRENAL MEDULLARY SYSTEM.
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The reflex effects of cutaneous mechanical or visceral baroreceptor stimulation on adrenal sympathetic efferent nerve activity and adrenal medullary catecholamine hormones; epinephrine (E) and norepinephrine (NE) were studied in anesthetized CNS intact rats. Noxious cutaneous stimulation (pinching the lower chest (LC) for 3 min) produced increases in both the nerve activity and secretion rate of E and NE. Non-noxious cutaneous stimulation (brushing the LC for 3 min) produced decreases in nerve activity and E secretion rate during stimulation, which were followed by increases in nerve activity and both E and NE secretion rates after stimulation. These reflex responses of secretion of E and NE were completely abolished after denervation of splanchnic nerves. Visceral baroreceptor stimulation (stimulated by pressor response after intravenous injection of phenylephrine) produced decreases in both the nerve activity and secretion rate of E and NE. These results suggest that both the adrenal sympathetic efferent nerve activity and adrenal medullary catecholamine hormone secretion can reflexly be controlled in either an excitatory or inhibitory way by noxious or non-noxious mechanical cutaneous, or visceral baroreceptor stimulation.

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NEURAL REGULATION OF ADRENAL CHROMAFFIN CELL FUNCTION

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The fate and distribution of exogenous [^{3}H]dopamine in denervated adrenal medulla was studied by the autoradiography. The left adrenal gland was denervated and the right adrenal gland was kept intact and served as the control of the left adrenal gland. The mice were sacrificed at certain time intervals after an injection of [^{3}H]dopamine.

In both denervated and intact adrenals, radioactivity increased from 7.5 to 30 min then decreased relatively slowly. At later time points (1h to 24h), denervated adrenal glands exhibited higher radioactivity than intact adrenals. Such dissociation was observed only in the marginal zones of the adrenal medulla. In the central zones, no significant difference was demonstrated between denervated and intact adrenals in the amount of radioactivity. The A:NA cell ratio for [^{3}H]dopamine uptake was increased by the denervation. At 24h the ratio was about 2.3 in denervated adrenals while in intact adrenals it was about 1.1. Gradient in radioactivity (higher radioactivity in the marginal zone than in the central zone) was reinforced by the denervation.

Thus, it seems likely that the neural regulation of chromaffin cell function is stronger in A cells than in NA cells and that it is intenser in the marginal zones than in the central zones.

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CERVICAL SYMPATHETIC,VAGAL AND PHRENIC EFFERENT DISCHARGES DURING THE THERMOREGULATORY RESPONSE.

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Changes in cervical sympathetic,vagus and phrenic efferent nerve discharges produced by increasing the body temperature and by injection of warm and cold blood or ringer into the internal carotid artery were studied in nitrous 65%, oxygen 35% anesthetized and vagotomized rabbits.

The increase of the body temperature from 36°C to 40°C produced a sustained increase of the cervical sympathetic,vagal and phrenic background discharges. This increase was due to an increase of the fundamental frequency of the bulbopontine pacemaker mechanism. The fact that decerabration produced a substantial decrease of these responses indicates that the so called bulbopontine fundamental frequency is influenced by a suprapontine acceleratory mechanism probably mediated by the hypothalamus.

Injection of warm (40-43°C) blood or ringer into the internal carotid artery produced a transient increase of the cervical vagus efferent discharges while cold blood or ringer produced mainly an increase of the sympathetic discharges which was followed by an increase of the arterial blood pressure. Decerabration made disappear the above mentioned responses. Thus, it is concluded that the latter responses are also mediated by supracollicular structures.

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PROJECTIONS OF CENTRAL NEURONS ONTO PREGANGLIONIC NEURONS OF THE CARDIAC VAGUS NERVE IN THE CAT MEDULLA OBLONGATA

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It is known that most of the preganglionic neurons of the cardiac vagus nerve (CVPNs) of the cat are present at the site in and around the ambiguus and retrofacial nucleus in the medulla. This site was identified by the following criteria : (1) stimulation of this site induces cardiac slowing; (2) evoked potentials are recorded by stimulation of the carotid sinus nerve (a major reflex input). Double concentric glass micropipettes were used: the inner pipette, used for stimulation and recording; the outer pipette, used for injecting HRP. A small amount of HRP was injected in situ. After 2 days' survival animals were perfused and brains were processed histochemically. From 4 experiments, 360 HRP-labeled neurons were identified and regarded as candidates for efferent neurons projecting to the CVPNs. They were distributed in the medulla (79.2%), midbrain (7.7%), pons (6.6%), upper cervical cord (4.7%) and hypothalamus (0.8%). In the medulla, the majority of them were found in the lateral tegmental field (39%) and the solitary tract nucleus (NTS, 21%). In the NTS, the labeled cells were evenly distributed in four subnuclei: (1) commissural (19%); (2) intermediate (14%); (3) dorsal (17%); (4) medial (24%).

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INTERACTION BETWEEN VAGAL AFFERENTS AND BARORECEPTOR IN THE CONTROL OF EFFERENT PREGANGLIONIC SYMPATHETIC NERVE ACTIVITY DURING MYOCARDIAL ISCHEMIA IN CATS.

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This study was designed to determine the relative influence of carotid sinus baroreceptor and of cardiac receptors with vagal afferents on efferent preganglionic splanchnic nerve(PSN) activity during left coronary artery occlusion in anesthetized cats with aortic denervation. Changes in blood pressure and PSN activity were determined during 60 seconds. Carotid sinus pressure(CSP) was controlled stepwisely to Medium, High and Low levels by means of the perfusion technique. In cats with vagal denervation but not baroreceptor, the occlusion resulted in marked increases in PSN activity, although the hypotension after vagotomy did not differ from that of the intact group. In cats with baroreceptor and vagal intact, PSN activity was not significantly altered despite a significant fall in blood pressure during the occlusion. In three controlled CSP, significant increase in PSN activity during the hypotension appeared only in Low CSP. These results show that vagal afferents exert an inhibitory influence on PSN activity during the ischemia, and that this influence limits the baroreceptor-mediated increases in PSN activity resulting ischemia-induced hypotension. In addition, it is suggested that inhibitory bulbospinal pathways minimize the influence of the spinal sympatho-sympathetic reflex during the ischemia-induced hypotension.

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UNIT DISCHARGES EVOKED WITHIN THE SOLITARY TRACT NUCLEUS OF THE RAT BY HEPATIC VAGAL STIMULATION

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Gluco and osmoreceptions in the liver have been analyzed by means of an electrophysiological method as well as behavioral experiments. To extend these observations, the medullary projection of the hepatic branch of the vagus was examined electrophysiologically. Afferent neurons in the solitary tract nucleus which innervate the liver were identified by the electrical stimulation of this nerve branch. A collision test was empolyed to separate the afferent unit discharges from the efferent. These afferent neurons are characterized by no abolition of the evoked unit discharges by the test. Deviation of the latency was always recognized. A suppression caused by repetitive stimulation of 5Hz or higher was recognized. After these examinations, hypertonic saline, water and isotonic glucose solutions were infused through the hepatic or jugular catheter. Some afferent neurons which innervate the liver were responsive to the portal infusion of isotonic glucose solution, hypertonic saline and water. Their response patterns were almost similar to those of the hepatic afferent nerves. All recording sites were histologically examined.

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RUNNING AND ENDING OF VAGAL AFFERENT NERVE FIBER IN RABBIT EPIGLOTTIS

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Various kinds of receptors in epithelial layer of epiglottis were reported by Storey. We examined whether or not majority of these receptors is free ending of vagal afferent nerve fiber by autoradiography.

After left supranodose vagotomy was made to degenerate efferent nerve fibers, about 1mCi of ^{3}H-leucine was injected into the nodose ganglion(NG). After 10 days of survival period, NG and superior laryngeal nerve(SLN), epiglottis were determined by autoradiography. Radioactive materials were extremely shown over the neurons of the injected NG, but few in contralateral NG as control, and transported from NG through SLN into the epiglottis. In lamina propria layer silver grains were seemed to be emerged from myelinated nerve fiber more than unmyelinated nerve fiber. In epithelial layer by light microscope silver grains were shown nearby taste bud cells which was seldomly found in epiglottis, and furthermore by electron microscope many silver grains were localized over structure like free ending positioned in intercellular cleft. From these observations we conclude that some of vagal afferent nerve endings seem to be formed a synapse with taste bud cells which perhaps function as chemoreceptor,and that water receptor and mechanoreceptor are free endings.

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TEMPERATURE EFFECTS ON BARORECEPTOR ACTIVITY FROM CAROTID SINUS NERVE OF RABBIT.
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The effects of temperature on the baroreceptor activity were examined under various pressures. The preparation was perfused with the modified Tyrode-solution. The pressure applied to the sinus area ranged from 30 to 120mmHg. The temperature changed from 25.0 to 40.0°C. In all experiments, afferent discharges were recorded from single nerve fibers.

We found that there are two groups of the baroreceptor fibers. One group is the fiber that is sensitive to low temperature and the discharge suddenly disappears with the rise of temperature in low pressure than 120mmHg. The setting point of temperature for sudden disappearance of discharge ascends with increasing pressure. The other group is the fiber of that the nervous activity is hardly restricted by increasing temperature over the pressure range 30 to 120mmHg. Both Q_{10} values of two groups increase with pressure, for example Q_{10} is 2.2 in 120mmHg and 1.1 in 30mmHg. The receptive areas are distributed on the walls of internal, external and common carotid arteries near the carotid body, but the difference of the receptive area between two groups is not recognized for the time being. It is relevant to know whether the difference of temperature characteristics between two groups is ascribed to the temperature properties of the organ innervated by the nerve fiber or that of the fiber itself.

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REFLEX ACTIVITY OF AUTONOMIC FIBERS IN THE INFERIOR ALVEOLAR NERVE IN RABBITS EVOKED BY MECHANICAL VIBRATORY STIMULATION.
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Two different types of efferent activity were recorded from filaments of the inferior alveolar nerve in rabbits. These units were considered to be parasympathetic and sympathetic units in the inferior alveolar nerve, from their conduction velocities and the effect of nerve section. The parasympathetic units responded spontaneously with reflex discharge evoked by mechanical stimulation to oral tissues. The properties of reflex discharge evoked by vibratory stimulus were studied.

1.Fifteen units out of 68 identified units responded with reflex discharges. Others did not responded to mechanical stimulation.

2.Two types of reflex discharge were recognized: whereas one stimulus evoked one discharge or less in the one type, in the other it evoked several discharges.

3.The optimal frequencies of the two types were 5-10 and 10-20 Hz.

4.The time courses of the excitability of reflex units were studied by electro-pair stimulation to afferent fibers in the inferior alveolarnerve.

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THE STRUCTURE OF THE RABBIT'S AORTIC NERVE.
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The neural elements of the aortic nerve trunk which is the main afferent pathway of the brain ischemic reflex bradycardia were electron microscopically studied on six rabbits. The tissues of cervical region were preliminarily perfused by Karnowsky's solution for 30 minutes under a constant pressure. The right and left aortic nerves were cut off to a length of 30 mm, and served for the electron microscopic study. An average of numbers of myelinated fibers contained in the aortic nerve trunk was 498 ± 215 in the left and 222 ± 78 in the right. The figure on non-myelinated fibers was 1026 ± 473 in the left and 968 ± 314 in the right. Each of myelinated and non-myelinated fibers was classified into large, medium and small groups respectively according to their diameters. The result suggests that the functional difference between the left and the right aortic nerve observed on elicitation of the brain ischemic reflex bradycardia in previous studies is due to the difference of the myelinated element between both sides.

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SOMATO-CARDIAC REFLEX AND DEPTH OF ANESTHESIA.

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In chloralose-urethane anesthetized rats, a nociceptive cutaneous stimulation of a hindpaw can produce a reflex increase in heart rate. The magnitude of this cardiac excitatory reflex response is influenced with the depth of anesthesia. Therefore, the present study was undertaken to find some correlations between the depth of anesthesia and the magnitude of the somato-cardiac reflex response by monitoring the EEG pattern, which was analyzed by the frequency analysis method. The frequency components of so-called δ,θ,α and β waves were indentified, and the percentages of each δ,θ and α frequency component to all frequencies components were continuously recorded. It was found that the somatically induced cardiac reflex response with relatively constant magnitude was obtained at the depth of anesthesia at which the δ wave's frequency component was kept steadily between 60 to 80% of the total frequencies components.

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THE OUTFLOW OF THE AUTONOMIC NERVES INNERVATING THE STOMACH DURING VOMITING IN DOGS.

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Retching activity (R) was elicited by apomorphine (0.2 mg/kg) injection or by intragastric infusions of $CuSO_4$ solutions in decerebrated or chloralose anesthetized dogs immobilized with Flaxedil. 1) Fine nerve filaments dissected from the antral and corpus branches of the gastric vagus showed a low frequency efferent discharge which was either increased or decreased by gastric distension with Tyrode's solutions (100-400 ml). Outflows in such two types of filaments increased markedly within a few minutes before R, and they were interrupted by a partial suppression during R. 2) Fine sympathetic nerve strands dissected from the nerve along the left gastroepiploic artery showed a marked spontaneous outflow which was transitorily increased by gastric inflation. No outflow was decreased by a cold block at the cervical spinal cord despite of a marked reduction of the splenic nerve outflow recorded simultaneously. Gastric sympathetic outflow increased with a rhythm of the powerful antral contractions which occurred immediately before R, and it was followed by a partial inhibition during R.

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CHANGES IN GASTRIC ACID SECRETION AFTER HEPATIC PORTAL INJECTIONS OF D-GLUCOSE AND ITS ANOMERS.

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Alterations in acid outputs from the stomach were examined following portal injections of D-glucose and its optical anomers in the bilaterally adrenalectomized rats with insulin hypoglycaemia. Significant reduction in gastric acid outputs was noted after portal injections of α-D-glucose, optically equilibrated D-glucose (OEDG) consisting of 36% α-anomer and 64% β-anomer and β-D-glucose. The effect of β-D-glucose was most potent in decreasing the acid outputs and the inhibitory response was disappeared by prior vagotomy at the hepatic level. The isotonic NaCl solution, however, caused no change in the acid outputs. Results suggest that oscillation in glucose levels in the hepatic portal vein may affect gastric acid secretion through hepatic vagal afferents and gastric vagal efferents and suggest that activation of hepatic glucosensitive mechanisms may be dependent on the anomeric stereospecificity of D-glucose in the blood.

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ACTIONS OF INTRACELLULARLY INJECTED Ca^{2+} AND EGTA ON THE SUBMANDIBULAR GANGLION CELLS.
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Intracellular injection of Ca^{2+} and EGTA, and recording of cellular responses were made in the hamster submandibular ganglion cells using single 3 M KCl glassmicroelectrodes partially filled with Ca EGTA buffer solution (60-100 MΩ). Effects of increased and decreased $[Ca^{2+}]_i$ on three types of hyperpolarization (HP) produced in these ganglion cells, which are spike- and EPSP-after HPs, and rhythmic HPs, were examined in order to analyze the electrogenesis mechanism of these HPs. Ca^{2+} injection inceased the amplitudes and durations of spike- and EPSP-after HPs of the ganglion cells. Ca^{2+} injection caused also rhythmic HPs and long lasting HPs (3-35 mV, 7-20 min) accompanied by a decrease and then an increase in R_m. EGTA injection decreased the amplitudes and durations of spike-after HPs of the ganglion cells. EGTA injection caused also long lasting depolarizations (DPs) (2-9 mV, 4-9 min) accompanied by a decrease in R_m. These long lasting HPs and DPs might be elementary components of slow rhythmic membrane potential change, the fourth cellular activity of the ganglion cells. Results showed that these three types of HP are generated by an activation of Ca-mediated K channels based on Ca^{2+} induced Ca^{2+} release mechanism.

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Effects of octopamine and serotonin on the ganglion cell discharges in the isolated heart of the lobster.
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Burst discharges of the large and small ganglion cells were separately recorded with suction electrodes after cutting the ganglionic trunk. Octopamine (OA, 10^{-7}- 10^{-5} M) inhibited discharges of the small cells (the bursts often ceased at 5×10^{-6} M or higher concentration). On the other hand, OA (10^{-6}- 10^{-5}M) often increased the large cell burst frequency (up to 2 Hz). When OA was applied to the intact heart, the heart rate often increased after initial decreasing although excitatory synaptic inputs to the large cells, provided by the small cell discharges, might increase. It could be confirmed by intracellular recording that the synaptic potentials seen in the large cell decreased and the large cell burst frequency increased after application of OA (10^{-5} M). Taken together, it is suggested that acceleratory effects of OA on the heartbeat of the lobster may appear through activation of the large cells. Serotonin (10^{-8}- 10^{-6} M) increased burst frequency of a small cell (e.g. the 6th cell) up to 1 Hz more or less, while burst duration of the other small cell (e.g. the 7th cell) increased by twice or more times under presence of 5HT (higher than 10^{-7} M). On the other hand, 5HT (10^{-6} M) decreased the large cell burst frequency, but the effect was very mild. It is suggested that the large cells may be activated in the intact ganglion by the two types of the small cell bursts in presence of 5HT. This also was confirmed by intracellular recording from the large cell.

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MECHANISM OF EXCITATION OF PANCREATIC EXTERNAL SECRETION CAUSED BY SPLANCHNIC NERVE STIMULATION IN DOG.
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The mechanism of pancreatic external secretion caused by splanchnic nerve stimulation after nicotinization of the celiac and superior mesenteric ganglia was investigated. Dogs were anesthetized with pentobarbital sodium and immobilized with gallamine triethiodide. The animals were artificially ventilated during experiments. The pancreatic external secretion (volume, protein, amylase and TAMe hydrolytic activity) was augmented by splanchnic nerve stimulation (10 V, 1 msec, 50 Hz) after nicotinization of the celiac and superior mesenteric ganglia and also in vestibulo-splanchnic nerve reflex. These responses of increased pancreatic external secretion were markedly inhibited by administration of atropine sulfate (0.1 mg/kg i.v.). The pancreatic external secretion was also augmented by exogenous gastrin (tetragastrin 0.1 - 0.2 μg/kg i.v.). However, the pancreatic external secretion was almost unaffected by endogenous gastrin released on stimulation of the splanchnic nerve. These results suggest that there are pancreatic cholinergic secretory nerve fibers in the splanchnic nerve and that these fibers pass through the celiac ganglion.

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EFFECTS OF ELECTRICAL STIMULATION OF THE TOOTH PULP ON THE RESPIRATORY ACTIVITY IN THE RAT. IKUNO, H.& SHIMADA, K. Dept. of Oral Physiol., Niigata Univ., Sch. of Dent., Niigata.

It has already been reported that trigeminal nerve afferents from orofacial region depress the respiratory activity. However the effect of stimulation of the intrapulpal nerve on the respiration was not yet precisely investigated. The rats were anaesthetized with α-chloralose & urethan, immobilized and artificially ventilated. Respiratory activity was indicated by phrenic nerve discharge. To stimulate the intrapulpal nerve bipolar electrode was inserted into the lower incisor and electrical pulses of 0.1 msec duration were used. Inhibitory effect on the respiratory activity was recognized with a latency of about 10-20 msec in the phrenic nerve discharge. The inhibitory effect increased with stimulus frequency. There was no effect in the rate of breathing during and after the stimulation.

The present results suggest that trigeminal nuclear complex to which the intrapulpal afferent fibers project have inhibitory polysynaptic relay to the respiratory center.

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VOLTAGE-CLAMP STUDY OF SLOW POSTSYNAPTIC POTENTIALS IN NEURONS WITHIN THE MYENTERIC PLEXUS OF GUINEA-PIG ILEUM. KATAYAMA, Y., MIHARA, S.* AND NISHI, S.* Dept. Autonomic Physiol., Med. Res. Inst., Tokyo Med. & Dent. Univ., Tokyo-101 and *Dept. Physiol., Kurume Univ. Sch. Med., Kurume-830

Slow postsynaptic currents (EPSC's and IPSC's) and substance P-induced currents were recorded from voltage-clamped neurons using double-barrelled electrodes. Changes in membrane conductance were detected by a jumping method. The slow EPSC's were accompanied by a decrease in membrane conductance and became small in amplitude when neurons were clamped at hyperpolarized levels of membrane potential. The reversal potential of the slow EPSC's was approximately at -90 mV. The slow IPSC's were associated with an increase in membrane conductance and were reduced in amplitude by membrane hyperpolarization, showing a reversal in polarity nearly at -90 mV. A decrease in membrane conductance occurred during the substance P-induced currents which were reversed in polarity as holding levels were more hyperpolarized than -90 mV. The results suggest that all of these responses are due to the change in resting potassium conductance.

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THE CELL-TO-CELL COMMUNICATION IN THE CULTURED AUERBACH'S PLEXUS CELLS. MARUYAMA, T. AND SUZUKI, T. Dept. of Applied Physiol., Tohoku Univ. Sch. of Med., Sendai.

The cultured Auerbach's plexus cells which were derived from the small intestine were studied in this experiments. The cells have maintained good cell proliferation in culture system. The cell-to-cell communication properties of Auerbach's plexus cells were characterized by the intracellular microelectrode and transfer of an injected florescein dyes. During the culture, the Auerbach's plexus cells connected with each other, some by their processes and other by cell bodies. The Auerbach's plexus cells were in electrical communication with each other. The injected florescein dyes in Auerbach's plexus cells passed to the connected cells. External calcium ions, ionophore A23187 and excess CO_2 produced the blockage of cell-to-cell communication. Injection of calcium ions in Auerbach's plexus cells also blocked the electrical communication and the transfer of florescein dyes. These results suggest that the intracellular calcium ions in Auerbach's plexus cells regulate the pathway of cell-to-cell communication.

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ELECTROPHYSIOLOGICAL PROPERTIES OF NEURONS IN THE SUBMUCOUS PLEXUS OF THE GUINEA-PIG CECUM. MIHARA, S., *KATAYAMA, Y. AND NISHI, S. Dept. Physiol., Kurume Univ. Sch. Med., Kurume and *Dept. Autonomic Physiol., Med. Res. Inst., Tokyo Med. & Dent. Univ., Tokyo

Intracellular recordings were made in vitro from submucous neurons. The neurons showed resting membrane potentials of 45 ∿ 70 mV, input resistances of 50 ∿ 130 MΩ and membrane time constants of 5 ∿ 10 msec. In a majority of neurons the action potential was followed by a relatively fast (10 ∿ 30 msec) afterhyperpolarization, while in a minority of neurons the afterpotential was composed of a fast and a slow (1.5 ∿ 2 sec) afterhyperpolarization. Three classes of post-synaptic potentials (PSP's) were observed: fast excitatory PSP, slow excitatory PSP and slow inhibitory PSP. The fast EPSP was nicotinic in nature and resembled the fast EPSP in sympathetic ganglia. The others were not affected by cholinergic antagonists. The slow IPSP was associated with a decrease in membrane resistance and was reversed in polarity at approximately -90 mV. On the other hand, the slow EPSP was accompanied by an increase in membrane resistance and was not successfully reversed. A voltage clamp analysis disclosed that slow EPS-currents were augmented by membrane depolarization but disappeared as holding levels were more hyperpolarized than -60 mV. These results suggest that the slow IPSP is due to an increase in resting K^+ conductance and the slow EPSP is due to a decrease in voltage-sensitive K^+ conductance.

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PROPERTY AND FUNCTION OF THE INTRAMURAL PELVIC NERVE IN THE COLON OF THE DOG. FUKUDA, H. AND FUKAI, K. Dept. of Physiol., Kawasaki Med. School, Kurashiki

It is commonly accepted that fibers of the pelvic nerve run in the colonic wall and innervate the proximal colon, however, no report has shown histological and physiological property of the intramural fibers.

It was found in this study that several nerve strands branched from the bilateral pelvic nerves ascended between the circular and longitudinal muscular layers of the colon of the dog. On the ascending pathway, the intramural pelvic nerves connected with each other by several communicating branches and with the myenteric plexus by numerous small branches. The longest one of the nerves ascended 70% of the colonic length, then fused into the myenteric plexus. Some of the fibers from the nerve ascended further in the plexus and reached the point of 94% of the colon. Afferent fibers in the nerve extended to the point of 87%. Distension of the distal colon caused reflex discharges of the intramural pelvic nerves and propulsive contractions of the proximal colon. After the intramural pelvic nerves were selectively cut, the contractions disappeared from the colonic part proximally situated to the cutting point.

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ROLE AND LOCALIZATION OF THE SUPRASPINAL SYMPATHO-INHIBITORY CENTER ON THE RECTO-RECTAL INHIBITORY REFLEX. TAKAKI, M., NEYA, T. AND NAKAYAMA, S. Dept. of Physiol., Okayama Univ. Med. Sch., Shikata-cho, Okayama

The rectal contraction was produced by local stimulation(50Hz,0.2-2ms,0.1-0.5mA) of the pons in spinalized guinea pigs(L4). This contraction was produced via lumbar sympathetic nerves, since it was abolished after division of lumbar sympathetic nerves. The points producing rectal contraction were located in the lateral reticular formation. Stimulation of many of these points in the rostral pons markedly reduced lumbar colonic efferent discharges. Accordingly, the supraspinal sympatho-inhibitory center appears to be located in that area. Pelvic afferent stimulation (20Hz,2ms,6-18V) produced rectal contraction and simultaneously inhibition of lumbar colonic efferent discharges after contralateral pelvic nerve section. These responses were abolished following Th 13 transection. The results show that lumbar colonic neuronal activities are depressed by the pelvico-lumbar sympathetic reflex via supraspinal sympatho-inhibitory center in the lateral reticular formation of the rostral pons and as a result sacral excitatory reflex occurs more dominantly than lumbar inhibitory reflex in defecation act.

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RESPONSES OF THE INTERNAL ANAL SPHINCTER AND LUMBAR SYMPATHETIC NERVES TO AFFERENT STIMULATION OF LUMBAR VISCERAL NERVES.
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Cats anethetized with pentobarbital sodium (35 mg/kg, i.p.) were used. Afferent stimulation of lumbar splanchnic, colonic and hypogastric nerves produced contractions of the internal anal sphincter. Transection of the lower thoracic segment did not abolish the contractile response, but pithing all the spinal cord caudal to Th 11, section of all efferent sympathetic nerves to the internal anal sphincter or administration of guanethidine (10 mg/kg, i.v.) abolished this response. Efferent stimulation of colonic nerve as well as hypogastric nerve produced contraction of the internal anal sphincter. Distention of the urinary bladder also caused contractions of the internal anal sphincter and an increase of efferent impulses of the hypogastric and colonic nerves after severance of bilateral pelvic nerves. These responses were also obtained after transection of the lower thoracic segment.

It is concluded that the efferents in sympatho-sympathetic reflex contraction of the internal anal sphincter are contained not only in the hypogastric nerve but also in the lumbar colonic nerve, and that its reflex center is located in the lumbar segments.

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AFFERENT INFORMATION DURING RECTO-ANAL REFLEX.
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The recto-anal reflex: transient fall of intra-anal pressure due to rectal distension, was described by Gowers. He stressed the following points; (i) this reflex was independent from sacral innervation and (ii) the 'rhythmic variation' was observed during recovery phase. We examined this reflex with recordings of intra-anal pressure and of electrical activity of internal anal sphincter (IAS) during last three years and obtained the following conclusions; (i) the fall of pressure was mainly due to the inhibition of sustained activity of IAS, (ii) the inhibition of IAS was wholly simulated by electrical stimulation of sacral roots, (iii) the recto-anal reflex was still elicited in chronically sacral denervated dogs, but the lengthening of recovery phase was noted.

Thus, we wished to know the afferent impulses in the sacral dorsal roots during this reflex elicited in decerebrate dogs and cats. The action potentials of sacral rootlets were picked up by bipolar electrodes and recorded on the CRO simultaneously with intra-anal pressure and activity of IAS. It was found that the 'rhythmic variation' of afferent discharges was observed consistently with rhythmic pressure wave and that this rhythmic discharge was mainly composed of 'pause of spindle unit', 'high-frequency burst of pressoreceptor unit' and 'sporadic firing of touch-pressure unit'.

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EFFECTS OF STIMULI OF THE LIMBIC SYSTEM, THALAMUS AND BRAIN STEM RETICULAR FORMATION ON THE BLADDER MOVEMENT
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We studied in rabbits how the bladder movements were affected when electric stimuli were given on the limbic system, the thalamus and the brain stem reticular formation(RF) which was closely related to the level of consciousness. As a result, inhibitory bladder responses were demonstrated with the 100Hz stimulation on the RF. With regard to the thalamus, facilitatory bladder responses were demonstrated predominantly with the 3Hz stimulation on the VL and the RET, and inhibitory responses with the 100Hz stimulation on the VA. But both effects, facilitatory and inhibitory responses, were observed when either the amygdala or the septum was so stimulated. The changes of the interval histogram of the pelvic nerve activity, the blood pressure and the heart rate simultaneously recorded suggested that facilitatory bladder responses were related both to the excitation of the parasympathetic nerve and to the inhibition of the sympathetic nerve, while inhibitory or no responses were related to the excitation of the sympathetic nerve.

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EFFECTS OF THE CEREBRAL CORTEX STIMULATION ON THE URINARY BLADDER VIA THE PELVIC NERVE IN DOGS YAMANE, M. Dept. of Physiol. (2nd), Kawasaki Med. School, Kurashiki

An intravesical pressure and the efferent activity of the pelvic vesical branch were recorded in urethane-chloralose anesthetized dogs. Electrical stimulation (2-5 mA, 1 msec, 20-100 Hz) of various areas of the cerebral cortex (anterior sylvian gyrus, pre- and postcruciate gyri and precuneus) elicited an excitatory or inhibitory response to the urinary bladder and the pelvic vesical nerve outflow. The excitatory response was abolished or reversed to an inhibitory response after ipsilateral partial lesion to the stimulated side in the ventro-lateral part of the medial reticular formation of the upper medulla oblongata and in the ventro-lateral part of the lateral funiculus (C_2-C_3). After cutting the dorsal roots (L_7-S_3), the excitatory response decreased but not abolished. As far as the micturition reflex remained after partial lesion of the medulla oblongata and the spinal cord (C_2-C_3), the inhibitory response could not be abolished. Train pulse stimulation (100 Hz, 10 pulses) to the inhibitory areas inhibited transiently the rhythmic outflow in the pelvic vesical branch and reset the rhythm.

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CHANGES IN DRUG SENSITIVITY OF THE RATS INTOXICATED WITH METHYLMERCURIC CHLORIDE. WAKITA, Y., MATSUOKA, Y., YONEMURA, K. AND TANAKA, I. Dept. of Physiol., Kumamoto Univ. Med. Sch., Kumamoto

Wistar rats (males) were chronically intoxicated with methylmercuric chloride (MMC), as reported previously. The total doses of administered MMC amounted to 120 mg/kg b.w.. Neurological symptomes such as ataxic gait and a crossing phenomenon in the hind limbs appeared when the total doses reached 105-120 mg/kg b.w.. Blood pressure (B.P.) measured at the rat tail artery increased with the intoxication; the values of B.P. were about 120 mmHg, 140 mmHg and 150-160 mmHg in the control rats, rats with MMC 60 mg/kg b.w. and rats with MMC 120 mg/kg b.w., respectively. In the aortic artery, right atrium and vas deferens isolated from the rats with MMC 60 mg/kg b.w., contractile responses to noradrenaline (NA) were found to be augmented. In the rats with MMC 100-120 mg/kg b.w., the following was also observed: (1) either responses of aortic artery to KCl or to NA and 5-HT were inhibited; (2) contractile responses of atrium to NA were slightly augmented but heart rates inhibited; and (3) both responses of vas to tyramine and to NA showed no apparent changes but those to KCl a slight inhibition.

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IDENTIFICATION FOR VASOMOTOR NEURONS IN THE BRAIN STEM OF THE CAT. NISHIKAWA, Y., MIYAKAWA, M. and HUKUHARA, T., JR.. Dept. of Pharmacol. II, Jikei Univ. Sch. of Med., Minatoku, Tokyo.

Twenty-five vagotomized and paralyzed cats were maintained by artificial ventilation under monitoring O_2 and CO_2 levels in expired air. Cross-correlation analysis between unitary discharges and the overall activity of the fractional components (500-700 Hz) of the efferent discharge of the renal sympathetic nerve, which was almost free from the respiratory modulation, revealed that spontaneous periodic fluctuations in unitary discharge frequency of some brain stem reticular neurons (44 cases out of 79) correlated to the spontaneous periodic changes in the activity of the fractional component of the renal sympathetic nerve activity. In 70 neurons spike number of their unitary discharges during each bin (1-4 sec) correlated to the peak value of the non-leaky integrated neurogram of the fractional components of the renal sympathetic nerve activity during respective bins in a fashion of the linear regression with correlation coefficients ranging from -0.59 to +0.68. Hereby in 47 neurons out of 70 cases the absolute value of the correlation coefficient was larger than 0.2. Fifty-six reticular neurons, which filled each or both of the either criteria based on the results of the cross-correlation or regression analyses were defined as vasomotor neurons. They were located in the entire area of the bulbar and pontine reticular formation in a scattered and intermingled distribution.

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HYPOTHALAMIC LOCALIZATION OF INHIBITORY AND EXCITATORY AREAS BY QUANTITATIVE ANALYSES OF CARDIAC AND RENAL SYMPATHETIC NERVE ACTIVITIES IN THE RABBIT.

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The hypothalamic area was stimulated in anesthetized rabbits at 73 selected points on each sides and the effects on the simultaneously recorded cardiac sympathetic nerve activity (CSNA) and renal nerve activity (RNA) were analyzed quantitatively. While various patterns of CSNA and RNA response could be observed, the most striking finding was the existence of opposite responses of CSNA and RNA at many hypothalamic stimulus points in addition to the conventionally observed CSNA and RNA responses in the same direction. By a new method, we drew the hypothalamic maps for CSNA and RNA response magnitudes. These maps show the excitatory areas of CSNA and RNA are surrounded by the respective inhibitory areas. Although the excitatory areas of CSNA and RNA are superimposable on each other on the ipsilateral side, they are not identical on the contralateral side. These data suggest that the heart and kidney are controlled by sympathetic nerve activities originating from the differnt hypothalamic neural groups in the rabbit.

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VASOPRESSIN-INDUCED PRESSOP RESPONSE ELICITED BY ELECTRICAL STIMULATION OF SOLITARY NUCLEUS AND DORSAL MOTOR NUCLEUS OF VAGUS OF RATS.

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Electrical stimulation of the solitary nucleus and/or dorsal motor nucleus of the vagus (a SOL-X complex) elicited a pressor response in vagotomized rat with spinal cord cut at C_1. Threshold current elevating mean arterial pressure (MAP) by 10 mmHg was 16 $\pm$ 2uA (mean $\pm$ SE, 19 rats) upon stimulation over 30 sec. Prolonged stimulatin over 4 min in 6 rats increased MAP from a prestimulus level of 100 $\pm$ 2mmHg up to 137 $\pm$ 6mmHg ($P < 0.005$). After a stimulation period of 4 min, plasma vasopressin (VP) concentration was 515 $\pm$ 133 pg/ml. This level was significantly higher than that determined in another control group of 7 rats in which VP level was 135 $\pm$ 32 pg/ml ($P < 0.05$). The pressor response was entirely accounted for by the increased release of VP upon stimulation of the SOL-X complex as evidenced by absent response in rats pretreated with a VP antagonist and in Brattleboro rats.

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ANGIOGRAPHIC STUDIES ON THE BLOOD SUPPLY TO THE BRAIN IN THE RABBIT.
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The mode of blood supply to the brain by each of carotid and vertebral arteries was studied by means of angiography on 11 rabbits. These four arteries were alternately occluded. A radiopaque material was rapidly injected into the aortic arch via a catheter inserted from the right femoral artery, and the cephalic vessels were visualized by successive roentgenshots with rate of 4 films/sec. for 4 seconds. When vertebral arteries were bilaterally occluded at a portion higher than the 4th vertebral bone in addition to occlusion of both carotid arteries, no vessels were visualized in the brain region. However, if the vertebral arteries were occluded at their origins, the radiopaque material was detected in the basilar arteries as well as internal carotid arteries. It was transported by some collateral vessels of subclavicular arteries. It is also shown that each of these four arteries has a capacity to supply blood to both side of the brain by itself. The cerebral circulation time seemed to be slightly prolonged by occlusion of these arteries as compared with the control.

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CORRELATION BETWEEN PLASMA VOLUME AND OTHER HEMODYNAMIC PARAMETERS.
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We clarified correlation between plasma volume and some hemodynamic parameters in men. In 43 hearthy men and 111 essential hypertensive patients, we measured cardiac index, hematocrit and totar peripheral resistance at the same time. And we classified essential hypertensive patients into three groups, namely low renin, normo-renin and high renin groups. Then we found that the plasma was more in the low renin hypertensive group than others.

Plasma volume and creatinine clearance were closely negative correlated ($r=-0.76$).

We could not find the correlation between the mean arterial pressure and the plasma volume in total subjects.

But the relationship between the plasma volume and the mean arterial pressure was positive correlated in the low renin hypertensive group ($r=0.52$, $p<0.05$).

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STATIC AND TRANSIENT CHARACTERISTICS OF ARTERIAL PRESSURE RESPONSE TO QUICK MILD HEMORRHAGE IN RABBITS.
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The purpose of this investigation is to estimate static and transient characteristics of arterial pressure response by the rapidly acting arterial pressure control system to a quick mild hemorrhage in anesthetized rabbits. Static characteristics can be expressed by an overall open-loop gain of the rapidly acting arterial pressure control system and transient characteristics by a time constant of arterial pressure response to the hemorrhage, respectively. We estimated the overall open-loop gain of the rapidly acting arterial pressure control system without surgically opening the reflex loop in rabbits. Animals anesthetized by intravenous injection of Nembutal (27.5 mg/kg body weight) and heparinized were bled by 2 ml/kg body weight within 1 to 2 sec through a catheter inserted into the aortic arch. Arterial pressure change after the quick mild hemorrhage was monitored via a catheter placed in the aortic arch for more than 2 min. The overall open-loop gain of the lumped arterial pressure control system determined from the mean arterial pressure response to the hemorrhage was 7.3. The arterial baroreflex system responded to the hemorrhage with the different time constants. The responses were classified into two response patterns, those with small time constant of 2.4 sec and those with large time constant of 9 sec.

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SOURCES OF ACTIVATOR CALCIUM IN BOVINE MESENTERIC LYMPHATICS. OHHASHI, T. AND AZUMA, T. Dept. of Physiol. and Res. Lab. for Cardiovasc. Dis., Shinshu Univ., Sch. of Med., Matsumoto 390.

Calcium dependence for bradykinin(BK)-induced contraction in isolated bovine mesenteric lymphatics was investigated by recording isometric tension and electrical activity in a sucrose gap apparatus. The addition of BK in concentrations ranging from 10^{-10} M to 4 x 10^{-6} M caused a dose-related contraction in the lymphatic segments. The BK-induced contraction was not significantly influenced by treatment with 10^{-6} M phentolamine or 10^{-6} M tetrodotoxin. A low concentration of BK elicited frequent discharges of action potentials and a rapid rise in smooth muscle tone superimposed by phasic contractions. BK was able to cause an additional rise in tension of smooth muscle which had already been depolarized in a high-K solution. The contraction induced by 4 x 10^{-9} M BK was abolished in calcium-free environment or in the presence of Ca-antagonist, 10^{-4} M D-600, but the contraction caused by 10^{-6} M was not reduced to less than 50 % of the control value in both circumstances. In a Ca-free solution containing 1 mM EGTA, 10^{-6} M BK caused a slight contraction, though high K-induced contraction was completely blocked. These results suggest that the BK-induced contraction may be closely related to an increased Ca influx through the membrane and release of membrane-bound and intracellular Ca.

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AUTONOMIC INNERVATION OF BOVINE MESENTERIC LYMPHATICS. OHHASHI, T. AND AZUMA, T Dept. of Physiol. and Res. Lab. for Cardiovasc. Dis., Shinshu Univ., Sch. of Med., Matsumoto 390.

Transmural stimulation and histochemical studies were carried out to analyse the autonomic innervation of bovine mesenteric lymphatics. The electrical stimulation with short train pulses (2 Hz, 0.3 ms and 50 V) elicited a brisk contraction which was followed sometimes by a short- or long-lasting relaxation. The contraction and two types of relaxation were abolished by tetrodotoxin (10^{-7} M). Phenoxybenzamine (10^{-6} M) caused a progressive block of the contraction. Propranolol (10^{-7} M) usually blocked the short-lasting relaxation. Bretylium (10^{-6} M) caused progressive reduction and final loss of the contraction and short-lasting relaxation. The long-lasting relaxation was reduced only slightly after perfusion of bretylium (10^{-6} M). Adrenergic nerve fibers demonstrated by the glyoxylic acid method were distributed in the smooth muscle layers as well as in the adventitia of the lymphatics. These findings suggest that the contraction induced by the electrical stimulation is due to the activation of excitatory postganglionic sympathetic nerves. It is concluded that the short-lasting relaxation is mediated mainly by the activation of sympathetic nerves via β-adrenoceptors and that excitation of non-adrenergic inhibitory nerves may play a major role in the appearance of the long-lasting relaxation.

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NEURAL CONTROL OF HEART RATE AND LEFT VENTRICULAR PRESSURE IN CONSCIOUS CATS NINOMIYA, I., NISHIURA, N. AND SHIRAHATA, M. Dept. of Cardiac Physiology, Research Inst., National Cardiovascular Center, Suita, OSAKA

It is known that the heart rate (HR) and left ventricular pressure (LVP) are controlled by the autonomic nervous system. However, in conscious and freely moving animals, no direct measurements of cardiac neural signals have been made. In this study we measured continuously the cardiac sympathetic nerve activity (CSNA), HR and LVP under both anesthetized and unanesthetized cats. The CSNA, HR and LVP changed from beat to beat associated with the respiratory cycle. In many instances, during resting and/or sleeping states, the variations of the HR and systolic LVP were negatively correlated to the CSNA. In some times, the HR increased without changes in LVP and CSNA. These findings indicated that the respiratory bradicardia was induced by an increase in vagal nerve activity while the sudden increase in HR without the enhanced CSNA was induced by a decrease in vagal nerve activity. On the otherhand, during body movements, walking, excitements or feeding, the variations of the HR and systolic LVP were positively correlated to the CSNA. Moreover, when the HR, systolic LVP and CSNA were sampled randomly during 24 hours, mean values of HR and systolic LVP were positively correlated to the CSNA. These results suggested that in daily life the CSNA originated from the higher central nervous system determined the mean HR and LVP.

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DISTRIBUTION OF VENTRICULAR RECEPTORS THAT MEDIATE INHIBITORY SYMPATHETIC RESPONSE DURING CORONARY OCCLUSION

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To investigate the distribution of ventricular receptors with vagal afferents that mediate the inhibitory sympathetic response, changes in renal nerve activity (RNA) were determined during 60-sec coronary occlusions in 17 dogs with carotid sinus denervated. In 12 dogs (group I), proximal portions of left anterior descending (LAD) and circumflex (LCX) coronary arteries were occluded. In the other 5 dogs (group II), proximal and distal portions of LCX were occluded. In group I, RNA decreased significantly more during LCX occlusion (-45 $\pm$ 7.0 (SE) %) than during LAD occlusion (-17 $\pm$ 4.6 %). The volume of left ventricular wall perfused by LCX was slightly greater (27 $\pm$ 4.5 %) than that perfused by LAD. In group II, the responses in RNA to proximal and distal LCX occlusions were essentially the same, and the decrease in RNA during proximal LCX occlusion (-33 $\pm$ 12.4 %) was not statistically different from that during distal LCX occlusion (-25 $\pm$ 7.8 %). The left ventricular wall perfused by distal LCX was almost equal to that perfused by LAD. These results suggested that the ventricular receptors that mediate the inhibitory RNA response to the coronary occlusion preferentially distributed to the inferoposterior wall of the left ventricle.

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CARDIAC OUTPUT IN UNANESTHETIZED HYPERTENSIVE RATS

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Cardiac index in the conscious resting state was compared among normotensive control rats (NCR), spontaneously hypertensive rats (SHR), and 1-clip, 2-kidney renovascular hypertensive rats (RHR). Cardiac output was measured by means of an electromagnetic flow probe chronically implanted around the ascending aorta. In reference to NCR, cardiac index was greater in SHR but not different in RHR. However, arterial pressure was lower in SHR with aortic probe than in those without it. In RHR, arterial pressure was not different with or without aortic probe. In SHR, the sum of peripheral flow indexes in the carotid artery, superior mesenteric artery, renal artery, and terminal aorta was similar to that in NCR, suggesting that cardiac index, under the condition without flow probe at the ascending aorta, was not different between SHR and NCR. One possible explanation for this discrepancy is that SHR with probe at the ascending aorta was in a hypodynamic state, which decreased vascular resistance or after-load, resulting in an increase in cardiac output. Presumably, this did not occur in RHR, because the hypertension was more stable than that in SHR.

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PERIPHERAL FLOW REDISTRIBUTION IN TRANSPOSITION RESPONSE

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Changes in blood flow in the common carotid,superior mesenteric and renal arteries as well as the terminal aorta in the transposition response of the rat, induced by transposing the animal from the home cage to a new cage, were studied by means of chronically implanted electromagnetic flow probes. In the response, hindquarter flow measured at the terminal aorta was increased by more than 50%, superior mesenteric and renal flows were decreased by about 30% and 15%, respectively, and common carotid flow remained almost unchanged. The increase in hindquarter flow in the response was much less after beta adrenoceptor blockade with propranolol. In adrenalectomized rats the flow changes in the hindquarter, mesenteric and renal areas were less marked in either direction. These findings indicate that the blood flow redistribution in the transposition response is characterized by a shift of flow from the viscera to the skeletal muscles, in which the alpha and beta effects of catecholamines released from the adrenal medulla in the response play a dominant role.

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HYPOTENSIVE EFFECT CAUSED BY RAPID TRANSFUSION INTO ARTERY

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The constant velocity blood transfusion (0.5 - 3.0 ml/sec., total 10 - 15 ml) into central artery was made on rabbits under urethane anesthesia and central arterial pressure (CAP), central venous pressure (CVP), heart rate and others were continuously recorded. The experimental data, however, indicated that the effect on CAP was considerably different from the theoretical result on the basis of circulatory dynamics.

At the beginning of the transfusion, CAP rised gradually but about 3 seconds later the effects reversed and CAP dropped gradually in spite of continuation of the transfusion. Sometimes this hypotensive effect was so marked as the CAP was fairly lower than before the perfusion. After the cessation of the transfusion, the drop of CAP continued and CAP reached its minimum value 1 - 2 seconds after the cessation, then it rised again gradually toward the higher level than before the transfusion. The drop of CAP during the perfusion were rather accentuated by the section of buffer nerves and vagi, but ganglion blockade (hexamethonium) suppressed these effects.

It is concluded that the hypotensive effect caused by constant velocity transfusion is not originated from barostatic reflex via buffer nerves and is not a kind of hemodynamic phenomenon. The existence of unknown circulatory reflex is suggested.

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DYNAMIC ANALYSIS OF CIRCULATORY EQUILIBRIUM THEORY APPLIED TO THE MEASUREMENT OF VENOUS COMPLIANCE

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Dynamic analysis of the transient responses of the closed circulatory system showed that stepwise shift of cardiac function causes mono-exponential change in the venous pressure with a time constant (τ) given by $\tau = Cv/(Gco + Gvr)$, where Cv is the systemic venous compliance and Gco and Gvr are the gradients of cardiac output and venous return curve, respectively. The validity of this model was experimentally confirmed by the identity between the transient and steady state venous return curves. The quite limitted effect of the carotid sinus baroreceptor refrex on the step response also supported the validity of assuming the constant cardiovascular parameters during the response.
The step response of the system were induced by the step changes in cardiac function using the right heart bypass and by inflation and deflation of balloon in the right atrium. The time course of venous pressure change showed almost mono-exponential function of time in both cases.

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ON THE METHODS FOR PERFUSION OF A WORKING RABBIT HEART IN VITRO.

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Kodama's method (1966) for perfusion of an isolated rabbit heart made it possible to record simultaneously internal pressure of aorta, vena cava, and pulmonary artery and vein. The activity of the heart, however, was not sufficiently high for the analytical investigation of working mammalian hearts in vitro. We have attempted to improve the activity, slightly modifying the original method and isolation procedure of the heart. By retrograde perfusion of a cold Krebs-Henseleit's solution from the aorta to the heart during isolation, the activity of the heart was well maintained. The perfusion system was changed to the more simple one, and the effects of noradrenaline (NA) on the heart were tested. NA produced increases in the pressure of aorta, left ventricle and pulmonary artery with an increase in dLVP/dt. These increases resulted in reduction of pressure in left and right atrium. However, our method is still necessary to be improved for a more precise analysis of working mammalian hearts in vitro.

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A THEORETICAL STUDY ON THE FOUR ELMENTS MODEL OF ARTERIAL SYSTEM

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A theoretical study led us to conclude that a modified Windkessel model was of high practical value, the model being composed of four elements, i.e. inductance L, compliance C, characteristic resistance Rc, and peripheral resistance R_p. The model proved to be equivalent to a simple network model of arterial system consisting of numerous symmetrical Y-junctions. Interrelations between the two models were investigated under a few assumptions. The values of L, C, and R_c in the four elements model were equal to those in the inlet vessel of the network model multiplied by respective numerical coefficients. The resistance R_c corresponded to the characteristic impedance of the central aorta. Based on the four elements model, an attempt was made to reconstruct aortic pulse wave from a measured aortic flow pulse. The contour of the calculated pressure wave was in good agreement with that of measured pressure pulse.

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HEART RATE INDEPENDENT RELATION BETWEEN LEFT VENTRICULAR SYSTOLIC PRESSURE VOLUME AREA AND OXYGEN CONSUMPTION PER BEAT

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We have previously shown that left ventricular systolic pressure volume area (PVA) linearly correlates with its oxygen consumption per beat (Vo2) regardless of contraction mode in a canine heart with stable inotropism. PVA is the area in the pressure-volume (P-V) diagram circumscribed by the end-systolic and end-diastolic P-V relation curves and the systolic segment of the P-V loop. We now studied whether primary changes in heart rate would affect the Vo2-PVA relation. In the excised cross-circulated canine heart whose left ventricular pre- and afterloads were controlled with a servo pump, we changed heart rate by pacing to compare the Vo2-PVA relations at low and high heart rates. The Vo2-PVA relation was found to be independent of heart rate. We therefore concluded that PVA can serve as a reliable predictor of Vo2 in a given canine left ventricle with a stable inotropic background regardless of changes in heart rate as well as changes in loading conditions.

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DYNAMIC RESPONSES OF CARDIAC OUTPUTS AND SYSTOLIC TIME INTERVALS TO SUPINE AND UPRIGHT EXERCISE.

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Transient and steady state responses of stroke volume (SV), heart rate (HR), cardiac output ($\dot{Q}$), left ventricular ejection time (LVET), pre-ejection period (PEP) and the ratio of LVET to PEP during bicycle exercises of 50 and 100 W were studied on four healthy men in a supine and upright postures. A computer based system in which impedance cardiography was incorporated served to determine the above parameters on a 10 sec interval basis. SV remained almost unchanged in response to exercise in a supine posture while it increased significantly in an upright posture although the individual differences among subjects were found to be large. The half response times of variables to step work load tended to increase with increasing work load but did not vary in response to postural stress. An approximate accordance was observed among the response times for HR, $\dot{Q}$ and LVET/PEP. There was an inverse relationship between LVET and HR, the slope of which was found to be steeper in the supine posture than in the upright posture, reflecting the postural difference of SV. LVET fell shortly after the cessation of exercise despite the decreasing HR. Inasmuch as the paradoxical reduction of LVET was also found in the case when SV remained unchanged in response to exercise, no change in SV can be the cause thereof. Thus, a transient increse in ejection rate, which is probably due to increased myocardial inotropy,may be responsible for the phenomenon.

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CHANGES OF ERYTHROCYTE VELOCITY AND FLUX IN MESENTERIC ARTERIOLES DURING THE MANIPULATION OF SYSTEMIC CIRCULATION.

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The effect of systemic circulatory changes on arteriolar erythrocyte velocity and flux was investigated in rabbit mesentery in relation to regulatory function. A new instrument for measuring erythrocyte velocity in arteriole was devised on basis of the dual-slit photometric technique. The photo-electric signal from a pair of sensors was simultaneously recorded with arterial blood pressure and cardiac output. The erythrocyte flux was determined by counting up the peaks of photo-sensor signal due to the passage of each erythrocyte. The manipulation of systemic circulation was induced by noradrenaline infusion or by loading Ringer's solution.

It was found that the erythrocyte velocity in small arterioles (20-40 μm diameter) was linearly inceased with the elevation of systemic blood pressure. When systemic pressure was elevated, the total peripheral resistance was increased but the arteriolar constriction was not evident. This indicates that the increasing rate of arteriolar resistance in the mesentery may be less than that of other organs.

The erythrocyte flux was also found to be linearly increased with the erythrocyte velocity within the physiological range of systemic pressure change.

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EFFECT OF VASODILATOR SUBSTANCE ON AORTIC BLOOD VELOCITY PATTERN.

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Blood flow was measured with EM flowmeter at the ascending, descending, and abdominal (proximal and distal of the renal artery) aorta, femoral and common carotid artery of the mongrel dogs under nembutal anesthesia and open heart surgery. Characteristic flow patterns with reflection wave and after-vibration were observed in the distal portion of aorta and arteries in 10 out of 11 cases under the control condition.

Sodium nitroprusside, a vasodilator substance, was then infused for 30 minutes at 5μg/Kg BW per minute. Mean arterial, pulmonary arterial, left and right atrial pressures decreased as expected with reduction in stroke volume and increase in heart rate. The oscillating pattern of the distal aortic and arterial blood velocity disappeared in 80% and reappeared 30 minutes after the infusion. Mechanism of the remarkable changes in flow pattern was studied by computer simulation with a lumped constant model of the arterial system. As the result it was concluded that only the capacitance increase of the proximal aorta probably due to the nonlinear pressure-volume relationship can account for the pattern change.

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NONUNIFORM SYMPATHETIC NERVOUS AND HUMORAL CONTROL IN INTERNAL DIAMETERS OF SMALL ARTERIES IN THE KIDNEY AND EAR OF RABBITS

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With angiography we measured internal diameters (ID's) of small arteries in the kidney and ear of anesthetized rabbits. Renal interlobar arteries under innervated condition or with 2.5 Hz electrical stimulation of peripheral cut end of renal nerve were constricted uniformly about 80% of those under denervated condition. In ear central arteries, ID's under innervated condition and with 2 Hz stimulation of cervical sympathetic nerve ranged from 25 to 100% of those under denervated condition depending on location, and in ear marginal arteries ranged from 34 to 125%. Administration of α-blocker (phentolamine mesylate) following sympathetic denervation caused no increase in ID's of renal interlobar arteries and ear marginal arteries but caused marked further increase in ID's of ear central arteries, especially in distal portion. We concluded that sympathetic nervous and humoral control in internal diameters of small arteries in the kidney and ear of rabbits is nonuniform.

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Effects of intravenous anesthetic agents on renal nerve activity

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The characterestics of the changes in renal nerve activity (RNA), arterial pressure (AP) and heart rate (HR) produced by intravenous injection of ketamine 5 mg/kg, pentobarbital 30 mg/kg and chloralose 10 mg/kg were studied in 21 Wistar rats in a control condition of 50 % nitrous oxide and pancuronium infusion. RNA initially decreased by all anesthetics, and then increased above control by ketamine, but returned to control without overshoot by pentobarbital and chloralose. AP first decreased by ketamine and pentobarbital, and gradual recoveries followed. On the other hand, AP did not significantly changed by chloralose. HR immediately decreased by all anesthetic agents and soon returned to control. Central arterial pressure was changed by a thracic descending aorta occlusion to observe effects of the three anesthetic agents on AP-RNA relationship. A slope of the AP-RNA relationship curve became steeper than control at 5 min after ketamine, and returned to control at 20 min. By pentobarbital the curve tended to shift to the left. On the contrary, the curve tended to shift to the right by chloralose.

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RESPONSES OF SYMPATHETIC RENAL NERVE ACTIVITY TO QUICK MILD HEMORRHAGE.

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Neural control of the arterial pressure was studied by recording activities of the sympathetic renal nerve (RNA) responded to quick hemorrhage in 6 anesthetized rabbits (Urethane 1g/Kg, i.v.). Instantaneous arterial pressure fall caused by the hemorrhage was followed by rapid recovery of the arterial pressure in 5 sec and then the arterial pressure recovered slowly and reached a quasi steady-state between 1 to 2 min. Instantaneous arterial pressure fall (ΔAP_I) and arterial pressure fall during a quasi steady-state (ΔAP_S) from the control level increased, when bled volume was increased (from 0.5 to 3.0 ml/Kg). Also rapid increase in integrated (every sec) RNA (first peak) was usually followed by instantaneous decrease in RNA within 5 sec and then RNA increased again (second peak). Increase of the first peak of RNA (ΔRNA_1) and second peak of RNA (ΔRNA_2) were measured as a percent of the control RNA before the hemorrhage. Both ΔRNA_1 and ΔRNA_2 tended to increase in almost parallel with increase in ΔAP_I and ΔAP_S. And then the greater ΔRNA_1 and ΔRNA_2, the smaller open-loop gain of the arterial pressure control system, which was estimated from ΔAP_I and ΔAP_S. The results suggest that RNA may reflect open-loop gain and gain of the system decreased due to parametric forcing when bled volume was increased.

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RESPONSIVENESS OF BAROREFLEX SYSTEM IN CONSCIOUS RABBITS.

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We attempted to estimate the overall open-loop gain (G) of the rapidly acting arterial pressure control system in conscious rabbits and the effect of anesthesia by Nembutal on G. Two catheters for arterial pressure measurement and for hemorrhage were chronically placed at the aortic arch via the left subclavian artery and via the left common carotid artery, respectively. The quick mild hemorrhage (2 ml/kg body weight and within 1 sec) was repeated 8 to 16 times under conscious state. The mean arterial pressure responses to the mild hemorrhage were sampled by a digital computer through an analog-to-digital converter every 100 msec and averaged for each animal by the digital computer. G was assessed as $\Delta AP_I / \Delta AP_S - 1$, where ΔAP_I and ΔAP_S are the immediate and steady-state falls in mean arterial pressure following the quick mild hemorrhage. The mean value of G (±SD) for 15 conscious rabbits was 7.35 (±2.94). To estimate the effect of Nembutal on G, the quick mild hemorrhage was repeated at intervals of 7 min after an intravenous injection of Nembutal (27.5 mg/kg body weight). G decreased to the lowest value of about 2.5 to 0.5 about 30 min after anesthesia and returned gradually to around the control level within 50 to 80 min after anesthesia.

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EFFECTS OF ATHEROSCLEROSIS ON MEAN LEVEL AND STABILITY OF ARTERIAL PRESSURE IN CONSCIOUS RABBITS. MORITA, H., BABA, M., HAYASHIDA, Y., AND HOSOMI, H.
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Seven normal rabbits and 8 Watanabe-heritable-hyperlipidemic rabbits (WHHL-rabbit), the strain with a consistently inherited hyperlipidemic trait produced by inbreeding from a mutant discovered in 1973, were chronically instrumented a pressure catheter at the aortic arch through the left subclavian artery. Mean arterial pressure under conscious state was recorded on the strip chart and sampled every second by a digital computer through an analog-to-digital converter over 6 hours. Mean level and standard deviations of arterial pressure were calculated for each animal, first. Secondly, mean (±SD) values of mean level and standard deviations of arterial pressure were calculated for 7 normal rabbits and for 8 WHHL-rabbits. Mean (±SD) values of mean level of arterial pressure for 7 normal and 8 WHHL rabbits are 86.1 (±8.0) and 116.8 (±10.7), respectively ($p<0.05$ by Wilcoxon's location test). Mean (±SD) values of standard deviations of arterial pressure for 7 normal and 8 WHHL rabbits are 6.53 (±1.40) and 9.62 (±1.86), respectively ($p<0.05$ by Wilcoxon's location test). We conclude that atherosclerosis increases mean level of arterial pressure and decreases stability of arterial pressure. Decrease in stability of arterial pressure is probably caused by a malfunction of the arterial pressure control system in the WHHL-rabbit.

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SEGMENTAL HETEROGENEITY OF THE VEIN : FROM THE FUNCTIONAL AND MORPHOLOGICAL POINTS OF VIEW. MURASE, K., OHHASHI, T. AND AZUMA, T. Dept. of Physiol. and Res. Lab. for Cardiovasc. Dis., Shinshu Univ. Sch. of Med., Matsumoto 390.

Pressure-diameter relationships of cylindrical segments isolated from various portions of the external jugular, femoral and saphenous veins were recorded to examine regional differences in distensibility characteristics. Activation of venous smooth muscle by noradrenaline hardly affected the relationships of the proximal part of the external jugular vein, whereas the procedure significantly altered the characteristics of the remaining preparations. No contraction was induced in the proximal jugular preparations by the application of electrical stimulation and noradrenaline which had elicited a remarkable rise in circumferential tension of the remaining preparations. Morphological observations suggested that the pitch angle of spiral smooth muscle layers in the wall of the external jugular vein was much greater in the proximal part than in the distal and that there existed an appreciable difference in the smooth muscle content between valvular and intervalvular segments of the lower extremity veins.

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POTENTIATED EFFECTS OF COLD AND LOW-PRESSURE ON SHEEP PULMONARY HEMODYNAMICS SAKAI,A., UEDA,G., SHIBAMOTO,T*, YOSHIMURA,K*, FUKUSHIMA,M*, KUBO,K* AND KOBAYASHI,T*. Dept. of Adapt. Physiol., Dept. of Internal Med*, Shinshu Univ. Sch. Med., Matsumoto, Nagano.

Standing awake sheep, weighing between 25 and 35 kg, were exposed to a simulated altitude of 5,500 m under 20 °C room temperature (A group). Other sheep were exposed to the similar altitude under 0 °C room temperature (B group). For these, some hemodynamic parameters such as pulmonary artery pressure P_{PA}, left atrial pressure P_{LA} and right atrial pressure P_{RA} were obtained. In addition, systemic artery pressure P_{SA} and minute cardiac output CO were measured. In B group, 20 hrs of cold exposure preceded the experimentation. In both A and B groups CO and P_{PA} increased according as the simulated altitude was elevated. By the pretreatment, the initial values of CO and P_{PA} in B became greater than those in A. This tendency was maintained in each stepwise-elevated level. This was thus reflected in the increased right ventricular work rate RVW of B. Consequently, cold and low-pressure applied simultaneously potentiated the RVW of B. This would be considered also as the basis for the right ventricular hypertrophy of rats found at high altitudes in winter.

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EFFECTS OF INTRAVENOUS INFUSION OF SALINE ON THE LOCAL BLOOD FLOW OF TAENIA COLI IN THE RABBIT. KAMIYAMA, A., SHIBAYAMA, R. AND SHIDA, T. Dept. Physiol., Teikyo Univ. Sch. Med., Itabashi-ku, Tokyo

The hydrogen-gas clearance method was used to observe the effect of fluid infusion on the local blood flow of taenia coli of the rabbit. By the intravenous infusion of saline 20 ml/kg, increase of the local blood flow in the taenia coli was significantly larger than that in the submucosal tissue of the colon. However, as the motility of colon was also enhanced very much by the infusion, electrical stimulation or local application of acetylcholine to the colon was carried out to exclude a possibility of increase of flow caused by the enhancement of colon movement. The local blood flow was not so much increased by mere enhancement of motility. Moreover, after the application of 10^{-5} g/ml atropine, the enhancement of motility by the infusion was eliminated, but the increase of local blood flow in the taenea coli was still significant. However, the taenia coli did not show any special vascularity except a existence of relatively large vessels running along the taenia both in the microangiography and the regin casting method to the vascular system of colon.

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APPLICATION OF IMAGE SENSORS TO MEASUREMENT OF DIAMETER OF ISOLATED SMALL BLOOD VESSELS. SAKAGUCHI, M.*, OHHASHI, T., FUKUSHIMA, T. AND AZUMA, T. *Dept. of Elec. Eng., Nagano Tech. College, Nagano 380 and Dept. of Physiol., Shinshu Univ. Sch. of Med., Matsumoto 390.

A photoelectric diameter gauge was designed and constructed by the use of two image sensors attached to a slide calliper placed on the screen plane of an universal projector. This device enable us to measure outer diameters of isolated blood vessels as small as 100 μm. The resolving power of the device was about 1.4 μm, and its frequency response was flat up to 20 Hz. Using this diameter gauge, responses of the proximal and distal saphenous artery of dogs to 10^{-7}M adrenaline and noradrenaline were recorded simultaneously with changes in perfusion pressure. The drugs induced a remarkable relaxation followed by a long lasting constriction in the proximal segments with a diameter of between 800 and 1000 μm. The relaxing response was suppressed completely by pretreatment with 10^{-6} M propranolol. The drugs, however, only induced a constriction in the distal segments of from 300 to 500 μm in outer diameter. The constrictive response vanished after pretreatment with 10^{-6} M phentolamine.

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ESTIMATION OF CARDIAC OUTPUT FROM VOLUME CHANGE IN LEFT UPPER ARM. OKAI, O., HIGUCHI, Y. AND MAKINO, K. Dept. of Physiol. Kyorin Univ. Sch. of Health Science

An estimation of cardiac output was made from volume change in the left upper arm noninvasively. In general blood vessel is distensible by increased blood pressure ΔP; ΔQ is $C\Delta P$ where C is a compliance. In the ascending aorta increased volume ΔQ_a can not be measurable so that increased volume ΔQ_b caused by pressure change ΔP in the left upper arm was measured. In this case ΔP is $R_1 F$ where aortic resistance $R_1 = k/S_a^2$; S_a and S_b are crossectional areas of aorta and brachial artery; k is constant.[1] Pulse propagation times t_a and t_b from R wave to hip and left elbow joints, respectively were measured. Cardiac output is given as $F = S_a \Delta Q_b/(S_b t_b^2)$. Taking an ratio of cardiac autputs between control and loaded conditions, we get $F/F_o = (t_a/t_{ao})^8 (t_{bo}/t_b)^2 \Delta Q_b/\Delta Q_{bo}$. From the cardiac output we calculated blood pressure. Namely, the validity of the calculated cardiac output was confermed by the agreement between the pressures calculated and observed. Subjects were loaded by runing at 4-6 miles per hour for two minutes. In compaison with control the pressures calculated were obtained within an error of 20 %. Therefore, the calculated cardiac output were also estimated within an error of 20 %.

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CHANGES IN STROKE VOLUME AND HEART RATE IN RESPONSE TO INCREASED VENOUS RETURN IN DOGS.

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We studied the relative importance of increases in stroke volume and heart rate in dealing with increased cardiac output caused by increased venous return. One of the most convenient methods for instantaneously increasing or decreasing venous return is to open or close an A-V fistula. The A-V fistula was made between the abdominal aorta and the inferior vena cava in 13 anesthetized dogs. The fistula flow volume measured using a magnetic flow probe was regulated (0-1.3 l/min) by a pump in the fistula circuit. Cardiac output and stroke volume were monitored using a magnetic flow probe instrumented at the ascending aorta. Arterial pressure was measured with a catheter-transducer system at the aortic arch. Heart rate was monitored. Venous return increases with increasing the fistula flow volume. But increase in venous return was less than increase in the fistula flow volume, because the blood flooded into the inferior vena cava through the fistula was pooled in the venous system. Increasing venous return by 0-75% of control, stroke volume increased by 0-55% and heart rate increased by 0-10%. We concluded that increase in cardiac output caused by increasing venous return (0-75% of control) was achieved by increase in stroke volume rather than heart rate.

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EFFECT OF NOREPINEPHRINE ON TENDON TISSUE TEMPERATURE OF TIBIALIS ANTERIOR IN RABBIT.

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Local blood flow of the tendon was observed by an indirect method measuring tissue temperature. A needle type of thermocouple-electrode with 0.6 mm in diameter was inserted into tendon and muscle tissue of tibialis anterior in rabbit anesthetized with urethane. Physiological sensitivity and response of the thermocouple in the tissues were checked by occlusion of femoral artery. With spontaneously stabilizing arterial pressure, tendon tissue temperature decreased with the increased severity of local exercise in M. tibialis anterior evoked by sciatic nerve stimulation at 1-20 Hz. The same decrease was observed in tendon tissue tugged mechanically from the peripheral side, as indicating -0.2°C at 20g and -1.0°C at 100g of tension force, respectively. Intravenous injection of norepinephrine produced the decrease of tissue temperature in muscle and tendon : 3.3ug/kg showed -0.42°C and -0.62°C, and 9.9ug/kg, -0.58°C and -0.80°C in mean(n=5), respectively. These results suggest that mechanical factor plays a role in regulation of tendon blood flow.

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EVALUATION OF THE CAHNGES OF BLOOD FLOW DISTRIBUTION IN THE LEGS AFTER LOCAL THERMAL STRESS

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The electrical admittance plethysmography and the Xe-133 clearance method were simultaneously applied to human legs to measure the changes of the blood flow distribution after local heating. In this study the total flow(Fy) of a leg segment was measured by the plethysmography, and the muscle and the skin flows(Fm and Fs) by the clearance method. The flow of other tissues(f) excluding the muscle and the skin was calculated from the equation; $Fy=0.65\cdot Fm+0.1\cdot Fs+0.25\cdot f$, where the constants 0.65, 0.1, and 0.25 represent the volume ratios of the muscle, skin, and other tissues to the total segment, respectively. Measurements were performed on healthy and diabetic subjects before and after warming the leg locally for 15 min in 42-44 °C water bath. The results of this simultaneous measurement seem to offer quantitative data concerning the difference in the microcirculatory function between healthy subjects and patients.

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Multi-compartmental analysis of "metabolic indices" of blood buffer system.
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Based on a two-compartmental model of blood buffer system, in which red blood cell membrane is assumed to be selective in permeating H^+ and HCO_3^- ions, a buffer curve equation was derived:

$$[HCO_3^-] = \frac{-((1-Ht)k_p + Ht\cdot K_c')(pH - 7.4) + 24(Ht(a-1-7.4b) + 1) + [BE]}{(1 - Ht) + Ht(a - b\cdot pH)}$$

where Ht is hematocrit; K_p and k_c', buffer slopes of plasma and red blood cells; and a and b, linear coefficients of partition of HCO_3^- between plasma and cell. This equation was led to explicit hyperbolic function of base excess and buffer base curves analytically which fit the observed points in the Siggaard-Andersen's nomogram. The parameter values obtained on the least square basis: K_p = 10.6, k_c = 40.0, a = 3.18, b = 0.324, p = 41.42, q = 5.5 ([NBB]=p+qHt). As the result, direct formulae were given to estimate the "metabolic indices" of blood acid-base status without reading the nomogram: $[BE]=0.03pCO_2 10^{(pH-6.1)}(Ht(a-1-bpH)+1)+((1-Ht)K_p+Htk_c')(pH-7.4)$ + const.

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EFFECT OF CO_2-RICH GAS PASSAGE THROUGH THE WHOLE BLOOD ON THE Na^+ AND HCO_3^- ION CONCENTRATION IN PLASMA .
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We have previously reported that Na^+ and HCO_3^- ions were released from red blood cell (RBC) to plasma when dog's whole blood was exposed to a CO_2-rich gas(60% N_2, 25% O_2, 15% CO_2) and concluded that,very probably, at least two channels were in operation;(1)chloride shift and (2)transport of Na^+ and HCO_3^- in one-to-one relations from RBC to plasma. In the present study, we investigated the reversibility of the ion "transport" under discussion. For this purpose, dog's whole blood was exposed to the above gas mixture for up to 30 minutes, then to the room air for another 30 minutes. Experiment was conducted on human whole blood as well. Results were as follows; (1)When dog's whole blood was exposed to a CO_2-rich gas,the concentration of Na^+ and HCO_3^- ions increased and that of Cl^- ion decreased in plasma, and these changes appeared to be almost reversible. (2) A similar result was obtained with human whole blood during the passage of a CO_2-rich gas.

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EFFECT OF ELECTROSTATIC INTERACTION ON TRANSGLOMERULAR PERMEABILITY OF FERRITIN.
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To clarify the electrostatic charge barrier in the permeability of glomerular capillary wall, polycationic protamin sulfate solution was infused to rats with a purpose of reducing the anionic charge of glomerular walls, and the permeability of anionic ferritin was compared to that of control animals, infused with negatively-charged egg-albumin solution. That of cationized ferritin was compared also with anionic ferritin in the rats infused with egg-albumin solution. The anioinc charge of the glomerular wall was stained with ruthenium red(RR). The permeation of anionic ferritin was greater in the rats treated with protamin sulfate than those with egg-albumin in all three layers of GBM. It was further revealed that much more cationic ferritin permeated into the three layers of GBM than the anionic. The RR positive particles decreased in protamin treated rats significantly in lamina rara interna but not in lamina rara externa, suggesting the significant decrease of anionic charge of the former by infusion of the polycation. These findings indicated that the electrostatic repulsion, interacting between the anionic charge of GBM and that of the macromolecules, induces a barrier-effect on the transglomerular permeation of the macromolecules.

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HORMONES AND ELECTROLYTES RESPONSES TO EXERCISE

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Effect of work intensity on hormone secretion and electrolyte balance were studied in normal males in age from 25 to 32 years. Work intensity was 100, 84, 61 and 43% of maximal oxygen uptake. Work duration was 20 min except exhaustive exercise(12 to 16 min).

Plasma angiotensin II and aldosterone levels post exercise was elevated in related to work intensity, and the higher the work intensity, the greater the increase in urinary excretion of catecholamines. The positive coefficient of correlation between urinary nor adrenaline and kallikrein excretion was observed following exhaustive exercise ($r=0.7670$, $p<0.001$), but negative correlation in 43% of $\dot{V}O_2max$ exercise ($r=-0.7595$, $p<0.001$). No relationship was observed between plasma aldosterone levels and urinary excretions of Na and K during 2 hours of recovery. Immediately after exercise, systolic blood pressure, plasma angiotensin II level and catecholamine excretion was found elevated. But, diastolic blood pressure was always found decreased after severe exercise.

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MODIFIED INTERPRETATION FOR RENAL AUTOREGULATION INCLUDING VISCOSITY THEORY

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Autoregulation of the renal blood flow has so far been explained from various aspects, such as myogenic, viscosity and many other factors, although the regulation seems to be affected by denervations and some drug agents. Here, this phenomenon was attempted to interpret firstly from the Starling hypothesis. Thus the equilibrium point of filtration and reabsorption was considered to be between afferent and efferent vessels. This corresponds to the efferent site in the case of glomerulus. The osmotic pressure can be a regulating force in the filtration fraction quantity. Also, a parameter used by Papenfuss, ε, which is an ultrafiltration coefficient, can be a regulating factor. It can be expressed as a product of filtration and longitudinal resistance. In the latter factor, a viscosity is included. When ε is increased, filtration begins to be weakened and reabsorption take place. Furthermore, a constant-flow generator circuit was assumed to be contributing to the autoregulation, as noticed from the anatomical similarity.

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EFFECTS OF ORAL ELECTROLYTES ADMINISTRATION ON URINARY Na,K EXCRETION IN JAPANESE YOUNG MEN(4)

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Natriuretic effects of potassium(K) was examined in this experiment. Latest study revealed that among several test solutions to drink(500 ml, the same osmolarities as 0.9% NaCl solution, containing only water, NaCl, and/or KCl), the solution whose Na/K ratio was 2 had the best natriuretic action. Our hypothesis is that Na/K ratio is essential in according for the results. However, sodium(Na) or K concentration in the Na/K=2 solution itself may lead the results. So, this experiment was conducted.

After more than 10 hours' overnight fasting and water depletion, test solutions(0.6% NaCl or 1.2% K-gluconate, 500ml) were administrated to Japanese young men (n=12, 19-22 yrs, male) under the condition of Ta=23$\pm$2°C, 40-65% RH. Serum Na,K levels were examined hourly, urinary Na,K excretion rate, by 30 min. for 5 hours.

Natriuretic effects of the both solutions were smaller than that of the Na/K=2 solution.

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EXCERCISE AND ELECTROLYTES METABOLISM IN MEN(5) YAMADA,T. AND NISHIMUTA,M.
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Effects of moderate physical excercise(intensity,120 beats/min. of heart rate; duration, 20 min.) after potassium (K) loading (drinking 1.2% K-gluconate 500ml, 2 hours before excercise, after more than 10 hours overnight fasting and water depletion) on Na, K metabolism were detected in Japanese young men (n=12, 19-22 yrs, male) under Ta=23±2°C, RH=40-65% conditions.

Serum level and urinary excretion of K were increased within 60 min. with the increase of urinary Na excretion, but without serum Na level by the K loading. Creatinine clearance was not changed obviously throughout the experiment. Moderate physical excercise decreased urinary Na excretion, without any obvious changes of urine flow, urinary K excretion, nor creatinine clearance, compared with the results of control (no excercise) experiment.

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COMPARATIVE STUDIES ON THE BEHAVIORS OF ISOLATED PERFUSED RAT-LIVER AND -KIDNEY BY THE ADMINISTRATION OF UROKINASE. SAKAI, J. SAEDA, Y. YAMAMOTO, H. AND SUGIE, I. Dept. of physiol.,Aichi Med. Univ., Nagakute-cho, Aichi.

To clarify the function of organs in fibrinolytic system, perfusion of rat-liver and -kidney were employed according to Miller and Weiss, respectively, using both open and closed circuit perfusion. Enzyme activities of perfusate and excreted fluid were measured by the synthetic substrates and the fibrin plate method following by perfusion of urokinase which was purified enzyme of plasminogen activator. 1) In the case of the closed circuit perfusion; UK-disappearance curves of perfusate in rat-liver and -kidney consisted of two components of initial sharp disappearance and following slow disappearance. These initial and following ones can be identified as take-in rate and metabolism rate. The rates of take-in and metabolism of rat-kidney were almost 3 times than that of liver but total disappearance of enzyme activities of liver were large than that of kidney. 2) In the case of the open circuit perfusion; UK activities in perfusate by rat-liver perfusion had not almost the changes except for the slight take-in, and in bile the plasminogen activator activities increased gradually. UK activities by rat-kidney perfusion had also the take-in, and continued in perfusate and urine after the end of administration period. From these results it is suggested that the physiological function of kidney in fibrinolytic system assumes a kind of regulator of UK.

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MECHANISMS FOR INDUCTION OF DRINKING IN THE EEL.
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In mammals, it has been suggested that three mechanisms are in function as physiological thirst stimuli; a volume stimulus (extracellular dehydration), a osmotic stimulus (cellular dehydration) and angiotensin II (AII). In contrast to those extensive studies, only little has been studied in fishes, although they do possess drinking mechanisms. Further, it is possible that fishes are more advantageous material for analyzing drinking mechanisms than mammals because of their aquatic life. We have obtained following results in the eel, Anguilla japonica; hypovolemia and injection of AII increased drinking but hypernatremia failed to increase it, and plasma AII concentration increased following hypovolemia or hypernatremia. These results show that hypernatremia is not a sufficient stimulus for drinking even with a conjoint action of increased plasma AII in the eel. This is in contrast to the result obtained in the quail, Coturnix coturnix japonica, that hypernatremia caused drinking though it decreased plasma AII. It seems that drinking mechanism activated by osmotic stimulus is not yet developed or has degenerated in the eel.

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REACTIVE SYNTHESIS AND RELEASE OF PROSTAGLANDIN I_2 (PG I_2) BY CORONARY VESSELS. OGAWA, K., MIKI, M., HIRATA, M., KITAGUCHI, H. AND FUNAHARA, Y. Dept. of Physiol., 1st Div., Kobe Univ. Sch. Med., Kobe

Using isolated dog heart perfused via aorta at 0.12 ml/g of heart weight/min with modified Krebs-Henseleit buffer (37°C in 95% O_2, 5% CO_2), the synthesis and release of PG I_2 by coronary vessels were examined. PG I_2 in the venous effuluent, collected every 10 sec and kept at -80°C, was determined by the platelet-aggregation inhibiting activity and the radioimmunoassay. PG I_2 activity was demonstrated transiently; at 40~50 sec after the start of the infusion (continued for 30 sec) of thrombin (25 u/ml) or bradykinin (5×10^{-6} M), the activity began to be demonstrated, reaching the maximum at 90~120 sec, then decreased to zero at about 210 sec. The appearance of the activity by bradykinin was inhibited by the presence of tranylcypromine (10^{-3} M) or pretreatment of the heart by indomethacin (preinfusion of 3×10^{-5} M solution for 30 min), indicating the PG I_2 release was coupled with the synthesis. From these experiments and others (Kitaguchi et al. Thrombos. Res. 16:159-173, 1979), blood vessels were demonstrated to have two kinds of reaction to bradykinin; the very rapid release reaction for F. VIII and plasminogne activator, and the slow reaction for PG I_2.

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ON HEMATOLOGICAL AND BIOCHEMICAL CHANGES IN PERIPHERAL CIRCULATING BLOOD DURING DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS IN THE RABBIT. ASANO, M., HIROKAWA, A., OHKUBO, C., OGAWA, S., SAWANOBORI, K., AND YONEKAWA, K. Dept. of Physiol. Hyg., the Inst. of Publ. Health, Minato-ku, Tokyo

Definite and marked atherosclerotic changes of aortas were observed macroscopically in male rabbits who were fed a cholesterol-supplemented(1.5%, w/w) standard diet for a period of 12 weeks. During the experimental period, decrease in counts of erythrocytes and platelets, hematocrit values, hemoglobin concentrations, and erythrocyte sedimentation rates and increase in leukocyte counts and carboxyhemoglobin concentrations of peripheral circulating blood were evidenced in association with enormous rise in plasma cholesterol levels. Although no appreciable atherosclerotic signs were observed on aortas after daily inhalations of cigarette smoke throughout the same period, increase in erythrocyte counts, hematocrit values and hemoglobin concentrations, in contrast to, and decrease in platelet counts and carboxyhemoglobin concentrations, in coincidence with the cases of cholesterol feeding, were noticed without plasma cholesterol changes. In comparison with the hematological and biochemical responses to cholesterol alone, no remarkable qaulitative alterations were noticed on the changes due to combined loading of cholesterol and smoke of cigarette(with or without glass-fiber filter for particulates), although slight quantitative modifications were observed on them.

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ROLES OF ALBUMIN, Na^+ AND K^+ IONS IN THE REGULATION OF RED BLOOD CELL DEFORMABILITY KIKUCHI, Y. AND KOYAMA, T. Div. of Physiol., Res. Inst. of Appl. Electr., Hokkaido Univ., Sapporo

Interactions between the red blood cell (RBC) membrane, plasma proteins and electrolytes and their effects on RBC deformability were studied using fresh human blood, radio-isotope labelled human serum albumin and an improved Nuclepore membrane filter method. An increase in the adsorption of albumin to the RBC membrane was observed with increasing ionic strength of the medium induced by an addition of NaCl. This increased protein adsorption, which would restrict the RBC membrane flexibility, was considered to be a main cause of the reduced deformability of RBCs exposed to hyperosmolarity (below 450 mOsmol). Further, an interesting specificity of ion species was found; no increase in the protein adsorption was observed when the osmolarity was increased by adding KCl. It was supposed that the distributions of Na^+ and K^+ ions around the RBC membrane might play a special role in controlling the protein adsorption to maintain the flexibility of the membrane. As the ion distributions are maintained by the Na-pump, the effect of blocking of the Na-pump was examined. A reduction in RBC deformability became prominent in four hours after an addition of ouabain.

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MEASUREMENTS OF INTRA- AND EXTRA- CELLULAR $_{p}H$, AND DONNAN EFFECT IN WHOLE BLOOD.
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For the purpose of analyzing the respiratory function of blood, it is necessary to understand the relation between intra- and extra- cellular environments. The change of pH difference(ΔpH) between intra- and extra- cellular pH in the normal blood was studied. ΔpH was measured by the freezed and thaw method. In this method, it was preferable for the extracellular pH_e to use the whole blood pH ; plasma pH which coexists with red cell rather than using the plasma pH after separation without red cell.

The change of pCO_2 in the whole blood from the CO_2 titration experiment did not affect MCHC change but did to shift the pH_e and pH_i. On the other hand, acid-base titration experiment elucidated that the change of base excess(BE) was influential to the change of MCHC. And also in these results it caused the disagreement of correlation between pH_e vs. pH_i at CO_2 titration. In case of using the whole blood whose BE was less than ±10, pH_i was calculated from the equation of $pH_i=0.785pH_e+1.436$. If BE was more than ±10, another equation induced by acid-base titration was used, and pH_i was estimated by using pCO_2 and pH_e. The relation between pH_e and pH_i at each oxygen saturation was derived too.

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RED CELL DEFORMABILITY MEASURED BY CENTRIFUGAL ELONGATION AND RHEOCYTOMETRY

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Capillary tube centrifugal (CTC) technique is based on the observation of the cellular deformation by the centrifugal elongation. The capillary tube was quickly spun at 12,000 rpm for 1 min at 37 C and the red cells in the top portion of the tube were subjected to the centrifugal fields which induces cell deformation. The resulting deformation of cells was fixed with the fixative in the bottom portion of the tube. Discocytes showed a good deformation into handbag-like forms; echinocytes and stomatocytes had reduced deformability with characteristic shapes of deformation. Rheocytometer consists of a flat cell and the helium-neon laser. The laser beam was made to traverse perpendicularly to the gap of the flat cell and the diffraction pattern was obtained on the screen. Deformability index (D.I.) was calculated by the quantity $(L - W)/(L + W)$, where L is the measured vertical dimension of the diffraction pattern and W is the horizontal dimension. Deformability measurement was sensitive to (1) viscoelastic properties of membrane, (2) cell geometry, including surface area to volume ratio, but is only marginally sensitive to (3) internal viscosity of the cells by the CTC technique. The rheocytometry is sensitive to all of these regulating factors of red cell deformability.

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KINETICS OF ERYTHROCYTE ROULEAUX FORMATION USING TV IMAGE ANALYZER. II. EFFECTS OF PLASMA PROTEINS.
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In order to determine the formation rate of human erythrocyte aggregates in a slow shear flow, a computer controlled TV image analyzer was combined with a rheoscope (consisted of an inverted microscope and a transparent cone-plate viscometer). The numbers and areas of moving particles (at shear rates ranging 0.4 - 35 sec^{-1}) on TV images were continuously fed, the time course of area/number was computed and the sequential means (of 22 TV images) of these data were plotted up to 2000 sec. The gradual increase in the means of area/number reflected the formation of one dimensional aggregates (rouleaux), while the increase in the standard deviation resulted from the development of three dimensional aggregates.

Fibrinogen augmented the rate of aggregation considerably: i.e., no aggregates was observed in diluted serum (60%), but the aggregation occured upon addition of fibrinogen. At shear rate of 7.5 sec^{-1}, the formation rate increased proportionally to the fibrinogen concentration up to 800 mg/dl.

α_2- and γ-globulin fractions also facilitated the formation rate: γ-globulin gave more aggregates rather than rouleaux.

The formation rates depended on pH: the rate increased at higher pH.

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EFFECT OF OSMOLALITY ON BLOOD VISCOSITY AND RED BLOOD CELL DEFORMABILITY

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Hypertonic media not only produce morphologic changes in red blood cells (RBC), but also affect the RBC deformability including its membrane rotation. The purpose of this paper is to evaluate effects of hypertonic media on the blood viscosity through the RBC morphology and deformability in relation to contrast media.

We used healthy human (or dog) blood and meglumine sodium diatrizoate (Urographin) as high osmotic media; the plasma osmolality was changed by controlling the volume ratio of NaCl and Urographin to blood. We measured the viscosity for two kinds of RBC suspensions in plasma with a cell volume concentration at various osmolality; normal RBC and hardened RBC treated with glutaraldehide at an osmolality. We measured the RBC deformability from the difference in the two shear-rate and shear-stress curves for normal and hardened RBC.

Normal RBC suspension increased in the viscosity with the increase in the osmolality, but hardened RBC suspension viscosity decreased with increasing osmolaity. This indicates that the increased osmolality may increase the effective fluid-dynamical volume of a normal RBC in shear flow but decreased that of a hardened RBC and also that the RBC deformability decreases with the increased osmolality.

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DISTRIBUTIONS OF HEMOPOIETIC PRECURSOR CELLS (CFU-mix AND BFU-E) IN NORMAL HUMAN PERIPHERAL BLOOD.

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Circulating pluripotent hemopoietic precursors (CFU-mix) and erythropoietic precursors (BFU-E) in normal human peripheral blood were studied by a clonal cell culture technique. Blood mononuclear cells separated with a modification of the Ficoll-Paque technique were cultured with erythropoietin in methylcellulose. The erythroid colonies (BFU-E) and the mixed colonies (CFU-mix) that contain both erythropoietic and granulopoietic cells were observed in culture. Blood mononuclear cells responded to erythropoietin, producing various numbers of BFU-E and CFU-mix with a dose-response relationship. The numbers of BFU-E and CFU-mix were proportional to the number of blood mononuclear cells in a range from 1.5×10^5 cells to 6.0×10^5 cells per culture. The serial observations revealed that BFU-E and CFU-mix reached a peak in number at 14th day of culture. The studies of in vitro erythroid colony and mixed colony formation were performed in 15 cases of normal adult subjects. The distributions of CFU-mix and BFU-E in the condition of 14 days culture and with 1.0 U/ml of erythropoietin were 5 ± 7 (Mean ± SD) per 9×10^5 cells and 43 ± 38 per 3×10^5 cells, respectively.

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THE RELATIONSHIP BETWEEN EXPIRED CARBON MONOXIDE (CO) CONENTRATIONS AND BLOOD CARBOXY-HEMOGLOBIN (COHB) SATURATION FOLLOWING CIGARETTE SMOKE INHALATION IN THE RABBIT.

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The study was undertaken to ascertain the validity of using expired air as an indicator of COHb in the rabbit. Thirty male rabbits weighing from 2.2 to 2.5 kg were used under unanesthetized conditions. A specially devised respirator for the rabbit was applied so as to enable a continuous collection of the expired air samples. The relationship of CO concentrations to COHb saturation was simultaneously evaluated during the administration of cigarette smoke. The expired CO concentrations were measured with an electrochemical CO analyzer (CM-2510,GASTEC) and the COHb saturation was obtained using the spectrophotometric technique according to Commins and Lawther (1965). A regression of the COHb saturation (Y,%) to the expired CO (X,ppm) was $Y=0.35X + 0.1$ and a correlation coefficient between them was 0.87 ($p<0.01$). This shows that the technique of the present study to estimate blood COHb saturation by expired CO analysis is also applicable not only to humans but also to rabbits.

In addition, half elimination time estimated from the expired CO concentrations between 15 and 30 min after cigarette smoke inhalation was 21 ± 14.9 min (mean ± s.d.).

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ISOLATION AND PROPERTIES OF GLYCOSYLATED HEMOGLOBINS IN CANINE BLOOD.
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Two minor and one major hemoglobins were isolated from canine hemolysate when chromatographed on Bio Rex-70 with 0.05 M phosphate-0.005 M NaCl(pH 6.7). The first and second minor hemoglobins(Hb A_{I-1} and A_{I-2}) were determined as 1.2±0.3 and 3.0±0.7 % of the total hemoglobin in ten normal dogs. Hb A_{I-1} was electrophoretically heterogeneous, constituting three hemoglobins. Hb A_{I-2} was homogeneous with faster anodal mobility than the major(Hb A_0) and the difference was localized solely in the β chains. Thiobarbituric acid reaction was negative for the α but positive for the β of Hb A_{I-2}, indicating the presence of glycosyl ketoamine linkage. Fingerprint of the β revealed that the peptide βTp-I was absent and all the rests were the same with those in β from Hb A_0. Intrinsic oxygen affinity of Hb A_{I-2} was trivially lower than that of Hb A_0, while their alkaline Bohr effects and Cl^- effects were not different. The effects of DPG, IHP and CO_2 were much depressed in Hb A_{I-2} compared with Hb A_0. These structural and functional results suggests that Hb A_{I-2} was derived from Hb A_0 by specific glycosylation at the β N-termini.

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Gγ AND Aγ GLOBIN CHAIN BIOSYNTHESIS IN INDIVIDUAL ERYTHROPOIETIC BURSTS FROM ADULT AND UMBILICAL CORD BLOOD.
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We cultured human adult and umbilical cord blood mononuclear cells in methylcellulose and measured the synthetic rates of globin chains in individual erythropoietic bursts. Globin chains were labeled with ^{14}C-amino acids in culture, separated by isoelectric focusing in slab gel containing 8 M-urea and 3 % Nonidet P-40, and quantitated by fluorographic methods. All bursts exhibited Gγ, Aγ, and β chains in varying ratios. Cumulative frequency distributions of individual bursts differing in the ratios of Gγ/(Gγ + Aγ) and γ/(γ + β) approached normal distributions. These results indicated that perinatal hemoglobin switching and the hemoglobin switching of cultured adult erythropoietic precursors do not involve population changes in the precursors. Further, a significant correlation between γ/(γ + β) and Gγ/(Gγ + Aγ) ratios of individual bursts existed in all of the samples. Switching between γ and β chains and switching of Gγ: Aγ ratios may share common regulatory mechanisms.

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ASSAY OF A NEW THIOL PROTEINASE INHIBITOR IN SERUM
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A new proteinase inhibitor has recently been found in human serum which specifically inhibits thiol proteinase such as ficin, papain, bromelain and cathepsin B_1. However, serum contains α_2 macroglobulin which also inhibits these thiol proteinases, and, consequently, interferes with the assay of the new thiol proteinase inhibitor. Therefore, assay of the inhibitor in the serum has not yet been established. Using the present method, α_2 macroglobulin is inactivated by preincubating the serum in methylamine solution at 55°C while the new thiol proteinase inhibitor retains its activity. The necessary amount of serum is about 10 μl. This inhibitor is also found in mammals such as cows, pigs and rats. Vitamin E deficient rats show a very high inhibitor level. This protein inhibits thiol proteinase more effectively than α_2 macroglobulin. Therefore, the present simple method will enable us to investigate the relation between diseases and the activity of the new thiol proteinase inhibitor.

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ACTIVATION OF GLU-PLASMINOGEN BY UROKINASE AND STREPTOKINASE IN THE PRESENCE OF FIBRINOGEN, FIBRIN AND SK-POTENTIATOR TAKADA, A. AND TAKADA, Y. Dept. of Physiology, Hamamatsu Univ., School of Med., Hamamatsu-shi, Shizuoka

Coagulation processes are closely related to fibrinolysis. We have indicated that clot formation enhanced the activation of native plasminogen (Glu-plg) by urokinase (UK) and streptokinase (SK). The enhanced activation rate in the presence of fibrin may be related to a conformational change of Glu-plg when lysine binding sites (LBS) of the heavy chain part of plg bind with fibrin, since binding of ω-aminoacids to LBS results in the change of conformation of plg. In the present study Glu-plg was converted to plasmin by UK and SK in the presence of fibrin, fibrinogen and SK-potentiator. SK-potentiator, which is contained in human plasma, is composed of peptide chains similar to β and γ-chains of fibrinogen. SK-potentiator enhanced the activation rate of Glu-plg better than fibrinogen, but fibrin formation was still more effective. The results of SDS-polyacrylamide gel electrophoresis indicated: (1) Glu-plg I (of higher molecular weight) was converted to plasmin more easily than Glu-plg II. (2) Fibrinogen and SK-potentiator enhanced the conversion, and the rate in the presence of SK-potentiator was faster than that in the presence of fibrinogen.

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CONVERSION OF GLU-PLASMINOGEN TO PLASMIN BY UROKINASE IN THE PRESENCE OF TRANEXAMIC ACID TAKADA, Y. AND TAKADA, A. Department of Physiology, Hamamatsu University, School of Medicine, Hamamatsu-shi, Shizuoka

When Glu-plasminogen (Glu-plg) was incubated with urokinase (UK) in the presence of various concentrations of tranexamic acid and S-2251, the hydrolysis of S-2251 increased in the presence of 1 mM of tranexamic acid, but decreased in the presence of more than 10 mM of tranexamic acid. Binding of tranexamic acid with lysine binding sites (LBS) of Glu-plg resulted in the conformational change of Glu-plg and increase in the activation rate. Decreased extent of conversion of Glu-plg by UK at the concentration of tranexamic acid more than 10 mM was due to competitive inhibition of the activator activity of UK by tranexamic acid. Use of SDS-polyacrylamide gel electrophoresis indicated: (1) Glu-plg was converted to plasmin better in the presence of 1 mM of tranexamic acid, but in the presence of more than 10 mM of tranexamic acid it was less converted. (2) Glu-plg I was more easily converted than Glu-plg II by UK, but binding of tranexamic acid with LBS of plasminogen caused almost equal rate of activation of Glu-plg I and II.

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SPECIES DIFFERENCES OF FIBRINOGEN SUBFRACTIONS.
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In order to elucidate the physiological significance of fibrinogen subfractions, species differences of the subfractions were studied. Samples of blood plasma of human adult, human fetus, mouse, rat, rabbit, dog and ox were subjected to SDS polyacrylamide gel electrophoresis, and fibrinogen subfractions were compared qualitatively and quantitatively. Compositions of the subfractions of mouse and dog resembled to that of human. Amount of the low molecular weight fraction decreased in the following order: human, mouse, ox, dog, rabbit, rat. Between the subfractions of human adult and of human fetus, no difference was observed in their chemical structure. But their coagulative functions were different significantly from each other. Work is under progress to elucidate the mechanism of the difference.

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FIBRINOLYTIC ACTIVITY IN TRACHEOBRONCHIAL SECRETIONS OF RATS

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It is known that a plasminogen activator (PA) exists in the secretions of various organs, i.e., PA has been demonstrated in milk, tears, saliva, seminal fluid, urine and bile. However, the presence of fibrinolytic enzymes in tracheobronchial secretions has not so far been reported. We developed a new method for collecting the tracheobronchial secretions of rats, and succeeded in detecting fibrinolytic activity in such secretions. Furthermore, it was found that the substance exhibiting the fibrinolytic activity was not plasmin but PA. The PA in the tracheobronchial secretions was very labile at alkaline pH and on heating. These properties resemble those of the plasminogen activator with low molecular weight which is obtained from the human paranasal mucous membrane. In addition, the effect of certain vasoactive drugs on this tracheobronchial activity was investigated in conjunction with blood pressure measurements. Administration of noradrenalin increased the fibrinolytic activity in the secretions accompanied by an increase in blood pressure, but atropine did not increase the fibrinolytic activity with no change in blood pressure. Although detailed studies on the physiological and pathophysiological significance of the PA in the tracheobronchial secretions of rats are now in progress, the results obtained so far suggest that this PA may serve to inhibit obstruction and adhesion of the tracheobronchial lumen.

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CHANGES IN COAGULATION, PLATELET AND FIBRINOLYSIS DURING HYPOTHERMIA,

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The changes in coagulation, platelet and fibrinolysis during experimental hypothermia were studied in mongrel dogs. The animals were anesthetized with pentobarbital sodium and premedicated with chlorpromazine (CP) or triflupromazine(TP) preventing the ventricular fibrillation. Blood samples were drawn from superior vena cava through the catheter. The results obtained were as follows: (1)Coagulation: Recalcification time, APTT and prothrombin time were measured. In both groups, coagulation times were progressively shortened, but there were no significant difference between control and hypothermia groups. (2)Platelet function: In the control group, no significant changes were observed. However, in the hypothermia group, platelets were progressively decreased during the hypothermia. These reductions were recovered by rewarming. (3)Fibrinolytic activity: In the control group, no changes of fibrinolytic activity were observed. On the other hand, in the hypothermia group premedicated with TP, fibrinolytic activity was slightly activated, but no fibrinolytic activity was observed with CP.

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EFFECT OF LONG-TERM TRAINING ON ERYTHROCYTE OSMOTIC FRAGILITY IN MICE.

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Physical exercise has been found to be associated with a significant increase in the sequestration and destruction of erythrocytes,suggesting a state of increasing fragility and decreasing the deformability of the cells. To further study this phenomenon,mice were used following a long-term training to examine the effect of training on the osmotic fragility of erythrocytes,by means of a precise and reproducible method for analyzing the osmotic fragility of erythrocytes. The osmotically fragile erythrocytes decreased significantly,whereas the resistant cells showed no significant changes for the trained mice with respect to the untrained mice. The results suggest that a long-term training accelerates the sequestration and destruction of aged fragile erythrocytes and must be an important factor in the maintenance of an efficient hematopoietic tissue.

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INHIBITION OF PLATELETS AGGREGATION BY PER OS ADMINISTRATION OF ASCORBIC ACID(AA) IN GUINEA PIGS

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Unusually high concentration of AA in circulating platelets and suppression of platelets aggregation by AA added to the in vitro PRP have led the authers to make the present studies on the inhibition of platelets aggregation by per os administration of AA by using guinea pigs.

AA determination in platelets was made according to Attwood et al. AA in control animal was 75.4±10ug (per 10^{10}platelets). In the guinea pigs to which AA of 80mg per animal per day for 8 days were per os administrated, platelets AA examined on 9th day was 186.5±40ug (ca. 2.5 times of the control values). Platelets aggregation test with ADP of 0.75uM and 1.5uM indicated, the secondary response showed ca. 85% inhibition with per os administration of AA, while the first response, only weak inhibition. Similar results were also obtained by using arachidonic acid.

Therefore, recalling the early studies by Born et al. on the significant decrease of platelets adhesiveness in scorbutic guinea pigs, dual actions of AA in the intrinsic mechanism of platelets would be considered; the first is essential role for the early reaction such as the adhesiveness or the aggregation, the second, inhibition for the secretion reaction.

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Cardiac pacemaker-like characteristics of the central respiratory neuronal activities in the adult lamprey.

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To examine the breathing rhythm-generation mechanism, medullary neuronal activities extracellularly and branchial movements were simultaneously recorded in the adult lamprey. Result 1: Respiratory bursts following immobilization (i.v. d-tubocurarine) continued without changing essentially in rate and duration. 2: Respiratory burst discharges simultaneously and bilaterally detected from the two sides of the medial medulla synchronously occurred (time lag, 0-5 msec), while after sectioning the midline of the brain, the bilateral synchronization was completely disturbed, revealing that the symmetrical halves of the medulla can behave as respiratory pacemaker, respectively. 3: Each of test pulses (0.2 msec, 5-8 V) extracellularly applied to the medulla produced, in almost all of the respiratory cycle phase, one-to-one burst discharge followed by branchial movement, which was similar in the shape to spontaneously driven one. 4: So-called diastolic slow potential preceding the burst discharge was seen after immobilization. These results favour the hypothesis that the respiratory burst generators in the lamprey are essentially similar in their physiological characteristics to a cardiac pacemaker.

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ASCENDING RESPIRATORY INTERNEURONS CONTROLLING MOTOR DISCHARGES IN DRAGONFLY LARVAE.

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Intracellular recordings revealed interneurons involved in ventilation control of dragonfly larvae, that is, ascending expiratory interneurons (AE neurons). The AE neurons exerted burst of spikes during expiration. Their burst evoked by current injection increased the spike number and duration of the expiratory motor discharges and suppressed the discharges of an inspiratory motoneuron. Stimulation of a sensory nerve of the terminal ganglion which reset the respiratory rhythm of the motor discharges also reset the burst rhythm of the AE neuron. But their burst evoked by current injection did not reset the rhythm. Intracellular stainings revealed that the AE neurons have their cell body in the terminal ganglion, send an axon anteriorly to the ipsilateral connective and extend branches bilaterally in each abdominal ganglion. These results suggest that the AE neurons are the coordinating interneurons which convey the expiratory signal from the main oscillator located in the terminal ganglion to the motoneurons in each abdominal ganglion.

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Dynamic characteristics of medullary switching neurons in rabbits.

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In bivagotomized, d-tubocurarine-paralysed rabbits, efferent phrenic discharge was recorded as an indicator of the respiratory cycle. Short electrical stimulus trains(250Hz,5-21pulses) were applied to vagi, superior laryngeal and intercostal nerve and to the rostral pons and medulla, at various predetermined times in the cycle. Phase response curves of respiratory cycle, inspiratory and expiratory duration were investigated. When stimulus trains were applied to intercostal and superior laryngeal nerve and to the medulla, which produced inspiratory-inhibitory effects, the curve exhibited a discrete point, which signified inspiratory to expiratory phase switching(I-E switching). When stimulus trains applied to vagus nerve and inspiratory-facilitatory or inspiratory -inhibitory points in the rostral pons, the curve indicated two discrete points, which corresponded to I-E and E-I switching. These discrete points and time course of the curves suggest that there are four functional pools of medullary respiratory neurons, which participate in respiratory switching and rhythmogenesis.

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FUNCTIONAL ORGANIZATION OF RESPIRATORY NEURONS IN THE BULBAR RESPIRATORY RHYTHM GENERATING MECHANISMS IN RABBITS. HUKUHARA, T. Jr., GOTO, K., KIGUCHI, Y., TAKANO, K., HATTANMARU, Y. and KIMURA, N.. Dept. of Pharmacol. II, Jikei Univ. Sch.of Med.,Minatoku,Tokyo.

Coefficient of variation(the ratio of the standard deviation to the mean as a percentage, CV) for the volley period of different types of respiratory unitary discharge varied over a wide range from 1.3 to 14.4%. Correlation coefficient of autocorrelation (CC) also exhibited a considerable range of variation from 0.23 to 0.92. Even among each of the inspiratory and expiratory neuron populations CV and CC varied over a wide range (inspiratory:1.3-14.4%, 0.21-0.94;expiratory:1.5-14.4%, 0.18-0.92). There was a significant correlation (r=-0.47, p 0.001) between the CC and CV of different types of 116 respiratory units.All of the inspiratory and expiratory neurons which were discharging stably with a small CV (1.3-2.1%) and a large CC (0.83-0.94) were located in the Nucl.ret. parvocellularis et ventralis of the medulla. Inspiratory and expiratory units discharging unstably ceased to fire by smaller doses (2-8 mg/kg i.v.) of thiamylal, while stable inspiratory and expiratory units as well as the phrenic burst activity continued to discharge. These experimental results indicate that the bulbar inspiratory and expiratory neurons having a higher stability may be playing an essential role as a member of the primary respiratory neuron group in generating the respiratory rhythms in the bulbar respiratory neuronal mechanisms.

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IS BRAIN EXTRACELLULAR FLUID (ECF) H^+ THE UNIQUE STIMULUS TO MEDULLARY CHEMOSENSOR? FUKUDA, Y. Dept. of Physiol. Chiba Univ. Sch. Med., Inohana, Chiba 280

To evaluate roles of the brain ECF H^+ in stimulating central respiratory chemosensor, effects of changing Pco_2 and/or $[HCO_3^-]$ on neuronal activity in the medullary chemosensitive areas were analyzed in brain slices obtained from the rat. Low ECF pH due to high Pco_2 increased discharge frequency in about 32% of the neurons, whereas 44% were activated by low $[HCO_3^-]$-low pH. However, only 12% of the neurons showed activation in response to both acidic solutions. Increase in discharge frequency were larger in high Pco_2 than in low $[HCO_3^-]$ condition. Activation of neurons caused by high Pco_2-low pH was not prevented by compensating reduction in the ECF pH with simultaneous increase in $[HCO_3^-]$. Numbers of neurons being activated and the magnitude of activation were the highest in high Pco_2-high $[HCO_3^-]$ (without ECF pH reduction) among various combinations of altered Pco_2 and $[HCO_3^-]$. The neuronal activation by low $[HCO_3^-]$-low pH was partially suppresses by simultaneous reduction in Pco_2 (pH compensated). The results suggested that actions of CO_2 and HCO_3^- on neuronal discharges might be independent of each other and that neuronal activation by high Pco_2 did not result from associated reduction in the ECF pH. Possible contributions of changes in transmembrane $[H^+]$ gradient in a particular activating actions of high Pco_2-high $[HCO_3^-]$ solution on the neurons were discussed.

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CHANGES IN THRESHOLD FOR INSPIRATORY-AUGMENTING REFLEX

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The effects of $PaCO_2$ and PaO_2 on the threshold for the inspiratory-augmenting reflex produced by hyperinflations of the lungs were investigated in anesthetized, paralyzed cats. Inflation threshold for elicitation of the inspiratory-augmenting reflex [V_T(IT)] increased with increasing levels of $PaCO_2$ while V_T(IT) decreased with decreasing levels of PaO_2. These effects of $PaCO_2$ and PaO_2 on V_T(IT) were observed even after bilateral sinus nerve resection. These results suggest that CO_2 and O_2 may act directly on central mechanisms which are responsible for elicitation of the inspiratory-augmenting reflex.

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INTERCOSTAL MUSCLE ELECTRICAL ACTIVITIES IN CHILDREN.
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Intercostal muscle activities were recorded from the upper inspiratory and the lower expiratory intercostal muscles in children. Two pairs of surface electrodes were planted unilaterally on the 2nd or 3rd (inspiratory) and 7th to 9th (expiratory) intercostal spaces. Signals were amplified, fed through an active filter and stored in a data recorder. When signals were analyzed, they were amplified and fed through an active filter again. Electrical activities in the inspiratory and the expiratory intercostal muscles during CO_2 rebreathing, increased with end tidal CO_2 concentration. Electrical activities lineally augmented with volume until a certain level of volume was reached. Inspiratory and expiratory electrical activities were also recorded and analyzed during the determination of forced expiratory volume (FEV). Upper chest wall vibration increased the activities of the underlying inspiratory intercostal muscles and inhibited expiratory intercostal muscles. Lower chest wall vibration increased the activities of the underlying expiratory intercostal muscles and inhibited the inspiratory intercostal muscles.

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EFFECTS OF STRYCHNINE ON EXPIRATORY ACTIVITY.
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In the previous study we reported changes of phrenic discharge pattern induced by administration of strychnine. In present study we investigated changes of expiratory activity. Rabbits and rats were vagotomized and anaesthetized with urethan or α-chloralose & urethan. Expiratory activity was recorded from the internal intercostal or abdominal muscles. Disinhibitory effect of strychnine on expiratory activity was easily induced. Impulse frequency of expiratory discharge increased and active expiratory period prolonged by i.v. applied strychnine. In early type of expiratory activity these effects appeared in late phase of preceding inspiratory period. In late type of expiratory activity simillar effects appeared in early phase of following inspiratory activity. Increase of dose of strychnine brought a depressing effect on expiratory discharge. Discharge frequency and duration of active period of expiratory unit decreased. Finally expiratory discharge disappeared in expiratory phase and remained only in inspiratory phase. Synchronizing excitation of inspiratory and expiratory activities was recognized.

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LOCAL OCCLUSION OF THE ARTERIES ON THE VENTRAL SURFACE OF THE MEDULLA AND THEIR EFFECTS ON THE INSPIRATORY ACTIVITY.
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The effects of occlusion of the arteria cerebelli inferior anterior (Arteria CIA) on the inspiratory activity were studied in anesthetized and paralyzed cats. The vagosympathetic nerves were bilaterally sectioned. The integrated phrenic nerve activity was used as an index of the central inspiratory activity. When systematic arterial blood pressure was below about 100 mmHg, bilateral occlusion of either the arteria carotis externa or Arteria CIA resulted in a decrease in the phrenic nerve activity, which finally disappeared within one minute. On a blood pressure of above 100 mmHg, occlusion of the arteria carotis externa did not noticeably alter the inspiratory activity. However, subsequent bilateral occlusion of Arteria CIA caused the disappearance of the phrenic nerve activity. During the period of occlusion, electrical stimulation of the carotid sinus nerves or an inhalation of carbon dioxide failed to produce an inspiratory activity. These results suggest that the arteria carotis externa acts primarily as channels of keeping the blood pressure in the arteria basilaris, and the function of the inspiratory activity is mainly dependent on the bloodflow of Arteria CIA.

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EFFECT OF HIGH DENSITY GAS(27 & 54g/l.BTPS) ON NEUROPHYSIOLOGICAL AND PULMONARY FUNCTION OF CATS UNDER HYPERBARIC ENVIRONMENT.

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Neurophysiology and pulmonary function were studied in three male adult cats(3.5-4.5kg) on two saturation dives in He-N_2-O_2 to the maximum depth of 51 ATA(27g/l.BTPS, PN_2=20bar, PO_2=0.21bar) and 101 ATA(54g/l.BTPS, PN_2=40bar, PO_2=0.21bar). EEG, EOG, EMG, Heart frequency(fH), Respiratory frequency(fR) and Brain Temperature from the chronically implanted electrodes were recorded during the pre-dive, compression, saturation, decompression and post-dive. In the latter experiment(101 ATA),Tidal Volume(VT), Minute Respiratory Volume($\dot{V}g$) and Oxygen Consumption($\dot{M}O_2$) were measured add to respiratory frequency during pre-dive and two hours of 101 ATA periods.

Results on Neurophysiological function: Two cats stayed at 51 ATA(27g/l.BTPS) for 72 hours. fH, fR and sleep function did not change in the presense of above high density gas. Body weight of one cat decreased in 13% and the other in 3% after decompression.

Results on Pulmonary function: One cat stayed at 101 ATA(54g/l.BTPS) for 2 hours. fR, VT, $\dot{V}g$ and $\dot{M}O_2$ did not change in the presense of above high density gas.

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The effects of running trainings at sea level and at altitude(2380 m) on the ventilatory responses to CO_2 and hypoxia.

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The effects of the programmed running trainings (20-30 km/day for 10 days) at sea level and at altitude (2380 m, 5th step of Mt. Fuji, P_B=570-580 mmHg) on the ventilatory responses to CO_2 and hypoxia were examined in the runners (n=17) by using the rebreathing (Read, 1967) and the progressive hypoxia (Weil et al., 1970) tests, respectively. The training at sea level induced the decrease in the hypoxic ventilatory response without any discernable change in the CO_2 responsiveness. On the following day after ascent to altitude, the increase in the hypoxic ventilatory drive and a simultaneous decrease in the CO_2 responsiveness were observed. After the termination of the training at altitude, the hypoxic drive was further increased to 80 % value of that measured at the first day at altitude though it was not statistically significant, while the CO_2 responsiveness did not alter. Since the initial ventilatory sensitization to hypoxia is known to diminish during subacute (several days) or chronic altitude exposure, the increment in the hypoxic drive seemed to be related to the effect of training at altitude.

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EFFECTS OF INACTIVATION OF SINUS NERVE BY COLD BLOCKING ON PHRENIC NERVE ACTIVITY UNDER HYPEROXIA, NORMOXIA OR HYPOXIA.

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Integrated phrenic nerve discharges were recorded as an output of respiratory activity in anesthetized, vagotomized cats paralyzed and artificially ventilated. The phrenic amplitude in the carotid sinus nerve (CSN) intact increased with increased P_{CO_2} and reached to the maximum level (Max) at the P_{CO_2}= 60 ~ 80 mmHg. The effects of cold blocking of the CSN on the phrenic activity at varying P_{CO_2} under hyperoxia, normoxia or hypoxia were investigated. The phrenic nerve discharges reduced during blockade. The reduction of the phrenic amplitude by blockade (P) were regarded as the contribution of the carotid sinus chemoreceptor to the respiratory system. The ratio of P/Max was 7 % under hyperoxia and 15 % under normoxia throughout the P_{CO_2} range that we studied. Under hypoxia (P_{a,O_2}= 57 mmHg), this ratio increased to 40 % at the P_{CO_2} level of threshold P_{CO_2}+ 5 ~ + 30 mmHg and decreased with increased P_{CO_2}. Then, P/Max became to be a slight difference at above + 40 mmHg compared with that of normoxia. These results indicate that the CSN conducts a constant respiratory drive, being independent of P_{CO_2}, at hyperoxia and normoxia, and hypoxic chemoreflex does not contribute largely to the respiratory system at high P_{CO_2} level.

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TWO TYPES OF SPONTANEOUS PERIODIC FLUCTUATION IN NEOCORTICAL ELECTROCORTICOGRAM IN THE CAT.

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Vagotomized and paralyzed cats being severed with the carotid sinus nerves were maintained by artificial ventilation under monitoring O_2 and CO_2 levels in expired air. Nature of two types of the spontaneous rhythmic activity in the electrocorticogram(ECoG) in the anterior sigmoid gyrus was studied. Correlation coefficient of cross-correlation (CC) between ECoG and the phrenic nerve activity was ranging from 0.072 to 0.429 (mean $\pm$ SD: 0.197 $\pm$ 0.111, N=21). The period (1.4-5.4 sec) of one type of the rhythmic ECoG activity synchronized with that of the respiratory cycles. The period (14.3-180.0 sec) of another type of the spontaneous ECoG rhythmic activity coincided with the period of a rhythmic fluctuation in the renal sympathetic nerve activity synchronizing with that of Mayer's wave in arterial blood pressure. CC between ECoG and the renal sympathetic nerve activity ranged from 0.092 to 0.305 (0.172 $\pm$ 0.055, N=19). After mid-pontine transection both of these two types of the rhythmic ECoG activity was abolished and an additional spontaneous rhythmic ECoG activity having a different period (19.2 $\pm$ 14.3 sec, N =11) from the both periods of the phrenic and renal sympathetic nerve activity was revealed, while the spontaneous rhythmic discharges in the phrenic (10.3 $\pm$ 7.4 sec) and renal sympathetic nerve activity (30.9 $\pm$ 17.2 sec) endured further.

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EFFECTS OF SITS ON THE ACID-BASE STATUS AND ELECTROLYTES DURING ACUTE HYPERCAPNIA IN SPLENECTOMIZED DOGS.

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Effects of CO_2(12%CO_2-30%O_2)inhalation on the acid-base status and electrolyte composition for blood and lymph were studied in SITS-treated (25mg/kg, iv) and untreated splenectomized dogs. The time-course of acid-base changes in SITS-treated group (n=7) was strikingly similar to that of untreated group (n=9) obtained previously. The in vivo buffer slopes for mixed venous blood and thoracic duct lymph showed no significant differences between both groups. On the other hand, the in vitro buffer slope of SITS(10^{-3}M)-treated blood (15.8 sl.) was significantly lower than that of untreated one (25.4 sl.)($P<0.01$, n=5). After in vitro SITS-treatment, both plasma bicarbonate increase and chloride decrease by CO_2 titration were inhibited. During in vivo CO_2 breathing plasma bicarbonate concentrations showed no essential differences between both animal groups. Decrease in plasma chloride concentration caused by CO_2 breathing was larger in SITS-treated group (n=7) than in untreated one (n=5). Chloride efflux from the various body cells may have been inhibited in SITS-treated dogs more than chloride influx occurred in the blood and brain cells. High bicarbonate concentration in SITS-treated dogs may have result from other possible processes such as Na^+-H^+ exchange and bone carbonate release.

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CHANGES OF CO_2-VENTILATION FEEDBACK REGULATION DURING SLEEP

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Seven healthy male subjects were studied in awake and slow-wave sleep conditions. Homeostatic control activity for $P_{A}co_2$ by the CO_2-ventilation feedback mechanism, which consists of restoring and driving systems, was evaluated by applying external dead space with 250, 500 and 750 ml, respectively. In the restoring system, the slope of the metabolic hyperbola , S_L, was steeper in the sleep than in the awake due to higher $P_{A}co_2$ and lower $\dot{V}co_2$ in the former condition. On the other hand, in the driving system slope of the CO_2-ventilation response curve, S, was nearly the same in both conditions. Accordingly, overall gain of the CO_2-ventilation feedback system (termed G), which is the product of S_L and S, was larger in the sleep than in the awake. S_L, S and G were 12.9 $\pm$ 3.2 (Mean + SD), 1.89 $\pm$ 0.94 and 20.5 + 7.4 in the sleep and 10.3 + 2.5, 1.90 $\pm$ 0.97 and 17.0 $\pm$ 8.9 in the awake condition, respectively. Since $P_{A}co_2$ was cosistently elevated by a few mmHg throughout the sleeping period from the equivalent conditions of the awake experiment, increased G would have worked to prevent further aggravation in increasing $P_{A}co_2$.

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ESTIMATION OF RED BLOOD CELL COMPONENT OF CO_2 DISSOCIATION CURVE.

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Blood from seven healthy male subjects was analyzed for component of CO_2 dissociation curve in RBC fully deoxygenated and oxygenated. The sample was equilibrated in sequence with each of ten CO_2 gas mixtures, and the CO_2 concentration of whole blood and true plasma was determined with Natelson analyzer. Simultaneously, pH was measured with IL meter for each sample and hematocrit (Hct), with micro-centrifuge. From the Hct which increased with increasing Pco_2 of equilibrating gas mixtures and the water concentration of RBC and plasma at a given Pco_2 which was measured by drying method, the water concentration of RBC and plasma at each equilibrating Pco_2 was estimated. From these values, RBC component of Cco_2 difference between deoxygenated and oxygenated blood at Pco_2 40 Torr was calculated to be about 60% of Haldane effect and the contribution of carbamate, less than 27%. The pH measured in the whole blood was on an average 0.014 lower than that in the true plasma. The pK' at pH 7.4 averaged out to 6.1 and 6.114 using pH values determined in the whole blood and in true plasma, respectively. The significant dependence of pK' on pH was observed in three subjects. The conventional value for pK'; 6.1 irrespective of pH, makes one overestimate the plasma buffer value and the slope of plasma CO_2 dissociation curve derived from Henderson-Hasselbalch equation.

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RELATIONSHIP BETWEEN HEMATOCRIT AND THE SHIFT-RATIO OF BICARBONATE PRODUCED WITHIN THE RED BLOOD CELL.

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When blood is exposed to a low Pco_2 atmosphere, CO_2 molecules diffuse out of the red cell and bicarbonate concentration decreases. When the lack in bicarbonate is not refilled by shifted bicarbonate, pH in the cell is lowered and the H^+ release from hemoglobin will be ceased. Thus, in a low hematocrit blood, where the shift-ratio of bicarbonate is thought to be greater than in a high hematocrit, the buffer action of hemoglobin should be greatly increased. In order to obtain the quantitative relation between the hematocrit and the shift-ratio, the changes in bicarbonate in whole blood, true and separated plasmas and the red cell compartment were measured by changing Pco_2 and hematocrit. Blood samples with three different hematocrits (ca. 25, 45 and 75 %) were prepared after separating true plasma anaerobically at 37°C, and then equilibrated with 12 mm Hg Pco_2. Before and after the separation the CO_2 content, pH and Pco_2 were measured in blood and plasma together with sodium and chloride concentrations in plasma. The bicarbonate change in the red cell decreased in parallel with hematocrit and was inversely related to the total bicarbonate change caused by hemoglobin buffer. The shift-ratios of 25, 45 and 75 % hematocrits were 0.76, 0.58 and 0.37, on an average, respectively.

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MEASUREMENT OF THE CO_2 DIFFUSION RATE IN RED BLOOD CELLS.

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A red blood cell (RBC) suspension and NaCl–bicarbonate solution equilibrated with CO_2 of about 8 and 65 torr, respectively and vice versa, were rapidly mixed in a stopped-flow apparatus equipped with a fluorometer. pH time course after the mixing was measured by recording fluorescence intensity of 4-methylumbelliferone. In order to facilitate the CO_2 hydration reaction, carbonic anhydrase was added in both the suspension and solution at a high ratio 20 mg/100 ml. The half-times of pH rise (CO_2-uptake by RBC) and fall (CO_2-release) were found to be 0.084 ± 0.015 sec and 0.12 ± 0.03 sec, respectively. Using the linear relation between $\log P_{co2}$ and pH and assuming that pH varies simultaneously with P_{co2}, the change in P_{co2} was calculated from the pH curve. From the final P_{co2} it was suggested that no HCO_3^- shift ocurred from RBC to plasma when CO_2 molecules diffused into RBC. Thus, applying a three dimensional diffusion equation, P_{co2} time course was calculated by referring to the observed CO_2 dissociation curves, and a good coincidence was observed when a CO_2 transfer coefficient across the RBC membrane was about 3×10^{-6} $cm.sec^{-1}.torr^{-1}$. This was comparable with the O_2 transfer coefficient.

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RESPIRATORY FREQUENCY AND BLOOD GASES DURING HATHA-YOGA

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In order to examine the respiration of Hatha-Yoga, we compared minute ventilation, respiratory frequency, oxygen uptake, heart rate and blood gases during normal and Hatha-Yoga respirations.
It was found in this study that respiratory frequency and minute ventilation during normoxia, hypoxia and hypercapnia were evidently lower in the Hatha-Yoga than that in the control, though heart rate and oxygen uptake were a little higher in the Hatha-Yoga. In addition, pH, P_{O_2}, P_{CO_2}, HCO_3^- and BE in the arterial blood were almost the same in both conditions.
From these results, possible mechanisms of Hatha-Yoga respiration was discussed with relation to chemical control of respiration.

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VENTILATORY RESPONSE TO HYPOXIA AND HYPERCAPNIA IN JUDO ATHLETES

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Fifty two active Judo athletes were examined by an isocapnic progressive hypoxia and a hypercapnic rebreathing tests.
The results were analyzed by hyperbola ventilation equation and least-squares method as follows: $\dot{V}_E = \dot{V}_o + A / (P_{ETO_2} - C)$ and $\dot{V}_E = S (P_{ETCO_2} - B)$.

Hypoxic sensitivity, A and hypercapnic sensitivity, S, were positively correlated with body weight. Such correlation in hypercapnic sensitivity, S, became insignificant when S values was recalculated after ventilation was normalyzed for 70 kg body weight, but in A, Judo athletes still exhibited significant correlation.
These results indicated that body weight plus obesity was determing factor to increase hypoxic sensitivity in active Judo athletes.

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PULMONARY MICROCIRCULATION

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Blood flow in microvessels on alveolar surface of exposed bullfrog's lung was observed by means of an intravital microscope. Arterioles distributing on alveolar surfaces were folded several times on the basal portion and then straightly stretched on the distal portion. They showed no sequential branchings but they had many direct outflow pores to capillary bed on the straight portion. The outflow pores were arranged at each 30 to 40 μm on terminal arterioles. It could be recorded on video tapes that elongated RBCs flew out through the pores, just like crowding gold fish. The diameter of alveolar cells was 39.8 ± 4.9 μm on an average and ± SD. Spaces among alveolar cells (width = 14.6 ± 2.8 μm) formed alveolar capillary network, whose geometeric structure could be well simulated with an hexagonal model. RBCs which were thin oval in the shape during the stationary state, were elongated to be 30 μm by the shear force acting on them in capillaries. Two RBCs strongly thinned by the deformation into a bambooleaflet shape could flow together in a capillary. Each hexagon of the capillary, therefore, contained one to four RBCs. Terminal lymph ducts (D = 15.1 ± 2.7 μm) contained small particles (D = 4.0 ± 1.1 μm). Their lymphatic valves seemed to be functionally a monocuspide. Small particles repeated flowing to and fro in lymph ducts.

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RENAL CONTRIBUTION TO ACID-BASE REGULATION DURING THE MENSTRUAL CYCLE.

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Menstruating woman exhibits a light but sustained hypocapnia during the luteal phase. To evaluate the renal contribution to acid-base regulation during this phase, we studied on the relationship between either end-tidal or arterial blood P_{CO_2} and net urinary excretion of acid in 5 subjects throughout the menstrual cycle, of whom in 3 subjects arterial blood pH and base excess (BE) were measured as well. During the follicular phase, the acid-base compositions of blood and the rate of net acid excretion remained virtually constant. After ovulation, significant decreases in P_{CO_2} (ranging from 2 to 4 mmHg) and BE (from 1.5 to 2 mEq/l) occurred until the end of the luteal phase, whereas pH remained at the preovulatory levels. A significant decrease in net acid excretion was seen only for 3 days in the middle of the luteal phase. The present results suggest that the decrease in net acid excretion is not a primary change in acid-base metabolism leading to a metabolic acidosis but a renal response to the fall in P_{CO_2}. The decreases in BE could not be accounted for solely by the renal response to hypocapnia.

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EFFECTS OF ELECTRICAL FIELD STIMULATION ON AMYLASE RELEASE FROM PAROTID AND PANCREATIC SEGMENTS IN VITRO. KATOH, K., SUZUKI, T. Y. AND NISHIYAMA, A* Dept. of Physiol., Yamagata Univ. Sch. of Med., Yamagata 990-23 and *Dept. of Physiol., Tohoku Univ. Sch. of Med., Sendai 980.

Employing electrical field stimulation method on superfused segments of parotid and pancreas isolated from rat and mouse, the autonomic nervous control on amylase release from each gland was investigated in vitro. Amylase release was continuously measured by an automated technique using fluorescent starch as a substrate. In all glands an increase in amylase release evoked by field stimulation (FS) was abolished by TTX (10^{-7} g/ml). In rat parotid the effect of FS was reduced by atropine (7×10^{-6} M) and abolished by further addition of propranolol (10^{-5} M). In rat pancreas the FS effect was completely blocked by atropine, while in mouse pancreas it was markedly reduced but not abolished. From these results it was concluded that the amylase release by FS from rat parotid or pancreas was evoked by activation of both adrenergic and cholinergic nerves or of cholinergic nerve respectively, while that of mouse pancreas might be mainly of cholinergic and partly of noncholinergic nerves.

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VAGUS NERVE CONTROL OF RAT PANCREATIC EXOCRINE SECRETION: EFFECTS OF ELECTRICAL STIMULATION OF ABDOMINAL TRUNKS OF VAGUS NERVE AND TRUNCAL VAGOTOMY. NONOYAMA, T., MURAI, K., KATOH, K. and *NISHIYAMA, A. Dept. of Physiol., Yamagata Univ. Sch. of Med., Yamagata. *Dept. of Physiol., Tohoku Univ. Sch. of Med., Sendai.

Functional roles of vagus nerve in control of pancreatic exocrine secretion were investigated in rat anesthetized with pentobarbital sodium. Electrical stimulation of both anterior and posterior abdominal trunks of vagus nerve at 10 Hz, 2 ms, 9 V for 10 min markedly increased the secretory rate of pancreatic juice rich in amylase. The maximum secretory response to vagal stimulation was comparable to that to intravenous injection of maximal dose of caerulein (100 ng). The secretory response to simultaneous stimulation of both anterior and posterior trunks of abdominal vagus nerve exceeded the sum of each response. The response to vagal stimulation was abolished by injection of atropine or hexamethonium. Ionic composition (Na, K, Cl) of pancreatic juice secreted by vagal stimulation was constant and independent on secretory rate. Truncal vagotomy did not reduce the secretory response to low doses of caerulein (3.1 - 12.5 ng). However, it significantly reduced the response to high doses of caerulein (100 - 200 ng). The results indicate that vagus nerve plays important roles in control of secretion of fluid and enzymes from the rat pancreatic acini.

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INHIBITORY ABILITY OF PHLORIZIN-COUPLED COMPOUNDS ON THE INTESTINAL GLUCOSE ABSORPTION OF RAT. KASAGI, T. Dept. Nursing, Tottori Univ. Col. Med. Care Technol. Yonago 683.

Inhibitory actions of phlorizin-coupled polysaccarides, named "dextran-phlorizin(MW. 60,000-90,000)' and pullulan-phlorizin(MW. 74,000)", were examined in the intestinal glucose absorption, using the circulating method in vivo. The state of the glucose absorption was detected by measuring the residual amount of glucose in the circulating solution (1 mM), to which dextran-phlorizin(equivalent to phlorizin of 0.083mM) or pullulan-phlorizin(0.25 mM) was added. These compounds showed the inhibitory effect on the glucose absorption at rates of 37% and 29% for 60 min after the start of circulating, respectively. It is concluded that both of present phlorizin-coupled compounds have the ability to depress the intestinal glucose absorption as well as single phlorizin. Also various glucosides related to phlorizin were tested to shown the variety of inhibitory effects among their chemical structures of aglicon.

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ON THE SENSITIVITY OF TRANSMURAL POTENTIAL DIFFERENCE INCREMENT PRODUCED BY SHORT CHAIN FATTY ACIDS IN THE RAT COLON

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Effects of short chain fatty acids (SCFA) on transmural potential difference (p.d.) across the proximal colon were studied in germ free (GF), gnotobiotic (GB) and conventionalized (CV) rats in vivo. Luminal or intravenous administrations of propionate and butyrate caused a transient increase in the p.d. across the proximal colon. There was a sigmoid relationship between the change in the p.d. and the logarithm of the dose of propionate i.v. This dose response curve of propionate was shifted to the right by atropine sulfate. Repeated administrations of the same dose of propionate i.v. evoked progressively smaller response. The p.d. increment caused by propionate was profoundly less sensitive in CV rats than in GF rats. The colon of GB rats (monocontamination of Fusobacterium varium) exhibited that its dose response curve of propionate was similar to that of CV rats. By chronic infusion of isotonic sodium propionate or butyrate (25.5 ml/day) into the proximal colon of GF rats for 7 days, the effect of propionate administered i.v. on p.d. increment was remarkably reduced. These results suggest that chronic exposure of the colon to SCFA produces refractoriness to the effect on the p.d. increment by SCFA.

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STUDIES ON PENETRATION PHENOMENA THROUGH INTESTINAL MEMBRANE — relationship between intestinal penetration and line of magnetic force —

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We performed a series of experiments on penetration of insulin as activepolypeptides and L-tryptophane as well as activetransport of glucose related to penetration efficiency, using a conventional circulation apparatus which was developed by our groupe. However, the conventional apparatus which uses everted intestine can define intestine length but cannot define its surface area accurately. To cope with this difficulty,we have devised a new apparatus which is able to define intestine surface area, as well as to examine the effect of magnetic force on penetration, applying magnetic force from mucosal side to serosal side or in reverse direction. The obtained experimental results with this new apparatus are as follows: 1) Penetration of substances through intestine was measured with higer accuracy than by conventional apparatus. 2) When intestine was irradiated from mucosal side to serosal side by 6000 Oe magnetic force, insulin and L-tryptophane penetration showed tendency to increase. 3) On the contrary, when irradiated from serosal side to mucosal side, the penetration of these substances were obviously inhibited. 4)This time, the experiment using magnetic force was performed under limited conditions. Therefore, the experiment should be continued hereafter, under other various conditions.

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The effect of colchicine on glucagon producing tumor cells(ITC-1).
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We have previously described the serial transplantation and the cell culture of glucagon producing tumor cell, which developed on Syrian hamster injected insulinoma(In-111) cells. Glucagon producing tumor cells(ITC-1) were cultured on plastic in RPMI-1640 medium supplemented with 10% newborn calf serum, at 37°C in a humidified atmosphere of 5% CO_2-95% air. In the present experiments, the effect of colchicine on glucagon secretion was studied. Glucagon was assayed based on the double antibody procedure using C-terminal antibody(OAL-123). ITC-1 cells were incubated in the medium containing colchicine at concentrations of 0 to 10^{-4}M for 3 hrs. The highest inhibitory effect of colchicine on glucagon release and the highest concentration in the cells were observed at a colchicine concentration of 10^{-6}M. The incorporation of ^{3}H-leucine into total protein was unaffected by colchicine. These results suggest that colchicine is acting only on the process of glucagon-release.

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CHANGES IN STEROIDOGENESIS OF RAT ADRENAL CELLS IN PRIMARY MONOLAYER CULTURE.
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It is well known that adrenals atrophy in hypopituitarism or after hypophysectomy. Atrophied adrenals does not restore steroidogenic activity instantly after administration of ACTH. The mechanism of this phenomena is not fully clarified. Therefore, we have studied how adrenal cells lose steroidogenic activity without ACTH and how they maintain it under ACTH *in vitro*, utilyzing rat adrenal cells in monolayer culture, which is as sensitive as 1.0pg/ml of ACTH. Results were shown by mean±SD. $^{1-24}$ACTH was used. (RESULTS) Basal corticosterone (B) secretion decreased time-dependently from 471±53 to 66±18ng/dish/2 days during 10 days of culture. B response to 2-hour loading of 10pg/ml of ACTH also decreased from 1845±130 to 30±8ng/dish. On the contrary, cAMP in cells did not decrease responding to 20-minute exposure to 50pg/ml of ACTH. Repeated 2-hour pulse exposure to 10 or 40pg/ml of ACTH every 2 days had no effect on the maintainance of basal B secretion or responsiveness to ACTH. 4-day culture in the medium with 160pg/ml of ACTH either had no effect. Basal secretion of B was maintained by changing medium with 100pg/ml of ACTH or more every 2 days. B response to 2-hour loading of 10 or 40pg/ml of ACTH was also maintained in the same experiment. (SUMMARY) These result suggest that decreased B response to ACTH is due to lowered function of the step(s) beyond adenylate cyclase, and that upper limit or supraphysiological concentration of ACTH is required to maintain adrenal cell function in our experimental condition.

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TRH-INDUCED MEMBRANE HYPERPOLARIZATION IN THE CLONAL ANTERIOR PITUITARY CELL.
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Thyrotropin-releasing hormone (TRH) elicited a transient membrane hyperpolarization followed by an enhanced generation of action potentials in the rat clonal anterior pituitary cell GH_3. The membrane conductance increased during the transient hyperpolarization. This response was due mainly to an increase in the membrane permeability to K ions, since it also occurred in Cl-free solutions in which Cl was replaced with methanesulfonate, acetate or propionate. Ca-ionophore A23187 caused a similar membrane hyperpolarization accompanied by an increase in the membrane conductance. The reversal potential of the A23187-induced hyperpolarization was identical with that of the TRH-induced response. Both TRH- and A23187-induced hyperpolarizations were markedly suppressed by injection of EGTA into the GH_3 cell. These results suggest the following mechanism on the genesis of the TRH-induced hyperpolarization: TRH mobilizes sequestered Ca into the cytoplasm of the GH_3 cell, and transiently elevates intracellular Ca^{2+} concentration, thereby activating the Ca-mediated K channels.

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EVIDENCE FOR SELECTIVE INFLUENCE OF THYROTROPIN-RELEASING HORMONE IN VAGAL EFFERENTS

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The efferent activity of the superior laryngeal nerve (n.sl) and cervical vagal trunk was recorded simultaneously and the effect of an i.c.v. injected TRH and SRIF was examined. The activity of the n.sl bundle and vagal trunk was composed of bursts of impulses generated rhythmically and of randomly distributed impulses. An i.c.v. injection of TRH (5 ug/100 g) consistently produced a rapid increase in the number of the randomly generated impulses of the n.sl bundle (456.1 + 90.0 %), while the density of the impulses of the bursts was not markedly affected. TRH may not centrally influence the generation of the respiration-related impulses but may stimulate the nerve activity which has been thought to affect the thyroid gland. On the other hand the response to TRH of the vagal trunk varied, showing a minute gradual increase, a transient increase or a transient decrease. In contrast to the diversity of the response to TRH of the vagal activity, SRIF (5 ug/100 g) inhibited all the nerve activity. These findings indicate the existence of a selectivity in the central effect of TRH on the particular vagal efferents.

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EFFECT OF ADRENAL HISTAMINE LIBERATION ON THE SECRETION OF ALDOSTERONE OF THE PERFUSED DOG ADRENAL GLAND

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The left adrenal gland of hypophysectomized-nephrectomized dog was perfused in situ with Krebs Ringer bicarbonate glucose solution (KRBG, saturated with 95% O_2 + 5% CO_2, K^+ = 3.6 mM). After perfusion with KRBG containing 200 µg/ml Comp.48/80 over 5 min, the secretory rate of aldosterone increased markedly and those of cortisol and corticosterone slightly. The secretory responses of 3 steroids were fairly depressed by continuous infusion of pyrilamine maleate.

The adrenal gland of hypophysectomized-nephrectomized dog which was sensitized by s.c. injection of horse serum 3.5 weeks before experiment was perfused with KRBG. After infusion of horse serum(20-fold diluted) into the sensitized adrenal gland, the secretory rate of aldosterone increased markedly and the increase continued for one hour or more.

These results suggest that mast cells in adrenal cortex may play a pathophysiological role in stimulating the secretion of aldosterone.

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CENTRAL ROLES OF ANGIOTENSIN II (AII) ON PLASMA VASOPRESSIN (ADH) RESPONSES TO THE BLOOD HYPEROSMOLALITY AND HYPOVOLAEMIA.

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We examined in conscious rats the effects of intraperitoneal (i.p.) injection (2 ml/100 g body weight) of hypertonic saline or polyethylene glycol (PEG; 250 mg/ml of 145 mM NaCl) on plasma ADH and AII and of intracerebroventricular (i.c.v.) administration of Sar^1-Ala^8-AII (a specific antagonist of AII) on the plasma ADH responses to the stimuli. Nine hundred mM NaCl produced increases in plasma ADH, sodium and osmolality and a decrease in haematocrit at 30 min after i.p. injection. Two h after i.p. administration of PEG, plasma ADH and haematocrit were augmented with unaltered plasma sodium and osmolality. The responses of plasma ADH induced by 900 mM NaCl and PEG were inhibited by 1 µg of Sar^1-Ala^8-AII injected i.c.v. at 5 min before blood samplings which unaffected those of the other plasma variables. Plasma AII was unchanged by 900 mM NaCl, but was markedly enhanced by PEG. From these results, we concluded that AII may participate in both the blood hyperosmolality- and hypovolaemia-induced ADH release by stimulating the brain.

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EFFECTS OF AMYGDALA STIMULATION UPON DISCHARGE ACTIVITY OF ADH-SECRETING SUPRAOPTIC NEURONES IN RATS

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Effects of amygdala stimulation upon discharge activity of ADH-secreting cells in the supraoptic nucleus (SON) were studied in male albino rats anaesthetized with urethane. ADH-secreting cells identified in the SON by their phasically firing pattern showed predominantly excitation after medial amygdala stimulation. In contrast, inhibition was mainly observed after basal amygdala stimulation. On the other hand, neurosecretory cells with randomly firing pattern showed mainly inhibition after medial or basal amygdala stimulation. The difference in percentage of cells which showed excitation and inhibition after medial amygdala stimulation is statistically significant between phasically and randomly firing cells. Stria terminalis lesion did not block these synaptic inputs. SON stimulation did not evoke antidromically conducted spike in any of 26 amygdala neurones tested. Thus it is concluded that amygdalar synaptic inputs to ADH-secreting cells in the SON are mediated by the pathway other than the stria terminalis, probably the ventral amygdalofugal pathway, and are polysynaptic.

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EFFECT OF TESTOSTERONE PROPIONATE ON THE EMBRYONIC GONAD OF THE JAPANESE QUAIL.

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Histologically, the embryonic gonad of quail contained two distinct component, the medulla and the cortex. It developed to ovary or testes depending upon either cortex or medulla had dominated by the genetic sex. The sexual differentiation of the gonad was first recognized in six day embryos where the ovarian cortex of left female gonad reached about 70 μm in thickness and was filled with many germ cells. On the other hand, when the cortex of right female gonad and the testicular cortex of male gonads grew to 10-15 μm in thickness, germ cells almost disappeared from the cortex. The embryo was injected with 0.25 mg testosterone propionate on the four day stage of incubation. All embryos were sacrificed on the eleven day stage of incubation. Histrogical observations of the gonad were carried out using the sex-linked marker gene, sw, for determinating genetic sex of the embryo. Testosterone propionate treated embryos were found to be larger than control embryos in the number of embryos that the cortex of right female gonad contained germ cells.

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FACILITATORY EFFECT OF CATECHOLAMINES ON THE EARLY PHASE OF ESTRADIOL BENZOATE (EB) INDUCTION OF LORDOSIS BEHAVIOR IN THE OVARIECTOMIZED RAT AND THE HYPERRESPONSIVENESS OF THE SEPTUM LESIONED (SL) RAT TO EB.

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Induction of lordosis behavior was expressed by lordosis quotient (LQ) after EB followed by progesterone (P) in the ovariectomized rat. Even as much as 10 μg of EB followed by 1 mg P required more than 22 h to produce an increase in LQ and 1.5 mg apomorphine (AP) given 48 h after the EB-treatment exerted an inhibitory effect on the LQ increase. However, the same dose of AP caused a marked facilitation on the increase in LQ, only when given within 4 h of the EB-treatment. The similar effect was also produced with 50 μg adrenaline (AD). In the ovariectomized SL rat that was behaviorally hyperresponsive to EB, 0.3 mg pimozide, 100 μg propranolol or 5 mg phenoxybenzamine given before EB depressed the increase in LQ. These results suggest that catecholamines facilitate the early phase of EB induction of lordosis behavior and that these mechanisms are involved in the hyperresponsiveness of the SL rat to EB.

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EFFECTS OF PRENATAL HYPOTHYROIDISM ON THE DEVELOPMENT OF RAT BRAINS.
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Thyroid states are known to affect the development of brains during the postnatal life. In our previous studies, thyroidectomy performed on 1 day old resulted in reduced tubulin content in 30-day-old rat brains.

The prenatal hypothyroidism, in the present study, increased the weight of the cerebrum and cerebellum from 1-day-old pups treated with 4-Methyl-2-Thiouracil(MTU).

In brains of 1-day-old pups from MTU-treated maternal rats, cerebral DNA contents (per wet weight) were decreased but tubulin contents(per wet weight) were not changed and, then, intracellular tubulin contents(per DNA) were increased. In the cerebellum, DNA contents(per wet weight) were increased by the MTU-treatment but tubulin contents (per wet weight) were not changed and the intracellular tubulin contents were not affected significantly. But in the hypothalamus, DNA contents(per wet weight) were not changed and since the tubulin contents(per wet weight) were decreased, the intracellular tubulin contents were also decreased in 1-day-old pups.

Most of these differences were not observed in 29-day-old rats treated continuously with MTU after the birth. Thyroid hormones might have some roles in the normal development of brains during both postnatal and prenatal life.

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ADRENAL MEDULLARY SECRETION IN RESPONSE TO DIENCEPHALIC STIMULATION IN THE RAT
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The effect of diencephalic stimulation on adrenal medullary secretion was investigated in pentobarbital-anesthetized rats. Adrenal venous blood samples were collected and analyzed for adrenaline and noradrenaline by the fluorometric method. Stimulation of the ventromedial and dorsomedial hypothalamic nuclei and posterior areas of the thalamus produced a significant increase in adrenaline secretion. Stimulation of the preoptic nucleus or adjacent area resulted in a significant decrease in noradrenaline secretion. It is suggested that in the diencephalon there exist two central nervous system mechanisms, excitatory and inhibitory, for the regulation of the adrenal medullary secretion.

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EFFECT OF ARGININE-VASOTOCIN ON LH RELEASE AND ITS INTERACTION WITH MELATONIN
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The possibility of any interaction occurring between melatonin and 5-methoxytryptophol (5-MTPH) or melatonin and arginine-vasotocin (AVT) at the pituitary level was examined ; the rate of secretion of 17-oxosteroids by the testes of anaesthetized dogs was used as an index of the luteinizing hormone releasing hormone (LH-RH)-induced release of LH. When LH-RH (5 μg/kg body wt) was injected into the carotid artery 3 h after intracarotid injection of 5-MTPH (40 μg/kg body wt) plus melatonin (10 μg/kg body wt), the testicular response to LH-RH was considerably inhibited. This effect was almost equally effective to 10 μg melatonin/kg in suppressing the LH-releasing activity of LH-RH. This indicates that the actions of 5-MTPH and melatonin are competitive.

However, dogs pretreatment with 0.005 μg AVT/kg plus 50 μg melatonin/kg had a testicular response to LH-RH that was not significantly different from that observed after treatment with either 0.01 μg AVT/kg or 100 μg melatonin/kg. This indicates that the actions of AVT and melatonin are additive.

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A RELATIONSHIP BETWEEN BOTH PERIPHERAL AND CEREBROSPINAL FLUID INSULIN AND GLUCOSE LEVELS IN FREELY MOVING RATS. SASAKI, K., ONO, T., NISHINO, H., FUKUDA, M., MURAMOTO, K-I. AND *STEFFENS, A.B. Dept. of Physiol., Fac. of Med., Toyama Med. and Pharmaceu. Univ., Sugitani, Toyama 930-01, * Dept. of Zool., Groningen State Univ., Groningen, The Netherlands.

A relationship between both peripheral and cerebrospinal fluid (CSF) insulin and glucose levels was investigated in freely moving rats with chronic brain cannulas and heart catheters for continuous glucose (CSF: 16 μg/min, blood: 10 mg/min) and insulin (CSF: 5 μU/min) infusion and frequent CSF and blood sampling. Results were as follows: 1) Increased peripheral insulin due to peripheral infusion of glucose did not change the CSF insulin level. On the other hand increased peripheral glucose resulted in an increase of CSF glucose with some delay. 2) Increased CSF levels of glucose elicited decreased peripheral glucose levels without an increase of peripheral insulin. 3) Increased CSF insulin did not change either peripheral insulin or peripheral glucose. 4) An increase of both CSF insulin and glucose did result in a gradual increase of peripheral glucose whereas peripheral insulin did not change. These results suggest that peripheral insulin does not penetrate into the CSF although peripheral glucose does and that a glucose dependent glucoregulator mechanism of which the function was modified by an increase of CSF insulin exists inside the central nervous system.

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DYNAMIC ANALYSES OF THE INTRAVENOUS GLUCOSE TOLERANCE TEST BY A MATHEMATICAL MODEL WITH TWO COMPARTMENTS OF INSULIN

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The dynamics of the blood glucose concentration and blood insulin activity during intravenous glucose tolerance test was mathematically identified with a linear differential equation model formulated with three variables, blood glucose, blood insulin and remote insulin. Six unknown parameters were estimated by a steepest descent method according to a least square error criterinn. The absurdity of parameter, the physiological paradox of positive sign of parameter found in the parameter estimation by so called Ackerman model, disappeared completely. The fitted to the model increased in number from 110 to 126 out of 134 total cases. The statistical difference of parameters between diabetics and non-diabetics was significant. The principal component analysis of five parameters yielded two components, representing insulin release and utilization of glucose.

This model is useful to estimate glucose tolerance and to identify clinical states of patients with diabetes mellitus.

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MECHANISM OF OPIOID ACTION ON SEXUAL RECEPTIVITY IN THE RAT. KUBO, K., TORII, M. and ANDO, Y. Dept. of Physiol., Inst. of Constitut. Med., Kumamoto Univ., Kumamoto 862.

The lordosis response to male mounting in the spayed rats treated with estrogen (E) and progesterone (P) was facilitated by the intraventricular injection of β-endorphin (EP) when EP was injected simultaneously with E-priming. The facilitatory effect of EP was observed exclusively during early stages of E action. The lordosis responses after local implantation of crystalline E into the septal-preoptic area and P injection were facilitated when EP was given at the onset of E implant. The response induced by E implant into the anterior ventromedial hypothalamus, however, was inhibited by EP, and the response after E implant into the ventral midbrain reticular formation was not affected. Moreover, the effects of EP upon lordosis response and LH release induced by a second injection of E or P 72 h after the first E injection were studied simultaneously in the spayed rats. EP 2 h after 1st E injection facilitated lordosis response but reduced E-induced LH release. EP following the 2nd E reduced LH release but did not affect lordosis response. EP on the day of 1st E facilitated P-activated lordosis response but did not affect P-induced LH secretion. The opioid actions may be closely related to the functional reorganization in the CNS induced by feedback of estrogen, and shows reciprocal effects on behavioral and secretory responses to estrogen actions. One of the sites of opioid actions may be estrogen receptive forebrain mechanisms.

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EFFECTS OF GONADECTOMY OR ANDROGEN-EXPOSURE DURING THE NEONATAL PERIOD ON THE SEXUAL DIFFERENTIATION OF THE RAT VENTROMEDIAL HYPOTHALAMUS.

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Existence of the sexually different groups of cells has been shown in the rat ventromedial hypothalamus by antidromic activation from the midbrain central gray. The differentiation depends largely, if not totally, on the hormonal environment during the perinatal period. Observed frequency distribution for the antidromic responses showed marked differences between the sexes. The distribution in the neonatally orchidectomized males resembled to that in the females, whereas neonatal treatment of female pups with testosterone made the distribution to bear some similarities to that of the males. Quantitatively, neonatal testosterone reduced the percentage of responses with latencies less than 11.0 ms from 41.4 % to 29.3 % (21.3 % in the males). An increase in the percentage of responses with latencies longer than 19.4 ms compensated for the loss of the faster responses. Neonatal orchidectomy had little effect on the percentage of the fast responses. The data demonstrate that testosterone interfered with development of the brain and suggest that this process may be set in motion before the birth.

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Modification of the circadian rhythm of corticosterone by restricted feeding regimen: differential effects of sodium pentobarbital

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Regulatory mechanism of corticosterone secretion induced by restricted feeding (RF) regimen was examined in male albino rats. Circadian rhythm of plasma corticosterone level was greatly modified by RF regimen, which was characterized by three changes: increased level of plasma corticosterone in anticipation of daily meal supply, a rapid decrease of the hormone level immediately after feeding and an attenuation of the circadian rise of the hormone level. The anticipatory secretion of corticosterone was much greater in rats whose feeding was fixed in the early dark period than in rats who had access to meal in the early light period. Decreased level of the hormone after meal was also higher in rats fed in the early dark period. Sodium pentobarbital injection suppressed the anticipatory secretion induced by RF regimen in dose related fashion but did not affect the circadian rise of the hormone level. The amount of the hormone suppression by the drug was as similar as that observed after feeding under RF regimen in both times of day. It is concluded that corticosterone secretion induced by RF regimen overrides the overt circadian rhythm of the hormone level and is differentiated from the secretion due to the circadian mechanism by sodium pentobarbital injection.

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RELATIONSHIP BETWEEN GROWTH HORMONE SECRETION AND SLEEP-WAKEFULNESS CYCLE IN THE IMMATURE FEMALE RAT.

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The correlation between growth hormone (GH) secretion and sleep-wakefulness cycle was studied in the 29- to 31-day-old female rat. They were bled serially through an intracardiac cannula at 10-min intervals from 1200 to 1800 h. EEGs recorded continuously during the blood sampling period were scored into wakefulness and sleep, and the amount of sleep for every 10 min was plotted against GH values as assessed by RIA.

The results were as follows; 1) The cross-correlation analysis performed between the time series of the amount of sleep and the GH value revealed a significant positive correlation between GH level and the amount of sleep during the preceding 10 min, and the power spectrum analysis followed by the least squares method showed that both time series had a 2.3-3.1 h periodicity. 2) Sleep deprivation performed between 1300 and 1500 h prevented GH secretory bursts, which otherwise should appear during this time period of a day. GH bursts occurred with the cessation of sleep deprivation. Analysis of the cross-correlation also revealed a significant positive correlation of GH values with the amount of sleep during the preceding 10 min.

It was indicated that GH secretion in the rat is sleep-related.

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SLEEP RHYTHM AND SEXUAL CYCLE IN JAPANESE MONKEY. YAMAOKA,S. Dept. of Physiol. Saitama Med. Sch. Saitama.

I have reported that the circadian rhythm of paradoxical sleep(PS) is correlated with an estradiol(E2) feedback in female rats, guinea pigs, cats and rabbits, and that the steroidal effects on sleep rhythm reflects the species differences of feedback system. These animals are not adequate as animal model for the study of relationship between sexual cycle and sleep rhythm in human ; because of short sexual cycle in rats, ultradian sleep rhythm in guinea pigs and copulatory ovulation in cats and rabbits. This study attempts to clarify the relationship between sexual cycle and sleep circadian rhythm in female japanese monkeys in relation to female baboons. Female monkeys were implanted with electrodes for recording EEG,EMG and eye movements. Slow wave sleep (SWS) and PS were monitored by visual inspection of long term records from sitting monkeys. Intact monkeys showed the circadian sleep rhythm with one peak at night. During menstruation, total amount of PS(0:00-24:00) maintained high level, while, around ovulatory period, tended to decline. On the other hand, in baboons, the daytime PS appearance increased during menstruation and ovulatory stage. Total amount of PS increased after E2 administration in ovariectomized monkeys and during the period of withdrawal bleeding. From these results, in female monkeys, E2 may partly be implicated with the sexual cycle dependent changes of sleep circadian rhythm.

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ETHOSECRETOGRAM - PHASE RELATION OF ULTRADIAN COMPONENTS BETWEEN SPONTANEOUS LOCOMOTOR ACTIVITY AND PLASMA CORTICOSTERONE IN FREELY MOVING INTACT RATS.
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Previously we showed (Neuroendocrinology 33, 52, 1981) that ultradian bursts of spontaneous locomotor activity were closely correlated with those of plasma corticosterone in rats with suprachiasmatic nucleus lesions. In the present study we have examined similar correlation, if any, between locomotor activity and plasma hormone levels in male rats freely moving on an Animex with implanted cardiac catheter. Sampling interval of 5 min was found suitable for effective resolution. Plasma corticosterone was determined by a competitive protein binding assay. 'Ethosecretogram' was examined in 31 rats, 9 of which were excluded from the results because of sustained high hormone levels, possibly indicative of stressful situation. The results showed that ultradian components were predominant even in conscious intact rats with periods ranging from 60 to 90 min. The bursts of locomotor activity were temporally correlated with those of plasma corticosterone level, the peak of the former preceding the hormone peak by 15 min. Further analysis revealed that the correlation was typical of the bursts due to feeding or scratching, whereas less typical in drinking behavior.

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PANCREATIC A CELL GENERATES ACTION POTENTIAL
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Pancreatic B cells of the isolated islet produce action potential characterised by bursting discharge superposed on a depolarisation plateau[1]. Pancreatic A, B and D cells have been identified morphologically in monolayer culture[2]. In the present experiments, A cells identified morphologically in monolayer culture of the newborn rat pancreas were found to produce simple spike action potentials in response to injected current pulse. They were also induced in Na-free solution. This simple spike action potential is not specific to A cell, since identified B cells also produced action potential of the same type. The simple spike is not due to immaturity of pancreatic cell membrane from newborn rats, since cultured pancreatic endocrine cells from adult rats also generated only simple spike action potential. The present experiments demonstrated for the first time that pancreatic A cells are electrically excitable. The difference of shape of action potentials between the isolated pancreatic islet and cultured cells is likely to be due to gap junctions between adjacent islet cells.

1. Dean PM & Matthews EK Nature 219, 389 (1968)
2. Kanazawa Y et al. in Diabetes (ed.Bajaj JS) 502 (Excerpta Med., Amsterdam, 1976)

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POLYAMINE METABOLISM IN THE HAMSTER EPIDIDYMIS

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Polyamines such as putrescine, spermidine and spermine have been shown to play a pivotal role in the regulation of cell proliferation and other cellular processes. When polyamines in the hamster epididymis were analyzed by high-performance liquid chromatography, several unknown peaks were observed. Three of them were identified as N'-acetylspermidine, N'-acetylspermine and sym-homospermidine by thin-layer chromatography, acid hydrolysis and gas chromatography-mass spectrometry.

The concentration of N'-acetylspermidine is much higher in the distal caput epididymidis than in other portions of the epididymis. Castration reduced the N'-acetylspermidine content in this region and its content was restored slightly by testosterone injection. The sym-homospermidine content was little affected by these treatments. The cytosol fraction of both caput and cauda epididymis had the ability to acetylate spermidine and spermine; spermidine was the better substrate for the acetyltransferase. The physiological significance of these compounds is discussed in relation to the function of the epididymis.

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GROWTH HORMONE RELEASE CONTROLED BY THYROID STATES TO CNS STIMULATION.

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Hypothyroid rats fail to release GH as the ultradian burst, while T_4 supplement to the rats recovers the characteristic secretion by 24 hr. Systemic administration of clonidine, α-2 agonist (15 μg/100 g body weight) to rats pretreated with α-MT (25 mg/100 g) for preventing the spontaneous GH burst stimulates the response which is affected by thyroid states. To analyse the hormone action, clonidine (1 μg/100 g) was given intraventricularly through the indwelling cannula to α-MT pretreated, conscious rats. Thyroidectomy 3 weeks prior to the experiment abolished the response to the drug, while T_4 (20 μg/100 g) also recovered the response by 24 hr. A minute amount of clonidine (67 ng/0.2 μl) was infused into the hypothalamus and the vicinity of conscious, normal rats through a double cannula fixed on the skull to find the area responsible for GH secretion. The localization was rather wide but there were two main area which resided in the POA-AH, and the upper part of mamillary body. Thyroid hormone may affect pituitary GH release through neuronal activities in these area.

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EFFECT OF OSMOTIC STIMULATION ON THE MILK EJECTION REFLEX IN RATS.

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On the day 9-11 of lactation, rats were anesthetized with urethane (1.1 g/kg b.w.) and a teat duct was cannulated for intramammary pressure recordings. Another cannula was inserted into the right atrium for blood sampling and oxytocin injection. The plasma osmotic pressure was altered by an i.p. injection of 1.5 M NaCl, 1 ml. Four hours after urethane injection suckling stimuli were given continuously by 8-11 pups. In 22 of 44 rats a milk ejection occurred with the mean latency of 33.2 ± 4.5 (S.E.) min and it recurred with the mean interval of 7.9 ± 0.6 min. The remaining 22 rats displayed no milk ejection more than 1.5 hours. The i.p. injection of 1.5 M NaCl brought about repetitive milk ejections to all the rats which exhibited no milk ejection until then. On the other hand, the rats which exhibited milk ejections during control recording increased the magnitude of milk ejections by 1.7 times but did not change their interval significantly. These results indicate that osmotic stimuli may alter the magnitude of oxytocin release during the milk ejection reflex but not the frequency of the reflex.

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RELATIONSHIP BETWEEN VOLUNTARY MUSCULAR EXERTION AND RESPIRATION BY MEANS OF RESPIROMETER (REPORT 2).

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By means of the same method of the report at the 58th Annual Meeting, the respiratory phase and volume in the voluntary muscular exertion were examined. This study examined particularly intra-subject changes of respiratory phase and volume in the voluntary muscular exertion. Subject was required to grip the hand-dynamometer voluntarily in three condition; 1) the quick motion, 2) the quick and powerful motion, and 3) the maximum speed motion.

The results were as follows: 1) In the quick motion condition , the pattern of respirogram could be devided into 4 types. And in the other conditions, the pattern could be devided into 3 types. 2) At the each onset of voluntary muscular exertion, the respiratory phase were almost inspiratory, and its volume (BTPS) ranged from 0 to 642ml at the quick motion, from 0 to 796ml at the quick and powerful motion, and from 0 to 1351ml at the maximum speed motion.

(The 0 indicates the expiratory end at the resting respiration.)

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VOLUNTARY SINGLE HUMAN MOTOR UNITS

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To study recruitment patterns of motor units (MUs), single human MUs of deltoid muscle were examined in various directions of force exertions using surface and coil-shaped inserted electrodes. Isometric forces detected by a force transducer at the elbow joint in the various directions were recorded simultaneously with action potentials of MUs. The subject exerted ramp forces at 1 kg/s at the direction of flexion, flexion-abduction, abduction, abduction-extension and extension. Burst discharges of anterior part of deltoid muscle were larger than other parts at flexion, those of middle part were largest at abduction and those of posterior part at extension. Threshold forces of MUs of anterior part were smallest at flexion-abduction, those of middle part were smallest at abduction and those of posterior part at abduction-extension. Threshold forces of MUs of middle part were decreased remarkably at abduction, although lesser changes were observed in low threshold units. Contraction times and peak tension of middle part MUs measured by averaging method were 45 - 75 ms and 3.1 - 79.4 g, respectively. Relation between the above firing characteristics and contraction properties of MUs was not clear. It is suggested that the activation of MUs in the deltoid muscle are finely localized at every direction of force exertions.

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THE THRESHOLD FORCE OF MOTOR UNITS IN FIRST DORSAL INTEROSSEOUS MUSCLE DURING VOLUNTARY ISOMETRIC CONTRACTION DEVELOPING FROM VARIOUS FORCE SPEEDS IN MAN.

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Subjects were required to make repeated ramp or ballistic contraction using first dorsal interosseous muscle. Force of abduction of the index finger was recorded using a strain gauge and a monopolar needle electrode (tangusten; tip 5μ) was inserted into the muscle for the motor unit recording. Each ramp contraction lasted about 1 to 5sec, and the time to peak of ballistic contractions were between 80 and 150msec or more. The contraction strengths were defined between 100 and 300g at each ramp or ballistic force developing.

The threshold force of almost ballistic contraction decreased in compare with the same strength of ramp contraction. The decrease was related to the force speed in most cases. Several modes of threshold changing were observed.

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ANALYSIS OF FACTORS EFFECT TO THE RELATION BETWEEN VOLUNTARY ISOMETRIC FORCE OF THUMB FLEXION MUSCLE AND INTEGRATED E.M.G. IN MAN. OISHI,K., YONEDA,T. & ISHIDA,A. Dept.of Physiol. Sch.of Physical Edu. Juntendo Univ. Narashino Chiba.

It had been reported that regression lines of the force-IEMG relation during voluntary rapid isometric contraction deviated for each trials and subjects, although the relations were almost linear. Some factors which might be doubted about deviation were tested as followed; 1) MVC % (instead of force impulses)-IEMG relations also revealed the deviation for trials and subjects. 2) MVC changed with angle of thumb joint reached maximum peak force in 20°-30°, however differences of IEMG were not so clear in any angles but for 0°. 3) To get the fact about effect of interelectrode resistance to IEMG, the constant muscle discharges elicited by electrical stimulation and special resistible electrode paste were used. Action potentials and IEMG were recorded constantly even though interelectrode resistance changed in the range from 18kΩ to 300kΩ. In conclusion, the effecting factor might be another more than MVC,joint angle and interelectrode resistance.

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URIC ACID METABOLISM AT EXERCISE (3th report)

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Hyperuricemia's serum uric acid(SUA) level decreased after light exercise(Ex.)(30-40 %Vo2max.).(1st report:1980) This Ex. also lowered hyperuricemia's SUA level on diurnal rhythm.The increase of SUA after exhaustive Ex. was controlled by being engaged in light Ex..(2nd report:1981) The purpose of these studies was the prevention and treatment of hyperuricemia.The purpose of the third study is to make a study of prevention methods for hyperuricemia on moderate Ex.For this purpose,there is a need to study the reason for the increase of SUA by exhaustive Ex..The reason for the increase of SUA by exhaustive Ex. was 1)the increase of synthesis 2)the decrease of excretion.This study is to investigate which reason is more significant.The result was as follows.1)SUA level didn't increase during and after exhaustive Ex. on taking an Allopurinol(SUA inhibitor, 200mg).2)SUA level didn't show remarkable decrease during and after exhaustive Ex. on taking a Benecid(SUA excretor,1g).The clearance of UA and excretion volume of UUA only increased.In case of giving a lot of water(4600ml/10hr.),he was absorbed high 02 gas(98%)for 90min. after Ex..SUA level was the same as his taking Benecid.Drinks for sports was more effective in the clearance of UA and the decrease of SUA level than water.

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EFFECTS OF BED BATH ON FUNCTIONS OF THE CIRCULATORY, RESPIRATORY, AND NERVOUS SYSTEM. *ABE, K. AND **TANAKA, I. Dept. of Physiol., Kumamoto Univ., *Coll. Med. Sci., **Med. School, Kumamoto

Bed bath is the nursing technique to wipe and clean a patient on a bed. The girl students aged 19–21 years were wiped with soap and warm water (50°C) for 25 min after a rest for 30 min on a bed. The duplicate examinations were carried out at two different room temperatures (17–21°C and 27–29°C), in course of time from 30 min before bed bath to 40 min after bed bath. Heart rate decreased by bed bath, most remarkable at 27–29°C. Mechanocardiography revealed shortening of ejection time, prolongation of isometric contraction time (ICT), and lowering of an index of heart strength (minimal blood pressure/ICT^2). These results indicate that work of the heart decreased by bed bath. By bed bath at 27–29°C, the skin temperature rose at the dorsal region of the foot, palmar and plantar region. By bed bath at 17–21°C, the skin temperature rose at the plantar region, but fell at the abdominal and palmar region. Pulse conducting time to the finger was prolonged by bed bath, but was shortened 40 min after bed bath at 17–21°C. Bed bath produced a decrease in oxygen uptake and no alteration in EEG, NCV, and plasma cortisol levels.

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SIGNIFICANCE OF SERUM TRIGLYCERIDE LEVELS FROM THE VIEWPOINTS OF A INDICATOR OF EXERCISE INDUCED STRESS.

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Serum triglyceride levels(TG) didn't increase during walking, when the man walked long distance and more over, when walked more speedy. In running, however, TG tended to increase after running, when run more longer. Relation between TG and catecholamine levels existed.

We supposed that TG seems to be a indicator of exercise induced stress.

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WATER AND ELECTROLYTE METABOLISM DURING AN ACUTE EXPOSURE TO A SIMULATED ALTITUDE BEFORE AND AFTER MOUNTAIN CLIMBING.

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To study pathophysiology of acute mountain sickness (AMS), alterations in water and electrolyte metabolism during acute altitude exposure were compared before and after mountain climbing (Pre-M,Post-M) of more than 4,000m for 3 weeks. 7 lowlanders (25-35 yr) were exposed to a simulated altitude of 6,000m (2hr ascent, 2hr sojourn, 2hr return) 1) Pre-M: Elevated Ht, serum protein and osmolality (Osm) was associated with decreased urine flow and elevated ADH at early phase of hypoxia suggesting fluid shift from extra- into intracellular space. Serum Na, Cl rose but serum K fell. Elevated aldosterone (Ald) was associated with plasma renin activity (PRA) and cortisol elevation suggesting both PRA and ACTH involved in Ald elevation. The elevated Ald may have acted on water retention and intensified serum electrolyte alterations. Serum Ca rose in parallel with serum protein, whereas serum P fell markedly. 2) Post-M: Most of responses became less marked except serum K. Osm, electrolytes, PRA and Ald were higher and urine flow was less in post-M suggesting an activated renin-ald system probably by plasma volume depletion. These results suggest that fluid shift and electrolyte changes may be important in AMS and hormonal change may modify these alterations either positively or negatively, activated renin-ald system may play adaptational roles under chronic hypoxia.

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CARDIAC OUTPUT RESPONSES IN MAN DURING REPEATED, SEVERE HYPOBARIC HYPOXIA.

MORI, S., SAKAKIBARA, M., TAKABAYASHI, A., TAKAGI, S., YAGI, T. AND MITARAI, G. Dept. of Aerospace Physiol., Res. Inst. Environ. Med., Nagoya Univ., Nagoya 464.

Cardiac output (CO) was measured by an impedance method for 8 subjects exposed to severe hypoxia in a decompression chamber. The peak of simulated altitudes was increased daily by an 1,000m step up to 8,000m (0.35 ATA) within 4 consecutive days. Two of the subjects who had climbed a 7,000m mountain 3 months before, showed the maximum CO above 7,000m (0.41 ATA), while most of the other showed at 6,000m (0.47 ATA), against a given workload which was a moderate exercise at sea level. The increase of CO was mainly due to acceleration of heart rate (HR). An ability to perform the exercise under hypoxia was improved in 3 subjects of the latter group, whereas it was lowered in the former two, in the last day of the experiment. In such cases where the exercise could not be completed, which happened above 7,000m, reduced CO responses had preceded; where the lowered stroke volume instead of HR was largely contributed. Effects of repeated exposure did not clearly manifest in the cardiac function, but a tendency that the large difference observed under severe hypoxia between the groups became small from day to day, breaking up again in the fourth day, might reflect a shift of the function.

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AUTOREGRESSIVE ACTIVITY OF STEP LENGTH IN LEVEL AND GRADE WALKING ON TREADMILL.
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Time series data of step lengths were analyzed by autoregressive method to study central mechanisms of rhythmic control of walking. Computerized system was employed to determine step length. The system consists of a position sensitive device, foot switches and a mini-computer. Subjects are six adults. They walked on a treadmill with inclinations of 0, 5 and 10%, at different speeds between 60 and 120 m/min with an increment of 10 m/min. A thousand step lengths were measured for each experiment. Mean values of step lengths increased and standard deviations decreased with increment of speed. These maximal values appeared in the steepest grade walking. Some subjects showed right-left asymmetry of step length, usually the length of right step being longer than that of left step. The autoregressive power spectrum revealed a dominant frequency of 0.5 cycle /step. By application of component analysis, percentage of power at different frequencies was determined, and the power at 0.5 cycle/step was found to reach 20-60% of the total. The two-step periodicity is partially due to the laterality of step length, although it was significantly dominant in subjects with right-left symmetry,too. These findings suggest that in free walking an endogenous periodicity mechanism is operating with a period of two steps.

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EFFECT OF MILD PHYSICAL TRAINING FOR 7 SUCCESSIVE DAYS ON BLOOD CONSTITUENTS OF WOMEN.
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Changes in blood constituents induced by mild exercise for 7 successive days were observed on 15 female university students in winter The subjects performed bicycle ergometer exercise at the constant work load of 1.25 Kp for 60 min. in the room temperature. The subjects with lower value of hemoglobin concentration showed rise in concentrations of hemoglobin and erythropoietin and no change in concentration of plasma iron after physical training when compared with those before the beginning of the training. The subjects with higher value of hemoglobin concentration showed fall in concentrations of hemoglobin and plasma iron and no change in concentration of erythropoietin after training when compared with those before the beginning of the training. These results indicate changes in blood constituents induced by mild exercise for 7 successive days were influenced by the level of hemoglobin concentration before the beginning of the training.

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RELATIONSHIP BETWEEN O_2 DEBT AND INTERMEDIATE METABOLISM — cholesterol metabolism in submaximal exercise —
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In recent years, it has reported that serum HDL-cholesterol of athletes with the aerobic training for a long time increased.

Subjects of which engaged the long-distance and sprint of track and field, and judo performed for ten minutes the exercise of 80 % $\dot{V}O_2$ max. using by bicycle ergometer. Oxygen uptake was determined throughout exercise and recovery. Quantitative analysis of chemical substances in blood were as follow: glucose, lactate, triglyceride and cholesterol(cho.)fraction (VLDL, LDL, HDL).

In the long-distance runner, amount of serum HDL-cho. was higher than that of other subjects at rest, and further increased with exercise. In general, the increment of serum HDL-cho. are refered to associate with the acceleration of cho. metabolism in tissues, and converte from chylomicron, VLDL- and LDL-cho. to HDL-cho.

The increment of HDL-cho. in long-distance runner might be owing to accelerate the activity of Lecithin Cholesterol Acyltransferase(LCAT)converting from cho. ester to HDL-cho.

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A STUDY ON THE VARIATION OF SKIN-TEMPERATURE BY THE STIMULATION FROM COLD WIND.

ITOW, H. AND ARAI, Y. Facul. of Litera. and Dome. Sci. Ichikawa

(Object) We study on the adaptation of children to the cold temperature. (Method) The subjects are the children of the nursery school on naked principle (♂ 32 ♀ 18). We gave the two subjects the wind from the fan (speed 2.2I m/s) on their baeks and measured the temperature of their backs forIO minutes (5 minutes during the ventilation, 5 minutes after the ventilation) (Result) I. We think that the state of drop of skin temperature by the wind relates the thickness of skin-fat. 2. We think that the recovery of drop of skin temperature relates the duration of naked nursing.

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THE BASIC MOTIONS OF TENNIS AND RESPIRATORY MOVEMENT.

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Respiratory movement, EMG of M. deltoideus and impact signals detected by strain gauge attatched on a racket were recorded during tennis motions by telemeter in 8 male subjects. The respiratory movement was recorded by thermister inserted into the nasal cavity. It was confirmed that the recorded temperature changes coincide with changes in respiratory flow volume and rate without time lag. For each type of tennis motion, subjects hit 20 balls supplied by automatic machine every 3 sec. In well-trained subjects, the motions were correlated with respiratory phase. On the forehand stroke, take back swings always took place in the inspiration phase. The onset of forward swing nearly coincided with the beginning of expiration and the expiration was transiently held at the moment of impact. These are commonly observed for all types of the motions investigated; that is, forehand and backhand stroke, forehand and backhand volley, smash and serve. On the other hand, beginners failed to adjust their respiratory movements according to the motions, so that the moment of impact fell on the different phase of respiratory cycle from shot to shot. These observations suggest that the muscular performance is influenced by respiratory phase.

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STUDIES ON THE SURFACE ELECTROMYOGRAM OF MASTICATORY MUSCLES ON THE MAXIMUM CLENCHING.

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EMG activities during substained the maximum clenching was studied. 5 subjects with no physiological abnormality in stomatognathic region were used.
Subjects were instructed to keep the maximum clenching on central occlusion untill they could not hold it for fatigue. Their EMG activities during clenching were divided into four parts, and power valuue, amplitude and interval of emg spike were analyzed separately.

The result is as follows: total power values and averaged amplitudes decreased gradually during clenching, while intervals with maximum number of spikes were unchanged appreciably.

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ON THE POSTURE SEEN FROM THE CURVE OF HEAD-TOP.

ITOW. H., OSADA. Y. AND RI. R. Body Designer School. Tokyo

(Object)We study the state of posture by the curve of head-top. (Method) We used the curve-meter of head-top. The mechanism of structure is the following.The subject puts on the hat which is fixed the recording apparatus and his movement is recorded as the curve on the recordingplate over the head. On the recording the subject closes his eyes, the recording time is I minute. (Result) I. We think that the type of the curve of head-top is classified in three types (before and behind, left and right and rotation) 2. We think that the curve of head-top relates the type of the rest posture.

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RELATIONSHIP BETWEEN EVOKED MYOELECTRIC SIGNALS AND TENSIONS ON THE PLANTAR FLECTION BY THE ELECTRIC STIMULUS.

NAGATA, A., HANBA, M., MIYAZAKI, M. AND MURO, M. Bio-dynamics Lab., Tokyo Metro. Univ., Dept. of Physiol. Sch. Dent. Showa Univ., Physical Edu. Waseda Univ. and Bio-dynamics Lab. Tokyo Coll. Pharma.

The purpose of this study is to discover relationship with evoked myoelectric signals and evoked tensions before and after the sustaining 45 min. standing exercise. Seven subjects were asked to lie on the bed and were accept electric stimuli on their tibialis nerves.These stimulus conditions were applied to them for square-waves of 0.5msec dura. and 5-80 Volt. Myoelectric signals(H and M-waves) in the gastrocnemius and muscular tensions from the plantar flection, which were measured by the special transducer of load-washer type, were evoked to storage in CRO.

Before this standing, the supramax. amplitude of M- and H-waves and tensions were shown as values of 4.7mV, 0.55mV and 8.8kg, while after this standing, those values were presented 6.66mV, 0.51mV and 8.3kg respectively. Those data indicate no effects on the excitatory state in the spinal cord and the amplitude of M-wave only had increased with the activated condition of muscle fivers after this exercise. On the contrast, the evoked tension had decreased with fatigabilities of FOG fiver type and FF motor unit. Efficiency ratio of M-wave/Tension had increased and especially untrained subjects had falled down into the level of fatigable exaustion after 45min. sustaining exercise.

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ON VARIANCE AND COVARIANCE OF WAVE PARAMETERS FOR COLD VASOFLUCTUATIONS
UEDA,G. TAKEOKA,M. AND KOSHIHARA,Y. Dept. of Adapt. Physiol., Shinshu Univ. Sch. Med., Matsumoto, Nagano.

When cold stimuli of -3 to -10 ℃ are applied to the rabbit's earlobe vessels, the vasomotor oscillation can be evoked. The fluctuations indicate irregular or regular patterns. The wave characteristics were examined by the wave heights X and the periods Y. First the standard deviation SDX and SDY were obtained. From these, coefficients of variation, CVX and CVY, were calculated. Thereafter, the new coefficient, CVXY, was obtained, while the coefficient of correlation RXY was calculated. These parameters reveal the absolute values of scatter (SD), the shift from the origin (CV) and the normalized property (R). Further, the pattern characteristics for CVX and CVY were expressed by a principal component. This was designated as W1. When this value increases, the regularity of the pattern is considered to be increasing. Lastly, the decreasing and the sequential orders were compared by rank correlation methods. The result indicated that most of the W1-series showed the lower coefficients. This means that the pattern regularity was generally steady for the observation periods of about 2 hours or so.

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PERMEABILITY THEORY ON HIGH-ALTITUDE PULMONARY EDEMA
UEDA,G. SAKAI,A, KOBAYASHI,T*, KUBO,K*, FUKUSHIMA,M*, YOSHIMURA,K* AND SHIBAMOTO,T* Dept. of Adapt. Physiol., Dept. of Internal med*, Shinshu Univ. Sch. Med., Matsumoto, Nagano.

When sheep were exposed for 3 to 4 hrs under a simulated altitude of 6,600 m or the left atrial pressures increased, lymphatic flow J_V increased remarkably. This phenomenon was considered as a model of highaltitude pulmonary edema. The regulating factors were reduced to the transcapillary hydrostatic and osmotic pressures. Also, the contribution of the permeability was estimated from the change of σ, a reflection coefficient. Between the σ and the ratio R of lymph to plasma protein concentrations, there exists Perl's relation. Here, dR/dJ_V becomes negative when σ=const. Thus when dR/dJ_V approaches to zero, σ must decrease to zero. This means that when R is constant, σ is decreasing, indicating the increased solute permeability. The σ-R relation is nearly hyperbolic when J_V is constant. The way to interprete the change of σ was considered from a three-dimentional standpoint, σ-R-J_V. Further, the calculation of effective permeability derived from Kedem-Katchalsky coefficients was made possible using colloid osmotic pressure data.

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SIMULTANEOUS EFFECTS OF VARIOUS TEMPERATURES AND LOW PRESSURES ON RAT OXYGEN CONSUMPTION.
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1. The controls (the Wister-strain adult male rats) indicated the greater increase of $\dot{V}O_2$ when the ambient temperatures were lowered. This increase was inhibited when measured under low pressures such as 2 to 4 km in terms of simulated altitudes. As for the rectal temperatures, both lowered temperatures and pressures decreased the values.
2. Rats raised in Yatsugadake, the altitude 2.1 km, for one month were compared with controls. As the combined effects of variable low temperature and constant low pressure (2 km in altitude conversion), Yatsugadake group indicated always greater relative rises of $\dot{V}O_2$ than those of controls, especially at 0 ℃ and 10 ℃. Here the response at a 20 ℃ - 0.6 km was set as 100%. Later the deadaptation took place.
3. However, outdoor acclimated rats raised in 0.93 ℃ on average indicated the less responses for the above-mentioned combined stimuli.
4. It is concluded that cold- and low pressure-exposure group induced greater responses in combined stimuli whereas cold-only exposure group induced lesser responses for combined stimuli.

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CHANGES IN VENTRICULAR WEIGHT AND BLOOD PARAMETERS OF WOOD MICE CAPTURED AT VARIOUS ALTITUDES THROUGHOUT FOUR SEASONS MOTOYAMA,T., SAKAI,A., YANAGIDAIRA,Y. AND UEDA,G. Dept. of Adapt. Physiol., Shinshu Univ. Sch. Med., Matsumoto, Nagano.

At three different altitudes in Nagano Prefecture [Tobira pass : 1,000 m, Shibunoyu at Yatsugadake (Y) : 1,900 m, Kuroyuridaira at Y : 2,400 m], wood mice (Apodemus argenteus) were captured every month throughout the year. It was then revealed that the right ventricular weight (RVW) / body weight (BW) increased significantly as the altitude increased whereas the left ventricular weight (LVW) / BW did not. Here, the total ventricular weight (TVW) / BW, ($\times 10^{-3}$), were 4.96 $\pm$ 0.62 for 1,000 m group, 5.55 $\pm$ 0.89 for 1,900 m, and 5.76 $\pm$ 0.95 for 2,400 m. The hematocrit values (Ht) showed a significant increase with the altitudes. The similar tendency was shown in hemoglobin concentrations. It followed from these that the positive correlation coefficient, $r=0.29$ ($p < 0.05$), was shown between the RVW/TVW and the Ht. In all three groups, RVW/TVW values showed respectively the minimum in summer and the maximum in winter. Corresponding to this, the blood viscosity data obtained from the mice of Tobira pass indicated lower values in summer than those in other seasons.

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SEASONAL RHYTHMS OF ENERGY METABOLISM IN THE DJUNGARIAN HAMSTER (PHODOPUS SUNGORUS), LIVING IN NATURAL PHOTOPERIOD AND AMBIENT TEMPERATURE. TOKURA, H. AND OISHI, T. Lab. Physiol., Dept. Clothing Sci., Nara Women's Univ., and Dept. Biol., Fac. Sci., Nara Women's Univ., 630 Nara

There existed remarkable seasonal changes in body weight, food intake, rectal and back skin temperatures and metabolism-temperature curves in the Djungarian hamster reared under natural photoperiod and ambient temperature throughout the year at Nara. At an ambient temperature (T_a) of 24°C body weight specific resting metabolic rate was highest in summer and lowest in winter. Although metabolic rate increased gradually both in summer and in winter, its slope was steeper in summer than in winter, and the lower critical temperature seemed to lie between 25° and 30°C in summer and between 20° and 25°C in winter. Food intake was lowest in summer and highest in winter, but body weight highest in summer and lowest in winter. Rectal and back skin temperatures measured immediately after 2 hrs' exposure to T_a of 0°C was significantly lower in winter than in summer. Short photoperiod seemed to be important environmental cue for enlargement of shell and decrease of body weight in winter.

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THE SEASONAL VARIATION OBSERVED IN THE INTERNAL MEMBRANE OF A TOAD SKELETAL MUSCLE FIBER

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The physiological properties of skeletal muscle of amphibians are influenced by the environmental conditions of its habitat. In order to obtain some information on the seasonal changes of some properties of the internal membrane system of skeletal muscle fiber, we observed the monthly changes in excitability of the skinned fiber of a Japanese toad which was placed in oil. Threshold voltage(Tv) of a twitch-like propagated contraction was used as an indicator of the excitability of the internal membrane. The threshold concentration of caffein(Tc) of intracellular ripple-like contractions was used also as an indicator of the properties of the internal membrane, particularly of sarcoplasmic reticulum. The seasonal changes of Tv and Tc recorded the min. point in the spawning season of a toad and the max. point in the beginning of autumn. Moreover, Tc recorded another min. point in the beginning of summer. The change of Tc is hardly dependent on the environmental temperature. These results indicate that the seasonal changes range over the intracellular property of a toad skeletal muscle.

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BLUNTED RESTRAINT-INDUCED THERMOGENESIS BY FASTING IN RATS.
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Physical restraint was made in fasted (F_3: fasted for 3 days, F_7: fasted for 7 days) and fed control (C) rats. In contrast to C group, F_3 and F_7 groups showed low resting metabolism and low calorigenic response to restraint. During restraint, plasma levels of norepinephrine (NE) and epinephrine (E) were markedly increased, but not significantly different between C and F_7 groups. Calorigenic responses to NE and E (0.6 and 1.2 nmoles$\cdot kg^{-1}\cdot min^{-1}$) were significantly less in F_3 and F_7 groups than in C group. Plasma concentrations of glucose were increased by infusion of catecholamines in C and F_7 groups. The values were not different between both groups. Plasma levels of free fatty acid in F_7 group were significantly higher than in C group before and during catecholamines infusion. Plasma concentrations of thyroxine and triiodothyronine were significantly less in F_7 group than in C group. The blunted restraint-induced thermogenesis in fasted rats may be attributed to the decreased calorigenic response to catecholamines. The decreased calorigenic response may be due to suppressed utilization of energy substrates in calorigenic tissues produced by lowered activity of thyroid hormone.

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CHANGES IN HEAT PRODUCTION AND HEAT LOSS IN FOOD-RESTRICTED RATS.
Y. SUGANO AND T. NAGASAKA Dept. of Physiol., Sch. of Med., Kanazawa Univ., Kanazawa

Food consumption, heat production (M) and heat loss (H_L) of two groups of rats, one was the nibblers and the other was the meal-eaters, were continuously measured in a constant dark environment of a direct calorimeter (T_a = 24°C). In the meal-eaters, food was supplied only for 2 hrs (10:00 - 12:00) daily. During the initial few days of food restriction schedule, the daily food consumption and body weight markedly decreased. However, the decrease in M and H_L was small and energy balance became highly negative. Three wks after the start of food restriction schedule, body weight began to increase but food consumption was not returned to the control value. M and H_L were significantly lower than those of the nibblers. These results indicate that rats may adapt to food restriction by decreasing energy dissipation as heat, that is, by increasing the efficiency of food.

	nibbler	meal eater	
		1 day	2 mo
M ($W\cdot m^{-2}$)	52.67	42.26	31.43
H_L ($W\cdot m^{-2}$)	53.56	42.77	31.46
Fin ($g\cdot kg^{0.75}$)	54.96	28.97	33.32
ΔBW ($g\cdot day$)	4.69	-9.92	3.69

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APPETITE FOR ESSENTIAL AMINO ACID IN GROWING RATS. KISHI, K. AND INOUE, G. Dept. of Nutr., Sch. of Med., Tokushima Univ., Kuramoto-cho, Tokushima.

It has been demonstrated from the self-selection experiment of protein diet and protein free diet that the rat regulates protein intake as well as energy intake. In the present study we examined the possible regulation of single amino acid intake. Growing rats of Wistar strain weighing about 80g were fed eight 10% single essential amino acid deficient diet with drinking water and the respective amino acid solution for 14 days. Control rats were given 10% complete amino acid mixture diet simulating egg protein with drinking water only. The sum of the intakes of drinking water and amino acid solution was significantly correlated to food intake and ranged from 15 g/day in Phe to 25 g/day in Lys. In all groups the intake of amino acid solution was greater than that of drinking water indicating that rats preferentially selected amino acid solution. Each amino acid intake expressed as dietary per cent was Thr 0.57, Lys 1.57, Met 0.80, Val 0.78, Trp 0.18, Leu 0.96, Phe 0.87 and Ile 0.61. These values are similar to amino acid requirements of growing rats (NRC) and resembles egg protein except Lys which was 1.9 times greater than egg protein. It is suggested that rats can adjust single essential amino acid intake to their body needs.

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INHIBITION OF SHIVERING AS A CAUSE OF METABOLIC SUPPRESSION WITH NOREPINEPHRINE IN RESTRAINED AND UNRESTRAINED RATS.

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Metabolic responses to infused norepinephrine (NE) or phenylephrine (PHE) were studied at 13°C in unrestrained or restrained rats. In the unrestrained rats, shivering was greatly inhibited and resting metabolic rate (M) was decreased by NE infusion in a dose of 2 - 4 μg/kg·min. After sinoaortic denervation, no such changes in M and shivering were observed. In the restrained rats, muscle activity including shivering and M were not suppressed by NE infusion. Vasopressor response to PHE (10 - 50 μg/kg·min) and baroreflex sensitivity were greatly reduced by physical restraint, which may contribute to the absence of metabolic suppression by NE infusion in the restrained rats. These results indicate that NE infusion inhibits shivering via the sinoaortic baroreceptor reflex in rats.

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BAROREFLEXIVE SUPPRESSION OF COLD THERMOGENESIS IN RATS.

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Metabolic response of warm-acclimated (WA: 25°C for 4 wks) and cold-acclimated (CA: 5°C for 4 wks) rats to infused norepinephrine (NE) (0.5 μg/kg·min) or phenylephrine (PHE) (5 - 30 μg/kg·min) was studied at temperatures below thermoneutral zone, either before or after sinoaortic denervation (SAD). At 13°C, shivering occurred consistently in WA but not in CA. NE (2 - 4 μg/kg·min) suppressed shivering and decreased heat production (M) in WA. Such a decrease in M was not observed after NE infusion in CA. The decrease of shivering and M was abolished by SAD in WA. At -5°C where shivering occurred in cold-acclimated rats, NE (4 μg/kg·min) decreased M accompanied by suppression of shivering in CA. The results indicate that infused NE suppresses shivering via the baroreflex in rats. At 13°C, PHE (15 - 30 μg/kg·min) caused a decrease in M and a fall in brown adipose tissue (BAT) temperature in CA. However, SAD abolished these changes in CA. These results suggest that alpha-adrenergic stimulants inhibit shivering and nonshivering thermogenesis in BAT via baroreflex in rats.

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THE METABOLISM OF ENERGY BY ARCHERS. - The relationship between energy metabolism and actual bow weights. -

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In order to investigate the relationship between energy metabolism and bow weight, the following experiments were performed. The subjects shot 6 arrows using bow weights of 32, 42 and 51 lbs (males) or 25, 35 and 45 lbs (females) and ran a distance of 30 meters and back to recover the arrows (Exp. I). The same subjects then shot 24 arrows without running to recover the arrows (Exp. II). Energy consumption levels during the experiments and at rest were recorded. In both experiments, relative metabolic rates increased linearly in proportion to increasing bow weight for all subjects. Energy requirements for each shot were 0.47, 0.59 and 0.73 kcal for males and 0.25, 0.39 and 0.47 kcal for females, following the above weight orders respectively. Energy requirements per 10kg of body weight for one shot were 0.08, 0.10 and 0.12 kcal for males and 0.04, 0.07 and 0.08 kcal for females. The values increased linearly in proportion to the increase in bow weights. Positive correlations were found between bow weight and energy requirements— 0.70 (males) and 0.84 (females). There were as well as between bow weight and energy requirement per 10kg— 0.68 (males) and 0.81 (females).

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MODIFICATION OF HYPOGRAVIC EFFECTS OF STATIC-ANTIORTHOSTASIS IN RATS. ABE,M., NAKAYA,M SUDOH, M. AND SAIKI, H. Space Medicine Lab., The Jikei University, Minato-ku, Tokyo

The comparison between the physiological effects of static orthostasis (S-OS) by horizontal suspension model (H. Saiki et al, 1970) and static antiorthostasis (S-AOS), body axis 20°, head down position, oblique suspension model (H. Saiki et al, 1980), in rats, for many parameters were investigated, and the following results were obtained. The data on the body weight, resting metabolism rate and urine volume, showed the clearer effects of hypodynamics at the case of S-AOS group, than that of S-OS group. Especially the resting metabolic rate were decreased in S-AOS group, in spite of the increasing in S-OS group. But in the mineral metabolism, especially urinary excretion of Na^+, Ca^{2+} showed a different tendency. No or less increasing of them were observed in S-AOS group than in S-OS group. From the characteristics of Ca^{2+} metabolism in S-AOS, some antiorthostatic effects were noticed related with the neuro-humoral blood-Ca^{2+} control factor. In the blood pressure, measured at tail arteria and urinary excretion rate of K^+, no change between AOS and OS group were observed during hypodynamics exposure and recovery time after the liberation. Such findings also seem to be useful for the solution of the physiological effects of the dynamic antiorthostatic suspension restraint, (D-AOS) (Morey et al, 1976).

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EFFECTS OF BILATERAL ADRENAL DEMEDULLATION AND 6-HYDROXYDOPAMINE ON COLD-INDUCED RESPONSES OF ENDOCRINE PANCREAS IN RAT. HABARA, Y., OHNO, T*, YAHATA, T. AND KUROSHIMA, A. Dept. of Physiol., Asahikawa Med. Coll. Asahikawa, and * Div. of Nutr. Physiol., Hokkaido Educational Coll., Asahikawa

In addition to catecholamines, pancreatic hormones, especially glucagon, have been reported to be closely involved in cold acclimation. The effects of bilateral adrenal demedullation plus chemical sympathectomy with 6-hydroxydopamine(ACS) were investigated in order to know the relation between glucagon and sympatho-adrenal system in the cold. Acute cold exposure to -5°C for 150 min increased plasma glucagon level in both ACS and sham operated and vehicle injected control (SV) rats, and it increased plasma insulin level only in ACS rats. Plasma insulin level in rat fed at 25°C was decreased by ACS. Insulin/Glucagon molar ratio (I/G) decreased by cold exposure in SV rats. In ACS rats, I/G decreased but was not affected by acute cold exposure. ACS rats responded to acute cold exposure with less increase in blood glycerol and more increase in blood free fatty acids. These results suggest that both sympatho-adrenal system and pancreatic glucagon are closely associated with cold-induced responses in rat possibly through regulating the fuel metabolism in a compensatory way.

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BROWN FAT INSULIN AND GLUCAGON IN TEMPERATURE-ACCLIMATED RATS. KUROSHIMA,A. AND HABARA,Y. Dept. of Physiol., Asahikawa Medical Coll., Asahikawa

In order to know a significance of role of pancreatic hormones in brown fat (BAT) in relation to temperature acclimation, the changes of the hormones in the interscapular BAT were studied in the temperature-acclimated rats. Glucagon level in BAT on the basis of g fresh weight was ca. 17 times higher than in the plasma. It increased in the cold-acclimated rats, while it decreased in the heat-acclimated rats. Cold acclimation induced the increase in protein content, while heat acclimation decreased it. Consequently, BAT glucagon content in terms of mg protein was not different among the groups. Plasma glucagon level was elevated in the cold-acclimated rats, but decreased in the heat-acclimated ones. Plasma insulin level decreased in both cold-acclimated and heat-acclimated rats. Insulin level was similar between BAT and plasma. Cold acclimation increased BAT insulin level in terms of mg protein as well as fresh weight. The submaxillary gland also contained considerably higher glucagon and insulin contents than the plasma. However, neither cold nor heat acclimation affected the submaxillary hormones.

These results suggest significant roles of pancreatic hormones in BAT function in temperature acclimation.

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EFFECT OF GLUCAGON ON THE BLOOD FLOW THROUGH BROWN ADIPOSE TISSUE. YAHATA,T., HABARA, Y. AND KUROSHIMA,A. Dept. of Physiol., Asahikawa Med. Coll., Asahikawa

Blood flow through brown adipose tissue (BAT) at various sites was measured with ^{113}Sn-labelled microsphere in the hexobarbitar-anesthetized warm controls (WC), cold-acclimated (CA) and heat-acclimated (HA) rats after 30 min infusion of glucagon (G)(0.2-4.0 μg/min). Blood flow increased maximally at 2 μg of G. G-induced increase in total BAT blood flow was significantly greater in CA and less in HA than that in WC. Noradrenaline (NA)(2 μg/min) caused greater increase in all groups than G. However, neither G- nor NA-induced increase in blood flow per unit BAT weight was modified by cold or heat acclimation. G increased cardiac output in both CA and HA, while NA increased it in HA but not in CA. The fractional distribution of cardiac output to BAT showed similar patterns to those of blood flow through the tissues.

The present findings suggest that increase in the blood flow through BAT would support, at least in part, the enhanced metabolic activities of BAT, but that the blood flow per se would not directly relate to the modified responsiveness of BAT to G or NA in temperature acclimation as previously reported.

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ENHANCED CALORIGENESIS IN BROWN ADIPOSE TISSUE OF PHYSICALLY TRAINED RATS. HIRATA, K. AND NAGASAKA, T. Dept. of Physiol., Sch. of Med., Kanazawa Univ., Kanazawa

Changes in blood flow to brown adipose tissue (BAT) and arteriovenous oxygen difference across the interscapular BAT in response to infused norepinephrine (NE: 4 μg/kg·min) were measured in physically trained (TR) and control (CT) rats. TR rats were trained by 2-hr swimming in agitated water at 36°C daily for 18 wks. The colonic temperature was maintained at approximately 37.5°C throughout the training period. The average weight of BAT was significantly less in TR than CT rats. With NE infusion, blood flow to BAT significantly increased and O_2 content of Sulzer's vein significantly decreased in both groups. The contribution of BAT O_2 consumption to whole body O_2 consumption was estimated as 36% in CT rats and 50% in TR rats. The increase in the O_2 consumption of other tissues was almost identical in both groups. These results indicate that physical training *per se* enhances the calorigenic response of BAT to NE supported by an increased cardiac output and blood flow to BAT in rats.

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CALORIGENIC RESPONSIVENESS TO TRIIODOTHYRONINE IN COLD-ACCLIMATED AND HEAT-ACCLIMATED RATS. DOI, K. AND KUROSHIMA, A. Dept. of Physiol., Asahikawa Med. Coll., Nishikagura , Asahikawa

From this laboratory glucagon has been previously suggested to serve thermoregulatory nonshivering thermogenesis in temperature acclimation as a calorigenic hormone through its action on brown adipose tissue (Life Sci., 1982). In the present study calorigenic effect of triiodothyronine (T_3) on whole body oxygen consumption ($\dot{V}o_2$), and interscapular brown adipose tissue (IBAT) and body temperature was investigated in the unanesthetized and non-restrained warm-acclimated rats (25°C)(WA), cold-acclimated ones (5°C)(CA) and heat-acclimated ones (34°C)(HA). $\dot{V}o_2$ was measured by using an automatic and manometric apparatus. IBAT and body temperature was continuously measured by means of telemetric transmitter system. Injection of T_3 (100 μg/100g, sc) produced significant elevations of $\dot{V}o_2$, and IBAT and body temperature in WA and CA for the period of 18-48 hr. Calorigenic response to T_3 was significantly potentiated in CA, but suppressed in HA as compared with that in WA. The extent of calorigenesis due to T_3 was significantly less than that due to glucagon or norepinephrine, but T_3-induced calorigenesis was maintained for longer period.

The present study suggests that T_3 may also function as a calorigenic hormone to be involved in the regulation of nonshivering thermogenesis in cold acclimation.

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EFFECT OF THERMAL ACCLIMATION ON VASOMOTOR CONTROL TO CHANGE IN ENVIRONMENTAL AIR TEMPERATURE. TSUCHIYA, K. (Dept. of Environment. Physiol., Inst. for Tropical Medicine, Nagasaki Univ., Sakamoto-machi, Nagasaki)

Adult male Wistar rats were thermally acclimated at 10°C and 30°C for more than 4 weeks with ad libitum food and water. During the experiment, conscious animals were kept in a wire-netting cage (5cm x 10cm x 22cm) individually. Environmental air temperature was changed at a constant rate of 1°C/3 min in the low temperature range of 10°C to 20°C and in the high temperature range of 20°C to 30°C and vice versa, respectively. An increase in heart rate was observed during a fall of environmental air temperature, while a decrease in heart rate was observed during a rise of environmental air temperature. When heart rate was plotted against the air temperature,in the low temperature range of 10°C to 20°C, the slope parameters of the regression lines were -3.9 ± 0.8 (n=7) and -2.7 ± 0.3 (n=7) (beats/min.°C, M.± S.E.) in cold- and heat-acclimated rats, respectively. On the other hand, in high temperature range of 20°C to 30°C, the slope parameters of regression lines were -5.0 ± 1.0 (n=6) and -10.4 ± 1.1 (n=8) (beats/min.°C) in cold- and heat-acclimated animals, respectively. These responses vanished in rats anesthetized with sodium pentobarbital (50mg/kg i.p. and 13mg/H.,i.v.). These results suggest that these vasomotor responses to changes in environmental air temperature are affected in the process of thermal acclimation.

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AORTIC ELASTIC ELEMENTS IN PHYSICALLY TRAINED RATS. MASUMURA, S., HASHIMOTO, Y., HASHIMOTO, M. AND SATŌ, T. Dept of Physiol., Shimane Medical Univ., Izumo 693

It has recently been shown that collagen and/or elastin production in mammalian arteries is stimulated by epinephrine, hypoxia and hypertension. These findings suggest that sympathetic activation and augmented secretory activity of adrenal medulla observed during daily exercise have an effect on arterial metabolism of these elastic elements. We estimated collagen and elastin in rat aorta after forcing the animals to run on a treadmill. In the experiments, female Wistar rats 4 weeks old ran at 10-25 m/min with a slope of 10° for 1 hr/day, 6 days/weeks. After 4 weeks, the animals ran continuously at 30 m/min for 1 hr/day. The total duration of experiments was 12 weeks. The estimated values of collagen and elastin were expressed in terms of percentage of dry defatted weight of rat aorta. In the controls (sedentary rats), collagen content was 28.6 % in the thoracic aorta and 42.2 % in the abdominal aorta, while elastin content amounted to 54.9 % in the thoracic aorta and 38.1 % in the abdominal aorta. In the trained animals, however, the estimated values of elastin decreased by 4-8 % in the thoracic and abdominal aorta ($p < 0.05$). Only collagen content in the abdominal aorta reduced by 5.2 % in this case ($p < 0.05$), indicating that physical training may prevent distal area of the aorta from its stiffness.

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EFFECT OF CA-CHANNEL INHIBITORS ON LIPOLYSIS INDUCED BY VARIOUS AGENTS. KOSHIMIZU, E., IZAWA, T., TSUBOI, M* AND KOMABAYASHI, T.* Lab. of Physi. Educa., * Dept. of Physiol., Tokyo Coll. Pharma.

The present work was conducted to determine the role of calcium in the lipolytic process. The lipolysis was stimulated by epinephrine, norepinephrine, caffein, and ACTH. The lipolysis by these agents was not observed when the adipose tissue was prepared with 2mM EGTA for 60 min and then incubated in the Ca-free medium. The response of the Ca-deficient tissue appeared by the addition of 0.75 mM Ca. The addition of La significantly inhibited the lipolysis. Verapamil and diltiazeme, which are Ca-channel inhibitors, also inhibited these lipolysis. Chlorpromazine and trifluoperazine, which are calmodulin inhibitors, significantly inhibited the lipolysis.

These results suggest that the flux and redistribution of Ca have an important role in the lipolytic process.

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EFFECT OF SUBSTANCE P ON THE OXYGEN UPTAKE IN RAT SUBMANDIBULAR SLICES

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The aim of the present work was to study the mechanism of the stimulation of respiration caused by substance P in rat submandibular slices. Substance P significantly stimulated the oxygen uptake in submandibular slices. The salivary secretion remarkably increased at 5 min after the intraperitoneal injection. The substance P-stimulated oxygen uptake was not inhibited by phentolamine, propranolol or atropine, but was inhibited by baclofen, a substance P-antagonist, in the central nervous system. These results suggest that the increase of oxygen uptake caused by substance P is not attributable to α- and β-adrenergic receptors, or muscarinic receptors, but rather to the stimulation of peptide receptors in the acinar cells.

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EFFECT OF HISTAMINE ON THE OXYGEN UPTAKE IN DOG SUBMANDIBULAR SLICES.

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The oxygen uptake in dog submandibular slices was measured by utilizing histamine. The addition of 5.5×10^{-9} - 5.5×10^{-7} M of histamine significantly increased the oxygen uptake, and also stimulated the amylase output from submandibular slices. The stimulant effect of histamine to the oxygen uptake was inhibited by neither of prometadine and diphenhydramine, H_1-antagonists, but was significantly inhibited by cimetidine, a H_2-antagonist. In addition, the histamine-stimulated oxygen uptake was not inhibited by phentolamine, propranolol or atropine.

These results suggest that the increase of oxygen uptake by histamine is produced by its stimulation to H_2-receptors in the acinar cells.

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DETERMINATION OF THE TRACE ELEMENTS IN HAIR OF NEW BORN BABIES AND THEIR MOTHERS BY NEUTRON ACTIVATION ANALYSIS.

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We examined for two purposes, then one determinated the trace elements in hair of new born babies and another investigated the correlations of the trace elements in hair between the new born babies (called B) and their mothers (called M). The hair were activated in atomic reactor (at Rikkyo Univ.) under two kinds of schedules, i.e. 3min irradiation and 4x6 hrs irradiation, and used as induced radionuclides. The measurements of γ-ray spectrum used a Ge (Li) spectrometer. The standard reference material was bovin liver (NBS). Results obtained are as follows: 1) The concentration (ppm/dry weight) of the trace elements in hair of B were Zn 197.18±12.03, Cu 12.78± 1.95, Br 7.21±1.51, Hg 4.02±0.92, Se 0.89±0.13, Mn 0.58±0.51, Cr 0.54±0.01, I 0.41± 0.05, Co 0.05±0.02 and V 0.04±0.01. 2) The concentration (ppm/dry weight) of the trace elements in hair of M were Zn 174.23±7.74, Cu 10.58±2.14, Br 8.84±2.20, Hg 4.21±0.91, Se 0.66±0.13, Mn 0.48±0.11, Cr 0.54±0.02, I 0.41±0.26, Co 0.04±0.02 and V 0.02±0.01. 3) The correlation coefficients of trace elements in hairs of B and M showed Cu 0.965, I 0.956, Cr 0.955, Co 0.935, Br 0.935, Hg 0.921, Se 0.907, V 0.882, Mn 0.830 and Zn 0.580. The concentration of these elements without Zn correlated to M and B.

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CLOTHING AND THERMAL COMFORT

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Four subjects each of the young and middle aged, males and females were exposed to ET* 16,18,20, and 22°C on the separate days in winter. R.H. was kept at 50% and the wind velocity was less than 20cm/s. The subjects kept rest on chairs for 60min, and then performed a mental work(addition of random numbers by electric calculator) and a light muscular work(stepping on and down from a 15cm high platform slowly) for 45min each. Clothes used were prepared previously for each subjects and the subjects were ordered to choose and change the assembly of clothes according to their thermal sensation to produce the thermal comfort condition. There was a close relationship between room temperature exposed and clo-value of the clothes assembly chosen by the subjects but warm sensation still arose by the increase of the room temperature especially during the muscular work. This might come from the increased skin temperature. From the relationships among the room temperature, clothing condition, and thermal sensation, clothing assembly necessary to obtain comfort in ET* 18°C was 1.0 to 1.5clo at rest and was 0.9 to 1.3clo at work. In ET* 20°C, comfort assembly was 0.8 to 1.3clo at rest and 0.7 to 1.2clo at work.

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STUDY AND DEVELOPMENT OF PORTABLE DEVICE FOR MONITORING OXYGEN CONSUMPTION.

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A system for making prolonged measurements of oxygen consumption without the use of a mask or mouthpiece is described. A hood is placed on the subject's head. The ambient air and expired gas in the hood are exhausted with a large pump continuously. A small pump bypasses small amount of sample gas from the main stream. The sample gas is dried by passing through molecular sieve desiccant and is delivered to in-line oxygen analyzer. The flow rate of main stream is measured by pulsed heat-wire flowmeter. The flow rate is essentially constant during a run. Both signals of flow rate and oxygen concentration are converted to digital quantities and then stores in memory devices at every five minutes. All measuring devices are assembled to small package which the subject wears on his back. The memory chip is removed from the device after experiment. Oxygen consumption is estimated by connecting the memory chip to a microcomputer. This measurement compares favorably with measurements with Douglas bag collection and gas analysis.

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EFFECTS OF SOMATOTYPE, HEIGHT RATIO, AND LEAN BODY MASS ON THE BASAL METABOLIC RATE

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The BMR in college normal female students (n=187) aged 21 - 22 years was measured with a electrometabolor (type 600, FUKUDA). The subjects were grouped conveniently by somatotyping devised by Sheldon (1940), namely,endomorphy,mesomorphy, and exomorphy. The actual somatotype ratings were made usually by experimenters from careful inspection of the subjects. Higher values of correlation coefficient between the BMR and the metabolic body size (that is, height, weight, surface area, height ratio, LBM, etc.) for the endomorph and/or the mesomorph were seen. Only in the ectomorph the values of the correlation coefficient between the BMR and the metabolic body size were low. It was found that the BMR was closely related to height ratio which is defined as height in centimeters devided by body weight in kilograms ($r=0.75$, $S_{yx}=1.0$ kcal / kg / day, n=49). The results indicate that somatotype,similar as age and sex, is a determinant of the BMR.

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THRESHOLD DISSOCIATIONS OF THERMOREGULATORY RESPONSES DURING LPS FEVER OF THE RABBIT.

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Changes in the threshold core temperatures for initiating thermoregulatory cold defence reactions, i.e. cutaneous vasoconstriction and shivering, during LPS fever were investigated in a warm environment (32°C) on rabbits. Core temperature in the retroperitoneum and ear skin temperature were measured with thermocouples, metabolic cold defence (shivering) was determined by measuring oxygen uptake.

The threshold core temperatures were determined by internal whole body cooling before, 1.5 h after and 4-5 h after i.v. injection of LPS (4ug/kg). 1.5 h after LPS, the threshold for vasoconstriction was significantly increased, whereas that for shivering was decreased. 4-5 h after LPS, vasoconstriction threshold was still elevated and shivering threshold had increased above that before LPS.

It is concluded that LPS, administered at the doses and experimental conditions of this study, leads to a dissociation of vasomotor and metabolic cold defence thresholds during the course of fever.

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CUTANEOUS AND RENAL VASCULAR RESPONSES TO SPINAL CORD THERMAL STIMULATION IN PCPA-TREATED RABBITS.

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The effect of p-chlorophenylalanine(PCPA)-treatment on the cutaneous and renal vascular responses to spinal cord warming was investigated in rabbits anaesthetized with sodium pentobarbital. 150 mg/kg or 300 mg/kg PCPA was injected intraperitoneally 72 and 48 hr prior to the experiments. Rectal and ear skin temperature were measured with thermister probes. Renal blood flow(RBF) was measured with an electromagnetic flow meter, and renal vascular resistence(RVR) was calculated from renal blood flow and blood pressure.

In control animals, spinal cord warming caused ear skin vasodilation and simultaneously a decrease in RBF accompanied by an increase in RVR. Spinal cord warming was repeatedly performed at different levels of the resting ear skin temperature, and thus the maximal ear vasodilation was determined. In PCPA-treated animals, the maximal vasodilation of the ear skin to spinal cord warming was obtained at significant lower resting ear skin temperature than control animals. RBF decrease also started at higher RVR in PCPA-treated animals. Amplitude of both ear skin vasodilation and renal vasoconstriction was enhanced in PCPA-treated animals.

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FREQUENCY-PROPERTY OF THERMOREGULATORY SYSTEM IN YOUNG AND OLD RATS INDUCED BY PERIODIC CHANGE OF AMBIENT TEMPERATURE

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The experiments were performed to investigate the frequency-property of the thermoregulatory system and the thermal property of the body in rats. The temperatures of rectum Tr and tail skin Ts of the free moving rats, and ambient temperature Ta were continuously recorded using themistors. The periodic change of Tr and Ts to the periodic change of Ta with 5 - 60 min cycles were analysed. $\Delta Tr/\Delta Ta$ was defined as a noise gain (ΔTr: the amplitude of the changes of Tr; ΔTa: that of Ta, indicating the intensity of artificial thermal disturbance).

The noise gain under short Ta cycle was lower than that under long Ta cycle. The noise gain 60 min Ta cycle was significantly higer 1) in old rats than in young rats at mean Ta 30°C, and 2) at mean Ta 30°C and 25°C than that at mean Ta 20°C in young rats.

It was concluded from results that the frequency-property can denote the dynamic characteristics of the thermoregulatory system including the negative feedback mechanism.

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EFFECTS OF INTRAVENTRICULAR ADMINISTRATION OF ENDORPHIN AND TRH UPON THERMOREGULATION IN THE RAT. TSUZUKI, S. and KUBO, K. Dept. of Physiol., Inst. of Constitut. Med., Kumamoto Univ., Kumamoto 862.

The effects of injections of β-endorphin (EP), naloxone (NLX) and TRH into the third ventricle upon rectal temperature (Tre) and thermoregulatory behaviors were studied in the adult female rats under various thermal conditions. EP raised Tre in warm environment (23°C), but lowered Tre under acute cold exposure (1-5°C). NLX and TRH did not affect Tre under those conditions. Any of EP, NLX and TRH did not affect the rising Tre under the heat stress. Either skin-cooling behavior against heat stress or skin-warming behavior on cold exposure was not inhibited by EP, and appeared the same as control. The behaviors, however, were not effective enough to recover the EP-induced temperature changes. After NLX injection, thermoregulatory behaviors appeared as well as control, and Tre was kept constant. TRH tended to decrease the skin-cooling behavior and increase skin-warming behavior during early stages of either heat or cold exposure. Present results suggest that opioid peptide may depress the central thermosensitivity and influence an autonomic fine-grade thermoregulatory responses, but may not affect deleteriously the behavioral thermoregulation based on peripheral thermal input. TRH, on the contrary, seems to facilitate the central thermosensitivity and maintain normal thermoregulation.

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CHANGES IN THE ACTIVITY OF THE MEDULLARY TEMPERATURE-RESPONSIVE NEURON WITH IONTOPHORETICAL APPLICATION OF ENDOGENOUS PYROGEN. SAKATA,Y., WATANABE,T. AND MURAKAMI,N. Dept. of Physiol., Yamaguchi Univ. Sch. Med., Kogushi 1144, Ube, Yamaguchi 755

It has been reported by our laboratory that the medullary temperature-responsive neurons responded to endogenous pyrogen (E.P.) injected systemically and locally. However, a question as to whether E.P. acts directly on the neurons per se still ramained. We partially purified E.P. by using of ultrafiltration and verified that this partially purified E.P. is transported through a five-barreled microelectrode with a positive current. Then, in order to examine the direct action of the E.P., the partially purified E.P. was iontophoretically applied on the medullary temperature-responsive neurons in rabbits. As a result, 10 out of 16 temperature-responsive neurons responded to the E.P., while no changes in the neurons were shown with application of the E.P. heated to 90°C for 30 min. All temperature-insensitive neurons (8 units) did not show any changes. This suggests that the E.P. acts directly on the limitted temperature-responsive neurons without any mediator.

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EFFECTS OF PYROGENS ON THERMOSENSITIVE NEURONS IN HYPOTHALAMIC SLICES. Nakashima, T., Hori, T., Kiyohara, T., Shibata, M. and Osaka, T. Dept. Physiol., Saga Medical College, Nabeshima, Saga 840-01

Single-unit activities were recorded from in vitro of preoptic and anterior hypothalamic (PO/AH) slices in the rat. A total of 22 PO/AH units were tested for their responsiveness to lipopolysaccharide (LPS) during perfusion of LPS-containing Krebs-Ringer's solution. Twelve of fifteen warm-units decreased and one cold-unit increased their firing rates with latencies of 20-30 sec. All the six thermally insensitive units and the remaining three warm-units were not affected by LPS. Two warm-units exhibiting inhibitory responses to LPS did not lose their thermosensitivites in a Ca^{2+}-free medium and were taken as primary thermosensitive neurons. Local application of LPS (1-30 μg) by the use of micropippettes depressed 14 of 15 warm-units with latencies of 20-30 sec and the responses were graded depending on the dose of LPS. Ten of them were also inhibited by local application of leucocyte pyrogen (LP) with similar latencies. Inhibitory effects of both LPS and LP on warm-units were blocked or attenuated by local application of sodium salicylate. The results demonstrate that PO/AH thermosensitive neurons respond to locally applied LPS and the neural response to LPS, like the response to LP, is blocked by salicylate.

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LOCAL METABOLISM, BLOOD FLOW AND TEMPERATURE OF THE HYPOTHALAMUS IN HEAT- AND COLD-ACCLIMATED RABBITS. OHWATARI, N., KOSAKA, M., INOMOTO, T., SOMEOKA, S. AND FUJIMOTO, H. Dept. of Environment. Physiol. and Epidemiol., Inst. for Tropical Medicine, Nagasaki Univ., Sakamoto-machi, Nagasaki

35 rabbits were subjected to surgical procedures for measuring local metabolism, blood flow and temperature of the hypothalamus (PO/AH) and mid-brain reticular formation (RF). Local metabolism of the brain was measured by an electrical method. Cerebral blood flow was calculated from the initial slope of the hydrogen clearance curves. In intact rabbits, local metabolism of PO/AH and RF increased during external cooling as well as during LPS-pyrogen induced fever. Blood flow of PO/AH and RF increased by 25% during external heating (Ta=40°C) as compared with external cooling (Ta=15°C) of thermally non-acclimated control rabbits (n=5). During LPS-pyrogen induced fever, blood flow of PO/AH and RF increased by 21% and 19% as compared with before administration of LPS-pyrogen in control rabbits (n=6). However, the increased blood flow of PO/AH and RF observed in thermally non-acclimated rabbits (control animals : n=11) diminished or vanished in heat- and cold-acclimated rabbits (n=24) during both pyrogen induced fever and external cooling. This suggests that blood flow changes in central thermosensitive tissues may play an important role in both thermoregulatory mechanisms and in the process of thermal acclimation.

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ACTIVITIES OF HYPOTHALAMIC WARM-SENSITIVE NEURONS AND TAIL VASOMOTOR RESPONSE IN RATS DURING BRAIN AND SCROTAL THERMAL STIMULATION. ISHIKAWA, Y., NAKAYAMA, T., KANOSUE, K. Dept. of Physiol., Osaka Univ. Med. Sch., Kita-ku, Osaka, 530

The effects of local warming and cooling of the preoptic area(PO) on tail vasomotor response were observed in urethane anesthetized rats at different scrotal temperatures (T_{scr}). Tail skin temperature(T_t) was measured at room temperature of 25-27°C. The threshold preoptic temperature(T_{po}) for tail vasodilation was about 39.6°C at T_{scr} of 25-33°C. The effects of lidocaine injected into PO(10ug/0.5ul) on vasodilated tail skin were observed while T_{po} and T_{scr} were kept constant at about 40°C. Immediately after the injection, T_t began to fall, indicating vasoconstriction. Tail vasomotor response to PO warming and cooling in rats desensitized by repeated s.c. or intrahypothalamic injections of capsaicin was reduced in comparison with that in control rats.

According to our previous observation, 3/4 of hypothalamic warm-sensitive neurons were facilitated by scrotal warming and the operating ranges of most warm-sensitive neurons were in T_{scr} of above 36°C. Tail vasomotor response is influenced by afferents from scrotal warm receptors.

These findings indicate that the hypothalamic warm-sensitive neurons, but not cold-sensitive neurons are responsible for tail vasomotor response.

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INFLUENCES OF SCROTAL THERMAL STIMULATION ON THE LATERAL HYPOTHALAMIC NEURONS IN RATS. IMAI, K., NAKAYAMA, T. AND ISHIKAWA, Y. Dept. of Physiol., Osaka Univ. Med. Sch., Kita-ku Osaka, 530

The effects of scrotal thermal stimulation(SC stimulation) and preoptic thermal stimulation(PO stimulation) were observed on neurons of the lateral hypothalamic area (LH) in rats. Twenty neurons out of 21 inhibited by iontophoretically applied glucose responded to SC stimulation, 12 were facilitated by cooling. In comparison, 23 neurons out of 46 which did not respond to glucose were not influenced by SC stimulation, but 15 were facilitated by warming. Changes in firing rate of these neurons responded to SC stimulation occurred within the scrotal temperature range of 33-41°C, and was usually less than 4°C. The neurons facilitated by SC warming were facilitated by PO warming, the neurons facilitated by SC cooling were facilitated by PO cooling, and the irresponsive neurons to SC stimulation did not respond to PO stimulation.

These results indicate that SC stimulation as well as PO stimulation influences LH neurons, especially those inhibited by glucose. This suggests that the feeding mechanism in LH is influenced by peripheral thermal stimulation.

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AUTONOMIC AND BEHAVIORAL THERMOREGULATION DURING CORTICAL SPREADING DEPRESSION IN THE RAT. SHIBATA, M., HORI, T., NAGASAKA, T., KIYOHARA, T., NAKASHIMA, T. AND OSAKA, T. Dept. of Physiol., Saga Medical College, Saga 840-01 and Dept. of Physiol., Kanazawa Univ. Sch. of Medicine, Kanazawa 920+.

Cortical spreading depression (CSD) in the frontal cortex of the rats inhibits the skin-cooling operant behavior. This behavioral change was explicable by inhibited activity of warm-sensitive neurons and facilitated activity of cold-sensitive neurons in the preoptic and anterior hypothalamus (PO/AH) during a functional decortication of the frontal cortex by CSD. In the present study, effects of CSD on skin-heating operant behavior and autonomic thermoregulation were studied in rats with unilateral PO/AH lesions by eliciting CSD contra- and ipsilateral to the lesion. Skin-heating operant behavior was facilitated for 5 min after CSD (elicited initially in the occipital cortex) had reached the frontal cortex, while the facilitation was small or absent in trials with CSD ipsilateral to the lesion. In the second experiment, oxygen consumption was measured with an open-circuit method. Heat production (Watt/Kg) increased significantly for 8 min after CSD contralateral to the lesion had entered the frontal cortex. A rise in rectal temperature was often observed in both experiments; it varied from 0.2 to 0.8 ^{o}C. The present results, together with previous findings, may suggest that the frontal cortex is involved in the central control of thermoregulation.

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MIDBRAIN NEURONAL RESPONSES TO LOCAL AND HYPOTHALAMIC TEMPERATURES IN RATS. SATO, H. AND OHARA, K. Dept. Physiol., Sch. Med., Nagoya City Univ., Nagoya

In urethan-anaesthetized rats, single-unit activity was recorded in the localized area of the rostral midbrain where the so-called 'efferent shivering pathway' is considered to pass through. 170 units were examined for their response to change in hypothalamic temperature which was controlled by an implanted thermode. 58 of these units (34%) responded to change in hypothalamic temperature with a high correlation between temperature and firing rate, and were classified into warm type(24), cold type(33) and wc type(1) according to their thermoresponse characteristic. In the 32 hypothalamic temperature responsive midbrain units(16 warm and 16 cold type), the response was also tested for the change in local temperature which was controlled by another thermode implanted closely to the recording electrode, and the type of response was similarly determined(2 warm, 13 cold and 17 N type). It was concluded that the responses of these midbrain neurons referring to hypothalamic temperature could be attributted to temperature signals derived from the primary warm and cold sensors in PO/AH. Furthermore, it was suggested that the hypothalamic output signals via this area might be caudally mediated through the locally cold sensitive(cold type) or thermo-insensitive neurons(N type), and that these neuronal connections might operate in processing of the efferent information for cold shivering.

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CHANGES IN METABOLIC ACTIVITY OF THE RAT BRAIN STEM FOLLOWING COLD OR HEAT EXPOSURE. MORIMOTO,A., TAKASE,Y. AND MURAKAMI,N. Dept of Physiol., Yamaguchi Univ. School of Med., Kogushi, Ube

The central nervous structures involved in thermoregulatory responses to peripheral thermal stimulation were investigated in conscious and unrestrained rats by the method of [^{14}C]-deoxyglucose autoradiography. When rats were placed in cold (10°C) or hot (34°C) environment from 26°C environment, tail skin temperature fell to about 12°C in the cold and rose to about 38°C in the heat with small changes (within ± 0.7°C) in rectal temperature. Exposure to cold increased metabolic activity in the lateral preoptic area (LPO), medial forebrain bundle (MFB), anterior part of ventromedial hypothalamus, mediodorsal thalamus, ventral thalamus (VE), red nucleus (RN), and substantia nigra (SN), while exposure to heat increased activity in LPO, MFB, the anterior hypothalamus and the stratum pyramidale of hyppocampus and decreased in VE, RN and SN.

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THERMOSENSITIVE NEURONS IN THE BRAIN ARE 'THERMOREGULATORS', NOT 'THERMOSENSORS'

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Warm- and cold-sensitive neurons in the brain have been assumed so far to be 'thermosensors'. On this assumption, several hypotheses have tried to explain the homeothermy but failed. We attribute their failures to the misunderstanding of these neurons. The aim of this study is to propose a new interpretation of them and to explain the homeothermy, with the automatic control theory. Our observation shows that warm-sensitive neurons fire only above their threshold temperatures. By analogy with an artificial thermostat, we interpret them as 'thermoregulators' against heat. When their firing rates elicit heat-loss responses, multiple negative-feedback loops with their threshold temperatures as reference temperatures appear. Similarly, when cold-sensitive neurons elicit heat-production responses, multiple negative-feedback loops appear against cold. These two types of loops together will automatically control the core temperature against heat or cold.

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SULCAL PREFRONTAL CORTEX INFLUENCES THE ACTIVITY OF HYPOTHALAMIC THERMOSENSITIVE NEURON

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In previous studies, we have demonstrated that cortical spreading depression (CSD) in the frontal cortex influences the behavioral and metabolic thermoregulatory responses in the rat, and that frontal CSD induces the inhibition of warm-sensitive neurons and the facilitation of cold-sensitive neurons in the preoptic and anterior hypothalamus (PO/AH) of anesthetized rat. In the present work, by the use of a "double CSD" technique, we found that the dorsal bank of rhinal fissure (sulcal prefrontal cortex, S-PFC) affected the activity of PO/AH thermosensitive neurons during CSD. Subsequently, we studied the effect of electrical stimulation of S-PFC on the PO/AH neurons. Following single pulse stimulation of S-PFC, PO/AH warm-sensitive neurons were either facilitated (40 %) or unaffected (50 %) and the majority (81 %) of cold-sensitive neurons were inhibited. On the other hand, thermally insensitive neurons showed various types of responses. The results, together with the previous findings on changes in thermoregulatory responses and in activities of PO/AH thermosensitive neurons during frontal CSD, suggest that the S-PFC has a role in the central control of thermoregulation in the rat.

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THERMOREGULATORY RESPONSES ASSOCIATED WITH TREADMILL RUNNING IN THE JAPANESE QUAIL.

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Four adult male Japanese quails were trained to run on a treadmill for 10 min at various speeds of 0.15, 0.27 and 0.50 m/s under ambient temperatures (T_a) of 10, 20 and 30°C. Metabolic heat production (MHP), respiratory rate (RR), core (T_c) and skin (T_s) temperatures were continuously measured.

MHP during rest period before exercise were 16.3, 12.2 and 9.0 W/kg at 10, 20 and 30°C respectively. T_c during rest period at T_a 10°C was slightly higher than other T_a conditions. T_s at T_a 30°C was always higher than other T_a conditions. During exercise period, MHP at T_a 10°C was higher than those of other T_a conditions regardless of running speed in the first 2.5 min, but the differences of MHP could not be recognized in the last 5 min. Shivering during rest period at T_a 10°C was depressed in the midst of exercise rather than at the onset of exercise. RR during rest period was 31-34 /min at any T_a conditions. Panting was typically observed only at a speed of 0.50 m/s at T_a 30°C.

The results indicated that MHP, RR, T_c and T_s of Japanese quail were effected by running speed and T_a condition in course of exercise.

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THERMOREGULATORY FUNCTIONS IN HYPOTHALAMUS IMPAIRED RABBITS.
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In an environmental control chamber, 6 unanesthetized rabbits of which bilateral preoptic anterior hypothalamus (PO/AH) had been selectively impaired by X-ray irradiation of three field technique (3000R : total) were exposed to general heating (Ta=40°C) and cooling (Ta=15°C). Rectal, PO/AH, reticular formation (RF) and ear skin temperatures were continuously measured with thermocouples. Respiration frequency was derived from temperature changes of inspired and expired air. Local metabolism of PO/AH and RF was detected by an electrical method. Cerebral blood flow was calculated from the initial slope of the hydrogen clearance curves. Mean blood flow and local metabolism of intact PO/AH were 37-42ml/100g/min and 15-20mmHg (Po_2), respectively. Although these hypothalamic blood flow and metabolic activities observed in intact rabbits vanished in PO/AH impaired rabbits, the time courses of the changes in various autonomic thermoregulatory responses induced by general heating and cooling of the skin in these PO/AH impaired rabbits closely resembled those observed in PO/AH intact animals. This suggests that the temperature regulation system is composed of hierarchically organized control loops located in thermosensitive tissues of central nervous axis.

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EFFECT OF LOW PARTIAL PRESSURE OF OXYGEN AND BAROMETRIC PRESSURE ON THE THERMAL REGULATION OF MAN.
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The effect of low partial pressure of oxygen in inspired gas ($P_I O_2$) and barometric pressure (Pb) on the thermal regulation of man was examined in a simulated high altitude. Ten healthy male volunteers were subjected to measure the partitional calorimetry in an altitude chamber at ambient temperature of 30°C. Normal and half $P_I O_2$ were simulated in 1 and 1/2 atmospheric pressure. Resting metabolic rate and rectal, mean body and mean skin temperatures did not change at all conditions tested. Convective heat loss decreased by 30% at 1/2 ATA. Water loss from the lungs increased in low $P_I O_2$, and evaporative heat loss from skin increased significantly in the environment of low-$P_I O_2$ plus low-Pb. The coefficient of conductive heat transfer from the core to skin slightly increased by lowering $P_I O_2$ and it increased by 16.3% at the low-$P_I O_2$ plus low-Pb condition. Vasodilation prevents that cutaneous heat losses cool the skin to a degree that will prevent evaporation. The high water loss from the skin at high altitude is explained by the physical factor (increased diffusivity of vapor) and physiological factor (cutaneous vasodilation).

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EFFECTS OF HEAT STRESS ON HEMODYNAMIC FUNCTION AND TRANSVASCULAR FLUID SHIFT
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The effect of heat stress on hemodynamic functions and transvascular fluid shift was examined on 8 splenectomized dogs. Elevation of core body temperature by 2 C over 90 - 120 min caused declines in total peripheral resistance and urinary output and an increase in cardiac output, while circulating blood volume, plasma colloid osmotic pressure and intravascular protein mass remained constant. To assess the effects of heat stress on transcapillary fluid shift, Ringer's solution was infused at a rate of 1.07 ml/min-kg under normothermia and hyperthermia. Under hyperthermia, the retention ratio of infused volume within the intravascular space was higher and transvascular fluid shift and urinary output were decreased during and after the infusion. The result suggests the moderate hyperthermia exerts redistributions of blood volume and flow within the intravascular space by the dilation of peripheral vascular bed such as skin and muscle. And this dilation accounts for the retention of the infused fluid within intravascular space and the decrease in transvascular fluid shift.

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INFLUENCE OF BAROMETRIC PRESSURE ON TEMPERATURE REGULATION: STUDIES AT 4 ATA.

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To clarify the physiological responses of the body temperature to high ambient pressure, experiments were done on 8 healthy male volunteers in 4 atmosphere absolute (ATA) in air. The ambient temperature (Ta) and the relative humidity were contolled to 30 or 32°c, 60%, respectively. To avoid the effects of increased convective heat loss (*C*) at 4 ATA, the air velocity of the chamber was changed as the dry heat losses of 4 ATA to be almost identical to that at 1 ATA. Body temperature, body weight loss and dry heat losses were measured. Partitional calorimetry was performed during the experiment. *C* and radiant heat loss (*R*) measured at 4 ATA were smaller than those expected theoretically. At a given Ta, evaporative heat loss was also decreased at 4 ATA than that at 1 ATA. Mean skin temperature was significantly low at 4 ATA despite decreased heat losses. The coefficient of conductive heat transfer from the core to skin was reduced significantly at 4 ATA, indicating vasoconstriction. Above facts and the observation of A-V block on ECG of one subject at the pressure above 3 ATA may suggest that the change in neural control mechanisms of circulatory system is induced by ambient pressure (via partial pressure of oxygen, or others).

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REEVALUATION OF SEASONAL VARIATIONS IN CHARACTERISTICS OF THE FREQUENCY OF SWEAT EXPULSIONS.

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Relations of body temperature to the frequency of sweat expulsion (Fsw) were examined in summer and winter on 6 healthy, sedentary students born and raised in the Mainland of Japan. Sweat rate from a forearm area was recorded continuously by capacitance hygrometry; whole body sweat rate (Sw) was recorded continuously. Ta was changed stepwise by 2-3 °C for each 60 min or more, with rh of 40%. Sweat expulsions for 15 min of a thermally equibrated state were counted and Fsw was calculated for each Ta. Fsw was related linearly with $\bar{T}b$ (=0.8Tre+0.2$\bar{T}s$) as well as Ta, the correlation coefficient being higher in the former. The regression lines of Fsw on $\bar{T}b$ showed no significant seasonal variations, indicating that there were no differences in the central sudomotor activity between summer and winter. Neither did the regression lines of Sw on Fsw showed seasonal variations, indicating that the secretory capacity of sweat glands was not increased in summer. These results, which are discordant with those of the previous study, reveal that heat acclimatization or deacclimatization is not apparent in our subjects. The cause of the discrepancy is discussed.

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CORRELATION BETWEEN TEMPERATURE OF FINGERS AND COLD PAIN DURING EXPOSURE OF LEFT HAND TO COLD IN AIR.

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Digital vascular hunting reactions during cold exposure of left hand in air were observed. Experiments were carried out on 30 male university students in summer at around 3 p.m. in a room of 27 °C and 70 % R.H. The left hand was inserted palm downwards and finger extended slightly up to the wrist into the cold chamber of -10 °C and a wind velocity of 20 cm/sec. for 30 min. The skin temperatures of the back of the distal phalanx of middle finger were recorded continuously with copper and constantan thermo-couples during cold exposure. The mean skin temperature of sixteen subjects were higher than 20 °C (H group) and that of fourteen subjects were lower than 20 °C (L group). Nine subjects experienced cold pain during cold exposure and were included in L groups. The subjects who experienced cold pain tended to show lower skin temperature before cold exposure, lower temperature of first rise and longer time for first temperature rise.

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EFFECT OF PHYSICAL CHARACTERISTICS ON SEASONAL VARIATION IN SWEATING REACTION OF MEN.

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Observation of physiological responses during hot foot bath were made on 4 subject groups of male university students (nonathletes including obese subjects, long distance runners, throwers, judoists) in summer and winter. The subjects immersed both legs just up to knees into a water bath of 42°C for 60 min. after staying for 30 min. in a room of 30 °C and 70 % R.H. Sweat volume per body weight tended to decrease in inversely proportional to body weight. The obese subjects showed greater seasonal variation in sweat volume than the lean subjects. Nonathletes and judoists showed greater sweat volume in summer than in winter. Throwers showed no seasonal variation in sweat volume. The long distance runners showed great sweat volume per body weight than other groups in both seasons and greater sweat volume in winter than in summer due to heavy training in winter.

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THE EFFECT OF ANESTHETICS ON THE THERMAL SALIVATION IN RATS.

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Sixty male heat tolerant rats were anesthetized with 130mg/Kg ketamine hydrochloride (ketaral, Park Davis & Sankyo), 1.2g/Kg urethane, 180mg/Kg α-chloralose, 40mg/Kg pentobarbital, 70mg/Kg amobarbital, or 150mg/Kg phenobarbital. Each group was divided into two groups and exposed to 25°C or 40°C. Amount of dropped saliva in liquid paraffin containing beaker was weighed. Rats did not drop saliva at 25°C, but salivated at 40°C except for α-chloralose anesthetized rats. The ketaral anesthetized rats secreted markedly larger amount of saliva (1462mg/100g b.w.) than rats which were anesthetized with the other anesthetics. The saliva was colorless and transparent watery. At 25°C ketaral anesthetized rats did not show hypothermia unlike the rats with the other anesthetics. The thermal equilibrium level of colonic temperature was near 36°C, slightly below the level in conscious state. In ketaral anesthetized Donryu rats, which is heat nontolerant, a small amount of thermal salivation (66.8mg/100g b.w.) was observed. The strain difference in thermal salivation in anesthesia reflect the strain difference in that function in the conscious state. It was shown in the present study that ketaral (dissociative anesthetic) do not prevent markedly the thermal salivation as compared with urethane and barbiturates.

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MULTIVARIATE AND AUTO-REGRESSIVE ANALYSES OF CHRONIC LIVER DISEASES BASED ON TIME-SERIESED CLINICAL LABORATORY DATA

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To clarify the characteristics of clinical states of liver disease, the principal component analysis and the multi-dimensional Auto-Regression model were applied to time-seriesed clinical laboratory data of patients with liver disease. A time oriented data-base has been developed for a follow-up study. The pathological diagnosis through biopsy performed to all 108 patients enhances the reliability of the data. The results are summarized as follows. 1) Three components, a component of damage of liver parenchymal cells, that of damage in protein synthesis and that of damage of biliary cells, were extracted from the data of 88 patients. The discrimination of the liver disease such as acute hepatitis, chronic active or inactive hepatitis, fatty liver, and liver cirrhosis was fairly satisfactorily achieved. 2) The simulation of the time course of CHE(cholinesterase),ALP(alkaline phosphatase) and GPT(glutamic oxaloacetic transaminase) by the A-R model described well the actual time course of measurement, on the other hand, the prediction failed to follow the time course. 3) The values of cross-covariance between the specified laboratory tests characterized these diseases better. This means that characteristics in time course may represent the clinical state more accurately.

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SEX EDUCATION FOR YOUTH

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Adolescence is the period for youth fully maturing both in body and soul and it may be said the second birth in life. At this time the youth becoming conscious of his or her being a member of society and at the same time self-consciously finds the value of being as one in the vast nature and space. Based on such consciousness of universal youth, what form of reasoning would be proper for starting his or her sex education both from body and soul? On this there are a number of proposals having been already made.

I have thought of a tentative plan. For a couple of opposite sexes to mutually come to one in body and soul and to cooperate each other complementarily, it can not be achieved without the spirit of love and service. I firmly believe that it can be understood by searching after the "Spiritual Food" in love and the "Creator of All Things". There may be some criticisms in introducing religion into education, but I think that the education can not fulfill its objective without the teachings and guidance in the spiritual phase. As I believe that it cannot be achieved without love and service, I want to make this clear and recommend it. In that manner, hoping for the understanding and practice in love and service, I would like to makeanadvance to a new rebirth and resurrection of my spirit and heart.

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THE EFFECT OF ISOPROTERENOL ON CHOLESTEROL METABOLISM IN MICE.

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The present study was conducted to determine the effect of isoproterenol (IP) administration on cholesterol metabolism (level and biosynthesis) .Ip (0.3㎎/mouse) was administrated toICR-JCL male mice for 58 or 31doys. The animal were injected with acetate-1-^{14}C,one hour before killing.The results are as follows:Iptreatment resulted in decreased cholesterol levels in salivary glands,kidney,carcass,increased cholesterol biosynthesis in salivary gland,kidney and salivary gland enlargement.Liver cholesterol levels and biosynthesis were not affected. 21days subsequent to the withdrawal of Ip resulted in normal salivary gland weight, whereas abnormal cholesterol metabolism asthe results above were not yet returned to normal condition.

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THE LAW OF REMAINDER IN ANIMAL EVOLUTION (1)

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The sponge has skeleton containing Si and the coral's skeleton contains Si + Ca. Shells have an outer shell containing Ca and crustacea's outer shell contains Ca + chitin. Fish have a skin containing Fe. Mammals have hairs containing I. On the assumption that, in the genesis of the earth, elements were formed in order of increasing weight and that various compounds were formed in combinations that allowed repetition of lighter elements rather than new heavier elements, one can imagine that animals, facing new elements, excreted them into their surface because they could not utilize them inside their body. This may be likened to the law of remainder in mathematics: in this simile the animal is analogous to the dividend, the new elements the divisor , the soft tissue of the animal the quotient, and the hard imtegmentum the remainder.

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THE LAW OF REMAINDER IN THE EVOLUTION OF PROCARYOTES (2)

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Nitrogen fixing bacteria release NH_3 and oxygen consuming organisms release CO_2. Halobacteria require Na^+ but release surplus Na^+. Phosphor released from photobacteria emits light on oxidation. Sulfur bacteria save S particles in or out of the cell. Chloroflexus requires Cl^- but releases its surplus. Among the organisms that require an element, the lowest one is considered to be the first that adopted the element. Thus the transition of the remainder elements of organisms with time reflects the locus of evolution. In higher animals, however, symbiosis of microorganisms is suggested since the speed of evolution is too fast.

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NECESSARY AND SUFFICIENT CONDITION OF DIET, NOT BY CALORIE (1)

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Diet D in one single ingestion contained animal protein P, vegetable protein V (seed a, leaf b, root and stem c), and cereal L. Nutritional health could be maintained if the quantity of P was approximately equal to that of V within the digestible range, on the assumption that the concentrations of P and V were equal. The lack of P induced a sleepiness, that of b an oppressed feeling, that of c a fatigue, that of a a malaise, and that of L a chill. Therefore, D = P+a+b+c+L. The diet should not contain denatured substances. It should contain fresh fat but not oxidized fat. Fermented foodstuffs, containing viable bacteria, should be limited below D/2.

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INDISPENSABLE FOODSTUFFS CONSTITUTING THE MINIMUM STANDARD OF DIET (2)

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1D was necessary and sufficient in a combination containing only each one kind from P, a, b, c, and L. However, physical condition was disturbed by deficiency of only one element, if it lasted for 14 days. Therefore, necessary foodstuffs were determined by eating D' which did not contain one particular element but contained each one kind of all the other elements. The number of combination of Pabc, listed below, is 6^4. It is considered that P+a+b+c+L=D satisfies the necessary and sufficient condition as the minimum standard of diet. P) milk, egg, fish, chicken, meat, shellfish, a) cucumber, tomato, bean, plum, orange, mushroom, b) lettuce, mentha, cabbage, spinach, tea, seaweed, c) potato, carrot, radish, onion, taro, burdock, L) rice, wheat.

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ERYTHROCYTE DYNAMICS IN THE RESPIRATION-CIRCULATION-URINE FORMATION-SYSTEM AND ASSUMED SYMBIOTIC BACTERIA (3)

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On the assumption that higher animals were formed in the course of evolution by symbiosis with microorganisms and that their life is maintained by a symbiotic coordination, the following speculation is possible: Erythrocyte, by the work of 1) a bacterium named Rhodopseudomonas, which functions under a dark aerobic-light anerobic condition 2) passes CO_2 and receives O_2 in dark aerobic pulmonary alveoli, 3) increases hemoglobin in the light anerobic skin as a result of Hamburger phenomenon by Cl^- issued from Chloroflexus, 4) in the kidney has Nitrosomonas receive NH_3, Bacillus pasteurii urea, Pseudomonas acidovorans sugars and amino acids, Halobacterium Na^+ and Hydrogenomonas H_2O, and 5) circulates by the work of Ectothiorhodospira in the heart.

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URINE FORMATION OF THOSE DOGS GIVEN LARGE AMOUNTS OF UREA AND GLUCOSE.

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Since 1980, we attempted to have dogs loaded with large amounts of urea by intravenous administrations of 30% urea solution containing 20% glucose such as to prevent animals from hematuria. Using 6 dogs weighing 10 to 15 kg, 25% of those amounts of urea given upto 30 to 150 g at the rates of 20 to 60 mg/min/kg were excreted into the urine by the volume of 0.02 to 2 ml/min/kg and by the concentration of 1 to 10 g/dl at the rates of 5 to 20 mg/min/kg during the period of 6 to 16 hours while the animals survived the respiratory and cardiac activities. Another 5 dogs weighing 8 to 15 kg each had 60 to 90 g of glucose given by intravenous injections of 20% and 50% solutions and had those 30% excreted into the urine at the rates of 0.02 to 3 mg/min/kg by the volume of 0.02 to 0.6 ml/min/kg and by the concentration of 0.2 to 10 g/dl during the period of 4 to 20 hours. In excretion into the urine, urea competed with glucose each other but accompanied Na and K with a large amount of water, while glucose did concentrate itself with economy of Na, K and water, after all, both resulting in polyuria of the diluted urine and into dehydration.

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RENAL TISSUE OXYGEN TENSIONS OF THE DOG GIVEN THE LARGE AMOUNTS OF UREA AND GLUCOSE.

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In those experiments of 11 dogs given large amounts of urea and/or glucose (6/5 cases) as above-mentioned by Tanaka et al, each under the thiopental anesthesia had the left kidney prepared to observe the renal tissue pO_2 of 5 different parts (outer & inner cortex, outer & inner medulla and pelvis, respectively, 3 & 5, 10 & 15 and 35 mm deep from the capsule surface) with Yagi's electrodes. Those pO_2 which were integrated into the figures of mV-hours every second for the time interval of 30 minutes taken to collect the urine, were found in a tendency for all parts to rise so much about 40% as the excretion rate of urea increased 10 times, but not so much as the glucose excretion rate increased even hundreds times.

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〔会　報〕

日本生理学会昭和57年度第1回常任幹事会議事録

日　時：昭和57年3月29日(月)午後2時～5時30分
会　場：慶応義塾大学医学部　カンファレンスルーム
出席者：広重　力，加藤正道，田崎京二，鈴木泰三，本間三郎，高木貞敬，新島　旭，本郷利憲，星　　猛，島津　浩，酒井敏夫，真島英信，塚田裕三，古河太郎，入沢　宏，内薗耕二，御手洗玄洋，永坂鉄夫，岩間吉也，中馬一郎，藤本　守，久野　宗，中山　沃，後藤昌義，大村裕，額縁教三
村上元彦（当番幹事），菊地鎌二（教育委員長）
欠席者：伊藤正男，竹内　昭，及川俊彦
議　長：塚田裕三（当番幹事），村上元彦（当番幹事）

Ⅰ．報　告

1. 庶務報告（伊藤幹事海外出張のため，星幹事が報告）

◦会員について

昭和57年1月より3月の期間，入会80名，退会75名の移動があり，会員総数3,186名(一般会員2,914，評議員865，特別会員21，名誉会員1，準会員250)である．

◦弔事

木村栄一評議員が昭和57年2月11日逝去された．ストックホルムのツォテルマン教授が逝去され，日本生理学会より弔電を呈した．

◦各財団研究援助の採択について

1) 山田科学振興財団援助Aに阪大，基礎工学部，塚原仲晃教授が「学習のシナプス機構の研究」で1,000万円採択された．

2) 日産学術研究助成に，横浜市大，医学部，竹中敏夫教授が，「界面活性剤の生体膜への侵襲機構の解明とそれに対する防御法の開発」で1,300万円(昭和57年から昭和59年まで）が採択された．3月16日の日産科学振興財団の贈呈式に伊藤幹事が出席し，お礼を述べた．

2. 会計報告（星会計幹事）

日本生理学会昭和56年度決算（昭和56年1月1日から昭和56年12月31日まで）について，星幹事から説明を受け，了承された（別表，総会・評議員会議事要旨の項)．また，この会計決算については，昭和57年3月3日に，真島，島津両会計監事の監査を受け，承認をえている旨の報告があった．

3. 日本生理学雑誌の編集報告（酒井編集幹事）

昭和56年度，日生誌43巻は，1号～12号（8，9号は合併）で11回発行され，総頁数は754頁であった．

昭和57年度日生誌44巻は，4号が初校刷で，5号も，3月29日に編集を終了した．

生理学論文表題集は昭和57年度より，日生誌と切り離して，印刷され，各研究機関で別途購入してもらうことになった．形式は，日生誌と同じ大きさで，オフセット版で発行される．現在250単位のうち約180単位からの原稿を受け取っているが，4月に編集を終り8月に刊行発送する予定である．

4. J. J. P. 編集・会計報告（入沢委員長）

昭和56年度決算と，昭和57年度予算の説明があった．昭和57年度予算で，文部省助成は昨年と同額を考えているので，増加頁数分だけ増額される旨の説明があった．(別表，総会・評議員会議事要旨の項)

J. J. P. 編集の現状の説明があり，投稿数は増加の傾向で，すでに，1,000頁を越えたこと，発行部数は1,600部で，国外購入数が減少していること，国内で未購入の機関や新設の機関で購入されるよう依頼を出したこと，投稿頁数に制限がないので，国内研究者の利用をお願いしたいこと，が報告された．

5. 教育委員会報告（菊地委員長）

生理学実習書の改訂と，英文版の出版を，大村，酒井両委員を中心に進めている．

教育委員会活動の資金は，生理学実習書の印税収入に依っている．

第59回日本生理学会大会の解説講演の一部について，ビデオテープを製作し，60分テープを25,000円で発売したい．このビデオ撮りは，独立採算制として企画したい．

6. 会則委員会報告（島津委員長）

臨時会員制度の新設について委員会で討議した内容につき説明があり，昭和56年12月の常任幹事会の結論

(提案1)と異なった提案（提案2）になった経過が述べられた．委員会としては，この新しい提案(提案2)を常任幹事会にあらためて提出したい．

提案1：「臨時会員は臨時会費を納めたもので，当該年度のみ，本会の主催する大会および地方会に会員と連名で業績を発表することができる．臨時会費は会費年額の2分の1とする．」を会則第5条の末尾に入れる（昭和56年12月の常任幹事会に提案したもの）．

提案2：「臨時会費として3,000円を納めたものは，当該年度のみ，本会の主催する大会および地方会に会員と連名で業績を発表することができる」を内規の第8項として加える．

7. 研究費委員会報告（御手洗委員長）

文部省学術審議会の資料について意見を交換した．昭和57年度の科研費総額は380億円が見込まれていること，および審査方法も，先駆的，萌芽的研究に重点をおく方針がとられているなどの報告があった．科研費の試験研究と総合研究(A)についてかなり高額のものも認める方針が新しくとられている．特別推進研究は，昭和57年度より恒常的に設けられることになるが，この申請は従来通り推薦制による．

注意事項として，一般研究の継続を途中で中断し，新たな申請をすると非常に不利になることが報告された．この注意事項を日生誌に掲載し，会員に注意を促したいとの意見が出された．

日本生理学会会員や各大学の生理学教室に対して，研究費の充足程度についての調査アンケートを行う用意のあることが報告された．

8. 評議員選考委員会報告（高木委員長）

今年度新評議員候補として57名の推薦があり，全員適格と判定された．

9. 生理学用語委員会報告（酒井委員長）

疑義のある用語については委員長に提案して欲しい．境界領域の用語も採り入れる方向で検討しており，3月中に用語の見直しを終了する予定である．

10. 日本生理学教室史編集委員会報告（酒井委員長）

第60回日本生理学会大会までに，是非とも出版したく，昭和57年6月1日を原稿の最終締切りとしたので，協力されたいとの要請があり，出版価格はその時点で概算を出したい旨報告された．

11. 生理科学研究連絡委員会および国際生理科学連合報告（勝木委員長欠席のため，本間委員より報告）

• 生理科学研究連絡委員会より内分泌研連が独立することになったため，研連定数は一名減となる．生理科学研連の委員長が勝木保次氏から本間三郎氏に本年5月から交替になる予定である．

• 1981年11月28日大阪大学歯学部（世話人，河村洋二郎教授）で生理科学研連主催のシンポジウム「感覚の脳機構」が開かれ，盛会裡に終了した．

• 現 IUPS 副会長勝木保次氏の任期が1983年 Sydney Congress で終了するので，後任理事候補として，伊藤正男氏を推薦することが，生理科学研連で了承されたとの報告があり，常任幹事会でも，これを了承した．

• IUPS Congress を再び日本に招致する件につき，伊藤正男氏を組織委員長候補として，1989年（フィンランドが立候補している），これが無理なときには，1992年に東京で開きたい旨，1982年6月の IUPS 理事会に申し入れることが，生理科学研連で認められたとの報告があり，常任幹事会でもこれを了承し，総会に報告することとした．

• 1983年1月に西アフリカ Abidjan 大学で，Sickle Cell Anemia のセミナーが，IUPS の後援で開かれる．参加希望者は勝木副会長まで申し出られたい．

• IUPS 分担金が3,000ドルから5,000ドルに増額になった．この件につき星幹事より，塚田，本間両幹事のご尽力に対し，謝辞が述べられた．

12. 第60回日本生理学会大会（昭和58年）に関する報告（岩間幹事）

第21回日本医学会総会が，昭和58年4月8，9，10日に大阪で開催されるので，第60回大会（昭和58年）は大阪地区で行う．

期日：昭和58年4月5日(火)，6日(水)，7日(木)

場所：大阪大学教養学部校舎（大阪市中心より約1時間程度）

発表形式：口演のみとする．

発表申し込み期日：昭和57年11月30日まで．

応募演題：講座およびこれに準ずる研究単位から3演題までとする．

第60回大会の案内は，第1報として日生誌44巻4号に，第2報は日生誌44巻7号に掲載予定である．生理学会のシンポジウムについて，「生理学の未来」などを考えているが，日本医学会総会のテーマなどを考慮して，検討中である．

第21回日本医学会総会の準備状況について，中馬幹事から報告があり，司会者，シンポジストを調整中で

あり，参加者の登録は6月から開始される．なお，第59回日本生理学会大会第2日目，総会終了後に，第21回日本医学会総会の紹介映画が上映される．

13. 第59回日本生理学会大会準備状況（村上当番幹事）

明日より3日間，慶応義塾大学日吉校舎で開催される．今回は演題数を制限しないで募集し，現在まで，演題総数682題（ポスター48題，口演634題）が集まり，12会場で行う．参加登録者数は1,333名である．尚，大会第2日夕刻より，食堂ホールで懇親会が開かれる．

Ⅱ．議　題

1. 昭和56年度第2回常任幹事会議事録を承認した．

2. 昭和57年度予算案について

IUPS資料の配布および連絡費を国際交流基金積立より支出することを認め，昭和57年度予算案を了承した．

3. 評議員推薦について

57名の候補者全員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とした．（別表，総会・評議員会議事要旨の項）

4. 特別会員推薦について

岩間幹事より吉井直三郎氏の，田崎幹事より三田俊定氏の，酒井幹事より名取礼二氏の紹介と推薦説明があり，それぞれ特別会員候補として承認された．前回の昭和56年度第2回常任幹事会（昭和56年12月19日）で特別会員推薦が承認された問田直幹，藤森聞一両氏を加え，5名を昭和57年度総会に提出し，承認を求めることになった．

5. 日本医学会評議員および連絡委員の選出

日本医学会評議員の真島英信氏，および日本医学会連絡委員の酒井敏夫氏の任期満了に伴い，常任幹事会として，再度両氏を評議員，連絡委員候補者として総会・評議員会に推薦することとした．

6. 臨時会費制度について

会則委員会島津委員長より，提案2（会則委員会報告の項参照）が提出され，現行規定の内規第8項として加えることが了承された．さらに，抄録演題の発表者に※印を付けること，会費は会員の1/2で，会費値上げにつれてスライドすること，会則改正の日付を会則に記入することが確認された．

7. 教育委員会昭和57年度活動計画

1) ビデオテープ作製について

版権は日本生理学会，企画製作は産業医科大学視聴覚センターとし，販売価格は1時間テープ25,000円とする．第59回大会での解説講演で，冨田，伊藤，浅沼，ビショップ4氏の講演を撮映する．以上を承認した．

2) 教育に関するアンケートについて

20万円の予算を昭和57年度予備費より支出することを了承した．

8. 生理学教室史の出版費について

出版価格は原稿締切り（昭和57年6月1日）以後に予定価格として算出することが了承された．

9. 其の他

1) 信州大，宮川教授から，International Symposium on Rhythmic Fluctuation of Systemic Arterial Pressure の開催にあたって，日本生理学会の後援を求める依頼があり，これを了承した．

2) 第62回日本生理学大会（昭和60年）の開催地について，意見の交換がなされた．

3) 生理学研究所が，基礎生物学研究所と共催で，昭和57年12月2日に披露を行うので参加されるよう内薗所長より要望があった．

4) 日本学術会議の改革について

本間幹事より，改革問題について現状の報告があり，種々意見の交換があった．

第59回日本生理学会評議員会および総会議事要旨

日　時：昭和57年3月31日(水)午後4時10分～5時30分
会　場：慶大日吉校舎A会場
出席者：450名
議　長：塚田裕三，村上元彦（当番幹事）

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

1) 庶務報告（伊藤庶務幹事が海外出張のため星会計幹事より行なわれた）：現在の会員数，評議員数および特別会員について報告がなされた．（表1）

2) 昭和56年度決算報告(星会計幹事)：昭和56年度の決算報告（表2）がなされ，承認された．

3) 日本生理学雑誌編集報告(酒井編集幹事)：日本生理学雑誌43巻（昭和56年度）の編集内容は次のとおりである．43巻1～12号，発行回数11回（8，9号合併大会号)，総頁数754頁(表3)．次いで44巻の編集進行状況につき説明があり，4号がすでに初校刷で，5号も3月29日に編集が終了したとの報告がなされた．また生理学論文表題集は昭和57年度より日生誌と切り離して印刷され，オフセット版で発行されることになっており，各研究機関で別途に購入されるよう要請があった．

4) J. J. P. 編集会計報告（入沢委員長）：昭和56年度 J. J. P. 編集決算，昭和57年度予算案，刊行状況などの報告があった（表4，5，6)．

5) 教育委員会報告(菊地委員長)：第59回日本生理学会大会における解説講演の原稿を順次日生誌に掲載し，さらにそれらの一部をビデオテープに収録し，60分テープを25,000円で発売したい旨の報告があり，会員各位の協力が求められた．

6) 会則委員会報告(島津委員長)：臨時会費制度の新設について，その必要性の説明がなされ，下記の如き会則改正が提案された．「臨時会費として3,000円を納めたものは当該年度のみ本会の主催する大会および地方会に会員と連名で業績を発表することができる」を内規の8）項として加える．

7) 選挙管理委員会報告(島津委員長)：文部省科学研究費審査委員の予備選挙（9月18日)，本選挙（10月5日）を行い，第1段審査委員候補者として8氏を推薦した旨の報告があった．

8) 研究費委員会報告(御手洗委員長)：昭和57年度の科学研究費の総額は380億円が見込まれ，審査方法に先駆的，萌芽的研究を重視する方針がとられているとの報告があった．また科研費のうち，試験研究と総合研究(A)は可成り高額のものも認める方針が新しくとられていること，研究費の中に人件費も計上できるよう，現在関係方面で検討されていることなどが説明された．

また研究費委員会として日本生理学会会員および各研究機関に対し，研究費の充足程度について調査アンケートを行う用意のあることが報告され，会員各位の協力が要請された．

9) 評議員選考委員会報告(高木委員長)：今年度新評議員候補として57名の推薦があり，全員適格であると判定された旨の報告があった．

10) 生理学用語委員会報告(酒井委員長)：カードの見直しが終了し，昭和57年度中に出版できるとの報告があった．

11) 日本生理学教室史編集委員会報告（酒井委員長)：第60回日本生理学会大会までに出版したいことから，原稿の最終締切りを昭和57年6月1日とし，再度協力の要請があった．

12) 国際生理科学連合および生理科学研究連絡委員会報告(本間幹事)：(i) 生理科学連絡委員会より内分泌研連が独立することにより，生理研連の定数が1名減となり，委員長が勝木保次氏から本間三郎氏に本年5月より交替の予定である．(ii) 生理研連主催のシンポジウム「感覚の脳機構」が河村洋二郎教授を世話人として昭和56年11月28日大阪大学歯学部で開かれた．(iii) 勝木保次氏の IUPS 副会長の任期が1983年シドニー大会で終了することから，後任理事候補として伊藤正男氏を推薦することが生理研連で承認され，常任幹事会でも了承をえた．(iv) IUPS の日本での開催の件につき，伊藤正男氏を組織委員長候補として，できれば1989年（フィンランドも立候補している)，不可能であるならば1992年に東京で開きたい旨，1982年6月の IUPS 理事会に申し入れすることが生理研連で認められ，常任幹事会でも了承された．(v) IUPS 分担金が3,000ドルから5,000ドルに増額された．(vi) 1983年1月に西アフリカ Abidjau 大学で Sickle Cell Anemia

のセミナーがIUPSの後援で開かれる．参加希望者は勝木副会長まで申し出られたい．以上の6項目の報告がなされた．

13) 第59回（昭和57年）日本生理学会大会に関する報告(村上当番幹事)：今日現在出席者1,569名，演題数687題（口演634題，ポスターセッション53題）であることが報告された．

14) 第60回（昭和58年）日本生理学会大会に関する報告(中馬，中山，岩間，河村幹事)：第21回日本医学会総会が，昭和58年4月8，9，10日に大阪で開催されるので，第60回大会は大阪で開催される．

期日：昭和58年4月5日(火)，6日(水)，7日(木)

ところ：大阪大学教養学部校舎（豊中市）

発表形式：口演のみ

発表締切：昭和57年11月30日

応募演題数：講座およびこれに準ずる研究単位から3演題まで．

Ⅱ．議　事

1) 昭和57年度予算案：星会計幹事より説明があり，評議員会の承認を経て，総会に附議し承認された(表7)．

2) 評議員推薦について：評議員選考委員会が提案した新評議員候補者はすべて評議員会において承認された（表8)．

3) 特別会員推薦について：藤森聞一氏，間田直幹氏，吉井直三郎氏，三田俊定氏，名取礼二氏の特別会員推薦につき，推薦の辞が述べられた後，評議員会より推薦することが承認され，さらに総会で承認された．

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英信氏が推せんされ総会で承認された．また，連絡委員に酒井敏夫氏が推せんされ評議員会で承認された．

5) 会則改正の件(臨時会費制，その他)：さきに常任幹事会で承認された臨時会費に関する会則改正案が提案され評議員会および総会において承認された．また，事務所所在地の変更，地方部会を地方会に訂正する件についても異議なく承認された．

6) 第61回（昭和59年度）日本生理学会大会に関する件：第61回日本生理学会大会は群馬大学医学部，高木貞敬当番幹事のもとで開催されることが報告され，了承された．

総会，評議員会終了後，松本前当番幹事より今回の当番幹事塚田・村上両氏に謝辞が述べられた．それに続いて第21回日本医学会総会（於大阪）の予告映画が上映された．

なお，午後6時より日吉校舎食堂ホールにおいて懇親会が開かれた（出席者410名)．

表1．日本生理学会庶務報告

(昭和56年12月末現在)

会　員	
一般会員	2,916名
特別会員	21名
名誉会員	1名
準会員	
学校図書館	136部（128件）
研究所，書店等	121部（71件）
寄贈及び交換	
外　国	20部
国　内	14部
合　計	3,229

名誉会員（1名）　Sir J. C. Eccles

特別会員（21名）

東　龍太郎	井上　清恒	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	冨田　恒男
長嶋　長節	西丸　和義	福田　邦三
福原　武	松田幸次郎	簑島　高
森　信胤	吉村　寿人	若林　勲

表2．日本生理学会昭和56年度決算報告

（自　昭和56年1月1日　至　昭和56年12月31日）

収　　入

	56年度予算	56年度決算	増減	備考
① 前年度繰越金	4,946,352円	4,946,352円		
② 昭和56年度収入	21,980,000	24,041,304		
(内訳)				
会費	16,550,000	17,179,000	＋	

購読料	1,840,000	1,763,600	−	
論文掲載料	1,500,000	1,657,426	+	
広告料	1,200,000	1,077,600	−	11件
会誌分冊売	20,000	42,150	+	15件
預金利子	120,000	149,748	+	第一勧業銀行
日本医学会奨励金	100,000	100,000		昭和55年度
印税	300,000	351,582	+	生理学実習書及び用語集
大会費立替返却	300,000	321,166	+	
雑収入	50,000	428,032	+	東洋文庫敷金及び徳島大会より入金
生理科学連合募金	0	971,000		
①+② 合計	**26,926,352**	**28,987,656**		

支出

(内訳)	56年度予算	56年度決算	増減	備考
会誌印刷代	9,914,000円	10,704,245円	+	42巻12号～43巻11号
会誌発送代	2,720,000	2,155,799	−	同上
編集会議費	80,000	81,480	+	日本生理学雑誌
校正料	250,000	246,000	−	同上
原稿査読料	30,000	28,500	−	同上
事務室使用料	732,000	1,945,387	+	事務室光熱費含む
通信費	800,000	821,270	+	
振替手数料	140,000	171,435	+	
事務費	850,000	930,955	+	
備品	80,000	91,300	+	書庫
会合費	300,000	307,711	+	常任幹事会，委員会
旅費	1,200,000	1,095,160	−	同上
人件費	2,500,000	2,491,780	−	アルバイト含む
職員社会保険	95,000	55,188	−	
職員退職金積立	100,000	100,000		
国際交流基金積立	250,000	1,221,000	+	生理科学連合募金含む
雑費	100,000	78,150	−	供花他
事務所移転敷金他	0	2,211,463	+	
予備費	6,785,352	162,239	−	
合計	**26,926,352**	**24,899,062**		
昭和57年度繰越金		4,088,594		
合計		**28,987,656**		

表3．日本生理学雑誌43巻（56年度）編集報告

43巻1～12号（8，9号合併）発行11回

43巻総頁数 754頁

(内訳)	編	頁	42巻総頁数
原著	8	94	676頁
短報	5	16	
業績表題		137	

口演演題	185
抄録	82
会報，その他（総目次，索引含）	166
広告	74

表4．JJP 昭和56年度決算および57年度予算　　(昭和57年3月4日)

	56年度 予算額	56年度 決算額(見込)	56年度 摘要	57年度 予算額	57年度 摘要
製作費	11,246,000円	11,662,000円	1,016頁	12,397,000円	1,080頁
審査・英文校閲料	1,630,000	1,478,225		1,628,000	
旅費・会議費	810,000	407,000		810,000	
通信・消耗品費	1,323,000	1,323,000		1,455,000	10%up
人件費	1,925,000	1,925,000		2,060,000	7%up
雑事務費	770,000	770,000		809,000	5%up
送料(外国)	3,600,000	3,348,000		3,600,000	
送料(国内)	1,968,000	2,428,820		2,820,000	
支出合計	**23,272,000**	**23,334,045**		**25,579,000**	
国内会員	5,320,000	5,399,800	812名 @7,000 5%未収	6,230,000	890名 @7,000
国内機関	1,800,000	1,800,000	120名 @15,000	2,250,000	150名 @15,000
外国購読	8,000,000	7,280,000	465名 80$ 40$ @200	8,448,000	480名 80$ @220
別刷バックナンバー	2,565,000	2,994,200		3,414,000	
文部省助成	5,360,000	5,400,000		5,400,000	
繰越金	475,400	475,400		15,355	
収入合計	**23,520,400**	**23,349,400**		**25,757,355**	
差引繰越		15,355		178,355	

表5．JJP 刊行状況 (Vol. 31, No. 6) 刊行部数 1,600部

個人購読	812	942	外国購読	452	465
団体購読	120		外国会員その他	13	
国内寄贈	10				

表6．JJP 論文投稿状況 (昭和57年3月8日現在)　　( ) 内はそのうちの S. C. 数

昭和56年3月末日論文手持ち数：80 …… a)

	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Feb.	Mar.		
受理数	10 (4)	5 (1)	6 (2)	13 (2)	12 (2)	9 (3)	9 (2)	16 (4)	5 (1)	4 (2)	9 (2)	2 (0)	100 (25)	b)
掲載数	15 (3)		15 (5)		15 (3)		15 (5)		13 (2)		15 (4)		88 (22)	c)
返却数		2 (1)		2 (0)	1 (0)	1 (1)	1 (1)	5 (1)	7 (0)	3 (0)	2 (0)	2 (0)	26 (4)	d)

昭和57年3月8日現在手持ち数：(a+b)−(c+d)=(80+100)−(88+26)=66

{ 審査・訂正中：33
Vol. 32, No. 2：16, No. 3：17

表7．日本生理学会昭和57年度予算

（自　昭和57年1月1日
　至　昭和57年12月31日）

収　　入

①	前年度繰越金	4,088,594円	
②	昭和57年度収入	21,985,000	
	(内　訳)		備　　考
	会費	16,675,000	(会費6,000円×3,088×0.9)
	購読料	1,800,000	(準会費8,000円×250×0.9)
	論文掲載料	1,500,000	
	広告料	1,100,000	
	会誌分冊売	30,000	
	預金利子	130,000	(第一勧業銀行)
	日本医学会奨励金	100,000	
	印税	300,000	(生理学実習書，用語集)
	大会費立替返却	300,000	
	雑収入	50,000	
①+②	合計	**26,073,594**	

支　　出

	(内　訳)		備　　考
	会誌印刷代	11,340,000円	(43巻12号～44巻11号)
	会誌発送代	2,500,000	(同上)
	編集会議費	100,000	(日本生理学雑誌)
	校正料	250,000	(同上)
	原稿査読料	30,000	(同上)
	事務室使用料	2,099,040	(12ヶ月)
	事務室光熱費	270,000	(同上)
	通信費	850,000	(会誌外通信)
	事務費	850,000	(印刷物，封筒，事務雑費)
	備品	80,000	
	会合費	300,000	(常任幹事会，委員会)
	旅費	1,200,000	(同上)
	人件費	2,600,000	(アルバイト含む)
	職員健康保険	70,000	
	職員退職金積立	100,000	
	国際交流基金積立	250,000	
	雑費	100,000	
	予備費	3,084,554	
	合計	**26,073,594**	

表8．日本生理学会新評議員（昭和57年度）

計57名（五十音順）

氏　名	所　属，現　職
赤川　公朗	慶大，医，助手
今泉　和彦	愛媛大，医，助手
今川美登里	東京医大，助手
臼井　支朗	豊橋技術科学大，助教授
遠藤　克昭	京大，医，助手
大塚　邦明	高知医大，助手
大羽　利治	名古屋市立大，医，講師
岡田　隆夫	順天堂大，医，助手
小笠原　卓	東北大，医，助手
奥山　博司	順天堂大，医，助手
金松　知幸	東邦大，医，助手
北村　邦男	埼玉医大，助手
昆　和典	愛媛大，医，助手
酒井　秋男	信州大，医，助手
榊原　吉一	千葉大，医，助手
佐々木和男	富山医薬大，医，助手
佐藤　義昭	防衛医大，講師
菅屋　潤壹	愛知医大，講師
鈴木　裕一	山形大，医，助教授
高木　芳夫	東邦大，医，助手
只木　英子	金城学院大，教授
田村　好弘	名大，環医研，助手
豊沢敬一郎	鳥取大，農，助教授
永井　正則	山梨医大，助手
長野みさ子	東医歯大，医，助手
中村　彰治	阪大，医，助手
永松　陽子	神戸学院大，栄養学部，助手
成沢　三雄	東海大，医，助手
二科　安三	生理研，助手
西野　卓	千葉大，医，講師
野崎　修一	東医歯大，歯，助手
波多江純真	福岡大，医，助手
浜田　生馬	都神経科学総研，研究員
林　良一	岐阜大，医，助手
日向　正義	東大，医，助手
平田　耕造	金沢大，医，講師
平野　鉄雄	新潟大，医，助手
福島真知世	日本医大，助手
藤本　正昭	聖マリアンナ医大，講師
藤墳　規明	名古屋工大，工学部，助教授
細井　和雄	城西歯大，助教授
堀　真一郎	都神経科学総研，研究員
	杏林大，医，講師
堀江　秀典	横浜市大，医，助手
本間　研一	北大，医，講師
松井　信夫	名大，環医研，教授
松村　道一	京大，霊長類研，助手
松本　逸郎	長崎大，医，助手
三上　章允	京大，霊長類研，助手
緑川　知子	光華女子短大，講師
森谷　恵	札幌医大，助手
柳平　坦徳	信州大，順応研，助手
山田　好秋	長崎大，歯，助教授
山本　健一	都神経科学総研，副参事研究員
山本　高司	中京大，体育学部，助教授
山本　喜通	名古屋市大，医，助手
吉崎　克明	秋田大，医，助手
吉田　繁	長崎大，医，講師

昭和57年度第一回日本生理学会教育委員会議事録

日　時：昭和57年7月17日（土）　午後1時
場　所：東京都文京区本郷5-24-12　百万石
出席者：中馬一郎（大阪大），塙　功(神戸大)，林　秀生(埼玉医大)，石河延貞(宮崎医大)，菊地鎌二(東京女子医大，委員長)，熊田　衛（筑波大），前川杏二（自治医大），村上　憲（山口大），中野昭一（東海大），小川哲朗（秋田大），山下　博（産業医大），
欠席者：本間三郎（千葉大），森　茂美(旭川医大)，富田忠雄(名古屋大)，鳥居鎮夫(東邦大)，（ＡＢＣ順）.

Ⅰ．昭和56年度第三回日本生理学会教育委員会議事録の確認

日本生理学雑誌：44(6)，236～237，1982のとおりに承認された.

Ⅱ．報告事項

1. 第59回生理学会大会の解説講演ビデオテープ作製状況と販売予定について

a．山下委員より「4つの解説講演のビデオテープは既に編集も終り，販売出来る状況になっている．コピーに時間を要するので，一部製作を開始している．要した費用は産業医大視聴覚センター技師2名の旅費116,000円，器材運搬用フェリー代とガソリン代83,000円，計199,000円であった」との報告があった.

また，産業医大視聴覚センターで各20本までは原価でコピーし，20本以上の分に関しては幾分の手数料を支払う必要があるむねの説明があった.

b．ビデオテープ有料配布に関して以下の3つの問題に関して菊地委員長より報告があった.

i）税金について――国税庁審理課の意見としては，3ヶ月間に限って配布するという条件ならば利潤に対して税金を支払う必要はないということであった.

ii）著作権について――講義に使用するスライド等に準じて教材として使用する，という条件で菊地委員長が文化庁著作権課に問合せることになった.

iii）ビデオテープ代の会計について――日本生理学会教育委員会代表者，星　猛，名義（第一勧業銀行本郷支店，075-1408-501）の新口座を設けて別会計とすることになった.

c．ビデオテープの申込書，注文書（案）につき検討した.

文化庁著作権課の了解が得られれば，申込書，注文書を印刷し，日本生理学会事務局に依頼して，注文をとり始めることを承認した．菊地委員長より臨床医学等の関連教室に広く紹介してほしいとの要望があった.

d．四人の解説講演者に対しては謝礼として各演者のビデオテープ1巻を贈呈することになった．また有料配布によりもし利潤が得られれば，解説講演者の原稿（下記）に対して謝礼を考慮することが討議された.

2. 解説講演の原稿について

菊地委員長より，解説講演の原稿は集まりつつあるので，日生誌7月号に2編を掲載し，8・9月号は生理学会大会号となるので，10月号より各2編づつ掲載の予定である．原稿未着の演者には8月末の期限で原稿依頼の願い状を発送するとの報告があった.

3. 日本生理学会編学生実習書の改訂について

菊地委員長より(鳥居委員欠席のため)，改訂は順調に進んでおり，今年秋に改訂版出版の予定，との報告があった.

4. 会計報告

中野委員より会計報告がなされた.

5. 従来の教育用ビデオテープに関して

ボブビジョン社から販売した教育用ビデオテープの著作者に対する支払いがおそいことが会員より指摘され，山下委員が調査したところ，ボブビジョン社としては支払いは年度末に一括して年1回にしてほしいとの要望があったむね報告了承された．この点および今後の取扱いに関して山下委員がさらにボブビジョン社と交渉して明確にすることになった.

Ⅲ　議　事

1) 生理学教育に関するアンケートについて

a．菊地委員長より，アンケート調査に関する作業グループ（菊地委員長，鳥居，冨田，林，熊田委員）の6月26日（土）の会合において，全委員から募集した調査項目の要望にもとづき，アンケート調査用紙

(案)が作製されたとの経過報告があった.

b．熊田委員よりアンケート調査（案）の説明があり，各項目を詳細に検討し，修正意見をいれて改訂した.

c．前回の教育委員会で提案された「ビデオ撮影設備の有無，製作意図の有無」等に関するアンケート調査（担当，山下，熊田委員）も，生理学教育に関するアンケート内にいれて同時に調査することにし，そのための調査項目を追加した.

d．今後の作業日程

本教育委員会で作製したアンケート調査（案）を電算器処理担当者の意見をいれて調整し，調査用紙原案をつくり9月に各委員に郵送する．承認されれば印刷して，全国諸大学生理学教室，付置研究所の関連部門および国立生理研に発送することになった.

2) 第60回日本生理学会大会について

当番幹事である中馬委員より，次回大会において教育委員会として特別の企画の有無につき質問があり，教育委員会としては，上記アンケート調査結果の集計，発表等の業務があるので，今回は特別の行事は開催しないことになった．この点に関し，中馬当番幹事より生理学教育関係として，「臨床医学との交流シンポジウム」を午前，午後の2回，演者10名程度で企画中であり，大会第3報に詳細は発表する予定である，との報告があった.

追記，前回議事録の訂正，日本生理学雑誌44(6)237頁右段，第5行および第17行，「第2回委員会…」を「第1回委員会…」と訂正する.

文化庁著作権に問合せたところ，講演者自身の図は殆んど問題ないが，完全に権利が移動してしまったものについては改めて了解をとる必要があるとの意見であった.　（菊地追記）

〔お知らせ〕

東京都神経科学総合研究所10周年記念講演会

――神経科学の今日的問題――

日　時　昭和57年11月5日(金)
午後　1時30分～5時00分

会　場　経団連ホール
(東京都千代田区大手町，経団連会館内)

挨　拶　研究所長　佐藤昌康

変貌する神経疾患　――将来への対策――
疫学研究室　近藤喜代太郎

ニューロピルの病理学
――神経病理学の新しい方向――
神経病理学研究室　藤沢浩四郎

神経に働く新しい生物毒
――生理・生化学的アプローチ――
病態神経生理学研究室　川合述史

脳と筋肉　――無機イオンを回って――
東京大学教授・日本学士院会員　江橋節郎

問い合わせ先：
東京都神経科学総合研究所　管理部調査課
〒183 東京都府中市武蔵台2－6
(電話　0423(25)3881，内線4104)

第21回日本医学会総会個人登録申込のご案内

会　期　学術講演：昭和58年4月8日(金)・9日(土)・10日(日)
総合医学展示：昭和58年4月3日(日)～11日(月)

会　場　学術講演：大阪市内　24会場
総合医学展示：大阪国際見本市港会場

登録費及び会誌費

登録費　昭和58年1月31日までに払込の場合……25,000円
昭和58年2月1日以降に払込の場合……30,000円
(昭和58年3月10日以降は総会当日の個人

受付のみとなります)

会誌費…… 10,000円

登録申込方法

・個人申込と一括申込の方法があります．いずれか一方でお申込み下さい．

登録に関するお問い合せ

第21回日本医学会総会事務局
登録係専用電話 06(453)3635
〒553 大阪市福島区福島 1－1－50
大阪大学医学部附属病院内

第14回(昭和57年度)内藤記念科学振興賞受賞候補者の推薦要領

1. テーマおよび候補者

(1) 人類の健康に関する自然科学の基礎的研究，なかんずく，健康福祉の増進，疾病の治療と予防に寄与する独創テーマに取り組み，自然科学の進歩発展に顕著な功績を挙げた研究者．

(2) 主たる研究者は原則として単独とするが，異なる研究グループによる協同研究の場合には，連名であってもよい．

2. 推薦依頼先

昭和57年度は，

(1)

高分子学会	日本生物物理学会
日本遺伝学会	日本生理学会
日本ウイルス学会	日本動物学会
日本栄養・食糧学会	日本農芸化学会
日本解剖学会	日本醗酵工学会
日本化学会	日本ビタミン学会
日本がん学会	日本病理学会
日本細菌学会	日本物理学会
日本獣医学会	日本免疫学会
日本植物生理学会	日本薬学会
日本生化学会	日本薬理学会

以上の22学会（50音順）の代表者に受賞候補の推薦を依頼する．

(2) 当財団の理事および評議員に，受賞候補の推薦を依頼する．

3. 候補推薦件数

1推薦者から1件に限る．

4. ほう賞の金額

昭和57年度の内藤記念科学振興賞（ほう賞）は1件とし，正賞（賞品）ならびに副賞(200万円)を贈呈する．

5. 推薦方法

所定の用紙に必要事項を記入し，当財団あて送付する．

6. 推薦書の締切日

昭和57年11月20日とする．

日本生理学会〆切　昭和57年11月6日

推薦書提出先および連絡先

財団法人　内藤記念科学振興財団
〒112 東京都文京区小石川4丁目6番10号
電話　(03)813-3005（直通）
(03)813-1151（内線511）

第9回(昭和57年度)日産学術研究助成候補推薦要領

1. 助成の趣旨

自然科学を主とする学術の有意義な研究であって，国の助成となるべく重複しないもの（その期間が長期に亘る研究，学際的なグループによって行なわれる研究等）に対し助成を行ない，わが国の基礎学術の向上，進展に寄与しようとするものであります．

2. 助成対象研究分野

助成対象を**資源・エネルギー，環境**の分野の基礎研究，および応用研究とし，本年度は当該分野のうち次のような研究を期待します．

必ずしも実験を伴う研究のみでなく所謂ソフトの研究も含みます．ただしその場合は調査研究助成として扱います．

① 開発に伴う環境の改変が生態系におよぼす影響に関する研究

② 生物指標による環境変化のモニタリングに関する研究

③ 新しい原理に基づく環境の分析方法と制御の方法に関する研究

④ 環境汚染物質の生体に対する作用機構に関する研究

⑤ 病害動物に対する無公害駆除剤の開発，並びに生物学的駆除法の研究

⑥ 人畜疾病の化学療法剤のスクリーニングに関

する方法論的研究

⑦ エネルギーの貯蔵に関する研究

⑧ 新しい機能材料に関する研究

⑨ 人間—機械系の解析による安全性向上の研究

⑩ 資源・エネルギー，環境の立場から見た

(イ) 海洋の利用，(ロ) 林産資源の利用，(ハ) 輸送システム，など

その他，資源・エネルギー，環境の分野における上記に類する研究

3. 助成対象の研究者

貴学(協)会に関する自然科学分野の研究に従事しており，2～4年の期間を要する上記の研究を行なおうとする研究者および研究グループで，その研究成果が学術の進歩，発展に貢献するところが大きいと思われるもの.

4. 研究助成金額と助成件数

(1) 研究助成金額

○総　額（研究全年度）約 200,000千円

○1件当り助成金額の上限

一般研究…… 30,000千円(研究期間2～4年)

調査研究…… 5,000千円(研究期間　2年)

○助成金の支払い

昭和58年度 (58/4～59/3) を研究第1年度とし，研究計画に従い年度毎にその所要額を支払います.

ただし，第1年度の交付額は原則として助成額の1/2以下とします.

(2) 助成件数

調査研究を含めて10件前後を予定しています.

5. 推薦件数

1学(協)会より1～2件とします.

6. 推　薦　者

学(協)会の代表者とします.

7. 推薦手続

所定の推薦用紙に必要事項を記入し，当財団宛に1部をご送付願います.

8. 推薦締切日

昭和57年11月30日（火）

日本生理学会〆切　昭和57年11月16日（火）

9. 選考方法

下記委員からなる選考委員会において選考の上，当財団理事会で決定します.

伏見康治(委員長)，向坊　隆，佐々　学，
田島弥太郎，沼田　真，藤永太一郎

(推薦書提出先・連絡先)

財団法人　日産科学振興財団

〒104 東京都中央区銀座6丁目17番2号

電話　東京 (03)543-5597

日本学術会議改革試案（ダイジェスト版）

1982年6月　　日本学術会議改革委員会編

本ダイジェスト版は，第85回総会で承認された「日本学術会議改革試案」を外部に提示して意見を求めるに際して，読者の便宜を図るため，改革委員会の責任で作成したものである.

I　改革を必要とする問題状況

日本学術会議は「日本の科学者の内外に対する代表機関として」，「科学の向上発達を図り，行政，産業及び国民生活に科学を反映浸透させる」（日本学術会議法第2条）という目的のために「(1)科学に関する重要事項を審議し，その実現を図ること，(2)科学に関する研究の連絡を図り，その能率を向上させること」（同，第3条）という職務を政府から独立して行うこととされている.

創設以来33年間，歴代会員はこの目的，職務の遂行に努力してきたが，この間における諸科学のめざましい発展，科学者数の激増とその分布状況の変化，科学・技術の社会的役割や影響力の増大，本会議以外の科学・技術関係審議・実施機関の設置とその機能の増幅，政府との信頼関係の低下など本会議をめぐるいちじるしい状況の変化が生じた．にもかかわらず，本会議はこれらの変化に十分対応することができず，そのため科学者の遊離，無関心をまねくに至った．とくに本会議の組織・運営面における改革，改善を十分断行し得なかったことは，本会議の活動，地位，役割等を

制約する主要な要因であった.

そこで，これらの制約要因を除去，縮減するための組織・運営の改革，改善をはかることは，本会議本来の目的，職務の充実強化のうえから緊要な課題となっている.(「試案」P1～P4)

Ⅱ 改革の基本的前提
—本会議の基本的性格，特色及び存在理由—

しかしながら，本会議の改革を図るに当たっては，本会議の存在理由，特色等を改めて確認し，それらに基づく基本的な任務の遂行上，いかなる組織，運営上の欠陥があるかを究明し，是正の方向を打ち出さなければならない．これは，改革の基本的前提である.

さて，本会議の基本的性格，特色，存在理由は以下の6点に要約することができる.

(1) 独特な国の機関であること.
(2) 科学者の自主的組織として機能していること.
(3) 政府から独立して職務を行う機関であること.
(4) 科学者の内外に対する代表機関であること.
(5) 公選制と重層構造性を特色とすること.
(6) 科学の全分野にまたがる総合的構成をもっていること.(「試案」P5～P12)

Ⅲ 改革の重点と個別的改革案

前述のⅠの問題状況に照らしてみると，Ⅱの存在理由等が十分発揮され，貫徹されていないことは明白である．そこで，以下，改革の重点及びそれに対応する改革案をかかげる.

ただし，個別的改革案は従来一応検討を行ったものだけをかかげてある．また，「改革試案」では，個別的提案は第2部に一括して記されているが，読者の便宜上，この要約では，各重点項目の箇所にまとめて記すことにした．なお，文中「提案○—○」の番号は，「試案」第2部所収の提案番号である.

1. 職務の明確化

本会議の目的，職務（法第2条，第3条）は基本的に堅持すべきである．しかしながら，審議，研究連絡の両職務をあらためて明確化する必要がある.

提案Ⅰ—1 審議活動

審議活動をあらためて以下のように明確化する.

(1) 科学の発達に資する本分に立ち，同時に科学の社会に及ぼす影響について深く配慮しつつ，科学的見地に立って審議する.
(2) 先見的，総合的，長期的視野に立って将来の科学・技術政策について提言を行う.
(3) 「縦割り行政」に陥りがちな科学・技術行政に対して大局的見地からの提言を行う.
(4) 学・協会，科学者等の意見を集約，反映できるような運営をする.(「試案」P17)

提案Ⅰ—2 国際的な研究連絡

従来とかく惰性に流れがちであった国際的な研究連絡については，研究連絡委員会を基盤とし，国内の研究連絡の充実強化を前提として，次のように明確化する.

政府諸機関が実施している業務との重複競合を避け，本会議独自の役割を充実，強化すると共に，これらの機関と適切な連携・協力を行う.

(1) 国際学術団体への加盟の範囲と数の急速な増大による国際協力の強化.
(2) 二国間学術交流，協力のための調査.
(3) 発展途上国との学術交流の重視.
(4) 国際学術団体，地域（とくにアジア，太平洋地域）学術団体，本会議に相当する各国アカデミー，カウンシル等との学術情報の交換.
(5) これによる国際的な学術団体に関する情報センターの役割の達成.(「試案」P17)

2. 会員のあり方

(1) 望ましい会員像

会員は①科学者の代表機関の構成員であるとともに，②その職務上，個別専門分野のみならず諸科学全体の発達を図り，また行政，産業，国民生活に科学を反映浸透させる目的のため，科学者の総意の下に，審議と研究連絡に当る責任を有する.

したがって，会員は以下の諸点を具えることが望ましい.

㋐ その専門分野で国内的・国際的にすぐれた業績を有するもの.
㋑ 自己の専門分野のみでなく他の分野の科学にも関心をもち理解に努め，科学の全般的発展のために総合的視野に立って，科学政策を審議しうる能力，見識をもち，責任を果す意欲を有するもの.
㋒ 科学者，とくに有権者と結びついてその意向や要望を汲みあげ，これを本会議の諸活動に組織的に反映させるよう努力するもの.

㊦ 科学者としての良心と本会議創設の精神，日本学術会議法，科学者憲章に基づいて適正な活動を行うもの．

なお，会員選挙に際しては前述の提案Ⅰと共に前記4項目の「望ましい会員像」が有権者に広報され，適切な会員が選出されることを期待する．

(2) 会員の活動の活発化のための保障

会員の地位と待遇（とくに旅費，調査費等の保障，国家公務員である会員にも手当を支給）の保障，本務軽減の条件づくり，また新陳代謝を図るための多選制限，任期の見直しなども必要である．（「試案」P13）

3. 会員の選出方法の再検討

従来，本会議の会員は有権者たる科学者の直接選挙によって公選されてきた（公選制）．これは本会議が科学者の代表機関であるという建前から民主主義の原則に基づいて取りきめられた制度である．

この公選制を全面的に廃止して政府による任命制にするというようなことは，代表機関としての存在理由を無に帰せしめ，また独立性にも悪影響を及ぼすであろう．したがって，基本的には公選制の原則は曲げられない．

しかし，選挙制度には当然弊害もあり，とくに立候補制度下では，前述のような望ましい会員の立候補，当選が実現しない可能性も存在する．

そこで，基本的には有権者の直接選挙を原則とするが，会員の一部について他の合理的な選出制度を検討する余地を残す．

直接選挙制を維持しつつも，co-option 制を併用するなどの改革を検討すべきである．

会員選挙制度改革の一環として，多選制限，任期の見直し，選挙制度としての地方区の見直し，有権者の資格基準の厳密化，同時に有権者の権利，義務の明確化をはかる必要がある．

提案Ⅲ— 1　会員選挙制度

会員の選出は有権者の直接選挙によることを原則とする．ただし会員の一部分については他の合理的な選出制度を取る可能性について検討の余地を残す．
（「試案」P19）

提案Ⅲ— 2　有権者の資格審査基準

科学者の有権者登録を促進することを前提として，上述の基準を現行制度より厳しくする．

(1) 大学卒業後2年以上の者（その他の高等教育機関4年以上の者）で過去10年以内に研究業績（口頭報告を除く）を有する者．

(2) 業績を10年（前後）ごとにチェックすることにし，現行の広範な除外例を原則として廃止する．

(3) 外国人研究者については別に検討する（同上）

提案Ⅲ— 3　地　方　区

地方区組織活動の重要性は認めるが，選挙制度としての地方区については全国区選挙との関係を考慮して抜本的に見直しを行う（同上）

提案Ⅲ— 4　任期・多選制限

任期を4年とし，通算3選以上を禁止することを検討する．（同上）

4. 内部組織の改編の必要

(1) 部制・専門別制の再検討

国際，国内の諸科学の発展の現状，とくに専門分野の細分化，学際化，総合化等に対比してみると，創設当時のままの7部制，44専門別制は明らかにアウト・オヴ・デイトになっており，科学の諸分野，科学者の分布の現況を反映していない．これは，日本の科学者の代表機関として構成されるべき本会議制度上の欠陥であろう．これは，また，当然，選挙の制度上の弱点にもなる（たとえば，家政学，体育学，経営工学等の分野の科学者は適当な登録の部，専門がなく，また，その分野の専門区の会員定員を欠く）．

(2) 各部の定員のあり方

有権者数の激増（創設時の約5倍）のなかで特定の部と部の間の有権者数のいちじるしい格差が増大した．また，文部省科学研究費の申請者数を見ても，研究者数の格差に差異はない．これを，同じく代表機関という視点から見てどう処理すべきか．もちろん，国政選挙等とはちがって，個々の専門分野の位置づけは単にその分野の科学者数の多少だけで行うべきではないが，それにしても，この問題を避けて通ることはできない．

提案Ⅲ— 5　部制・専門別制の再編と定数

現在の科学の発達に即応するよう現行の部制・専門別制を見直し，また各部同数制についても検討する．
（「試案」P20）

(1) 会長・副会長の地位，職務，権限等の明確化

（未検討）

(2) 総会，部会のあり方の検討（未検討）

(3) 常置，特別委員会等の組織，運営の見直し（未検討）

(4) 運営審議会の性格，権限，構成，運営方法の検討（未検討）

5. 研究連絡委員会の地位の確立とその役割の増強，抜本的整備と拡充

すでにⅡ－5でもふれたように，研究連絡委員会（「研連」）は本会議の重要な内部機関であり，国際，国内の研究連絡という本会議の重要な職務にもっぱら対応する組織である．

しかも，「研連」はそれぞれの分野の第一線の科学者を学・協会の推薦や選出に基づいて委嘱された委員として擁しており，本会議の内部機関の中では，学・協会，科学者と直接結びついている唯一の機関である．したがって，「研連」の地位，役割等の拡充強化は決定的ともいうべき改革上の課題である．

提案Ⅲ－6　研究連絡委員会

研究連絡委員会を充実し，またこれを法律上正規の組織として位置づけ，その組織規定を整備する．
(「試案」P20)

6. 予算および事務局のあり方の検討（未検討）

(1) 予算について

(2) 事務局について

7. 科学者，学・協会との結びつきの強化

本会議が名実ともに「わが国の科学者の内外に対する代表機関」たりうるためには会員選挙の制度と機能が適切であることも当然必要であるが，同時に本会議の運営が常に科学者の総意を反映できるような当を得たものであることが不可欠である．

このためには，①本会議の諸活動が常に科学者の総意を反映し，その意向を汲みあげつつ行われること，また，②本会議の諸活動が広く科学者の間に周知せられるようにすることが最低限必要である．

科学者との結びつきは，本会議の存在，活動にとってアルファでありオメガである．科学者との結びつきは，①「研連」を通じての結びつき（前項），②学・協会との結びつき，③科学者との一般的な結びつき（全国的および各地方区における）の諸レベルのものがある．最も本質的なものは審議，研究連絡の場におけるそれであるが（「研連」，シンポジウム，ヒアリング，アンケート等および国際交流，協力など），同時に科学者，学・協会への情報伝達（いわゆる広報活動）も重要であり，このため広報活動の充実，強化のため全力をあげる必要がある．

提案Ⅱ－1　科学者との結びつきの強化の方策

当面の方策として，全科学者に対して，学・協会誌等を通じて，本会議の活動状況を随時報告する．

学・協会を通じ，科学者の意見を求める方策を講ずる．（「試案」P18)

提案Ⅱ－2　学・協会との結びつきの強化の方策

「第○部学・協会連絡協議会」を早急に設置する．身近な問題を協議し，学・協会間の研究連絡を計るため，各部毎に学・協会連絡協議会を設置する．ただしその実施方法は各部の判断に委ねることにする．
(「試案」P18)

8. 他の学術関係機関等との関係の改善

科学技術行政機構全体のなかでの本会議の位置づけ，現存の科学技術関係機関との相互補完の関係は重要な点であり，科学技術会議，日本学術振興会等との関係の改善について具体案を作成し，実現に向って努力する．

提案Ⅳ－1　外部からの意見聴取制度

科学技術会議その他学術関係機関，国公私立大学学長・研究所長会議等，日本学術会議の外部の人からの意見を聞くためのなんらかの制度を設ける．
(「試案」P21)

提案Ⅳ－2　連絡会議等の充実

現行の科学技術会議内の日本学術会議連絡部会，日本学術振興会との三者連絡会の運営の充実を図る．
(同　上)

提案Ⅳ－3　他の機関等への委員としての参加

科学技術会議の各部会の構成員（専門委員）に本会議が推薦した者（会員または研連委員長等）が参加し得るようにする．学術審議会等の関係審議会についても同様な考慮を求める．（同　上)

提案Ⅳ— 4　行政省庁との関係

本会議の勧告の取扱いについて行政省庁との懇談ルートを設ける．（同　上）

この改革案についてのご意見をぜひ8月末までにお送り頂ければ幸いです．

送付先　〒 106　東京都港区六本木 7 -22-34

日本学術会議改革委員会

—学会事務局より—

本会の主催する大会および地方会に会員と連名で業績を発表するものは，入会（一般会員），もしくは臨時会費を納めたものと規定されております（日生誌44巻5号会則参照）．次回大会の発表者で該当する方は，参加費とは別に上記会費のいずれかを下記の日本生理学会振替口座にお払い込み下さい．入会申込書，一般会費および臨時会費の振替用紙は，事務所にお申出下さい．

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布施ビル内

日　本　生　理　学　会

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論文表題集　日生誌第44巻号外の残部がありますので，お求め下さい．

定価　1 部　5,000円也

日本生理学会編集委員会

〔編集後記〕

桜花爛漫の下で行われた第59回日本生理学会大会号をお送り致します．ゲラ刷りを手にして感ずることは，今さらとはいえ，会員皆様の研究への高いポテルシャルとエネルギーには驚くばかりであります．部厚な大会抄録集が第44巻8，9号として納められることは，一つの大きな歴史的現実として価値あることと思います．第56回大会から始められた英文抄録も，次回の大阪大会にも踏習されることが決定しており，このスタイルも定着しつつある感が致します．

この英文化に対して，全く批判が無いわけでもありません．日本生理学会における発表の priority の問題として，国際的立場の措置でもありますが，はたして日生誌という和文雑誌の中でのこの様な形がどれだけ国際的役割をはたしているだろうかという疑問です．J. J. P. へのドッキングが出来ないものかという質問もあります．以前にも述べたかと思いますが，J. J. P. の編集委員は日本生理学会々員で構成されてはいるものの，出版・会計等は学会組織とは別の運営であり，今ただちにドッキングすることは実現不可能と思われます．いづれ，J. J. P. 編集委員長からの解答があることになっております．

さて，昨年打ち出した編集方針に沿って，本誌も一年を経過しました．計画の骨子でありました論文表題集も本誌号外として出版することが出来，約500部の購入をして頂き，学会々計に迷惑を掛けずに終始出来ました．誌上を借りて御協力いただきました皆様にお礼を申し上げます．それにしても，全会員くまなく本表題集を御覧頂けないことには，編集子として心残りがあります．論文表題集に対する批判を“生理学の広場”にお寄せ下さることを希望致します．

第44巻第7号の通し頁は270ページで，第42巻，第43巻のそれは，それぞれ178ページ，258ページでありました．後者の両2巻には論文表題集分がありますので，機関誌としての実質は140ページ，216ページとなることになります．これらの数字からお解り頂けると思いますが，編集計画が何んとか実現の軌道に乗りつつあるといえましょう．しかし，10年前の第34巻7号においては，通し頁が467ページで論文表題集の掲載は無く，誌面充実があった事には感無きを得ません．

(酒井敏夫)

編集委員

酒井敏夫(幹事)	馬詰良樹	上山章光
田中励作	中村嘉男	平野修助
菅野富夫(北海道)	中浜博(東北)	新島旭(関東)
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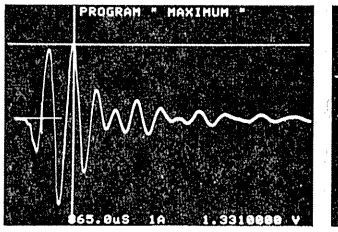
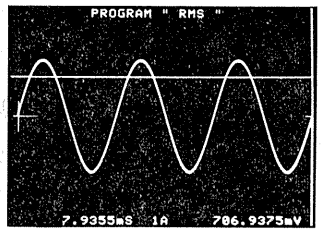
プログラム・リスト

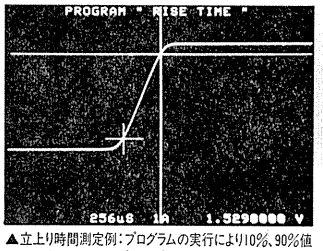
●カタログ(バージョン1.0)●絶対値●加算●面積●平均値●アベレージ回数●センタリングOV●微分●ディスケット・コピー●水平シフト●積分●積分(AC)●反転●最大値●最小値●掛算●立上り時間●RMS(実効値)●スケール(0-200%)●3点スムージング●減算 X−Y=Y●タイトル●垂直シフト



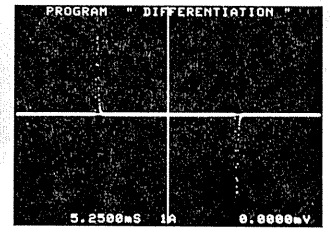
▲最大値測定例：最大値プログラムによりこの波形の最大値が1.331Vであることがわかります。



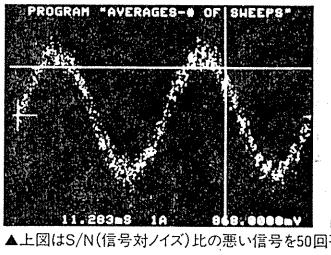
▲実効値測定例：RMS計算結果は水平カーソルと共に数値(706.9mV)で表示されます。

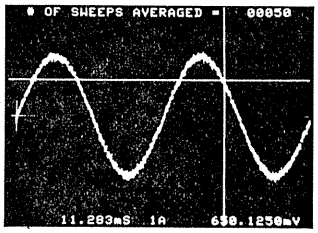


▲立上り時間測定例：プログラムの実行により10%、90%値の電圧差と立上り時間(256μs)を計算します。

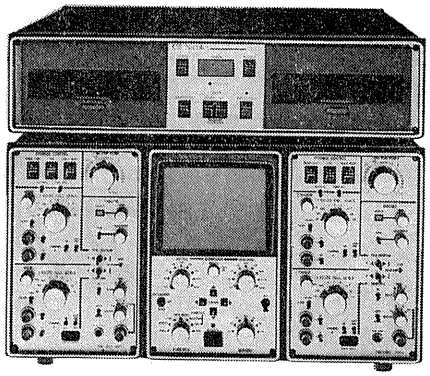


▲矩形波の微分を実行し、垂直軸の拡大率を16倍とし表示させました。





▲上図はS/N(信号対ノイズ)比の悪い信号を50回平均加算したものです。



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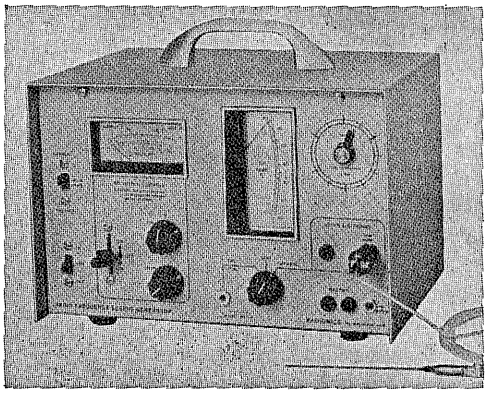
動物用 高周波 RF リージョン ジェネレータ



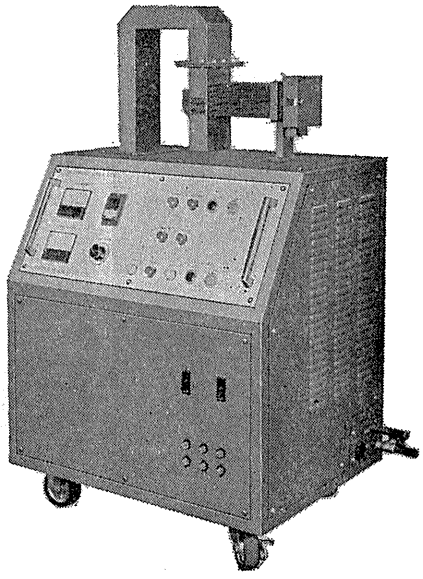
主な特徴

- 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℃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。

実験動物脳内酵素不活性化装置

東芝マイクロウェーブ アプリケータ

TMW-6402A型



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。

最近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用いられるようになりました。とくに照射後は凍結法で行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。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ご活用いただけます。

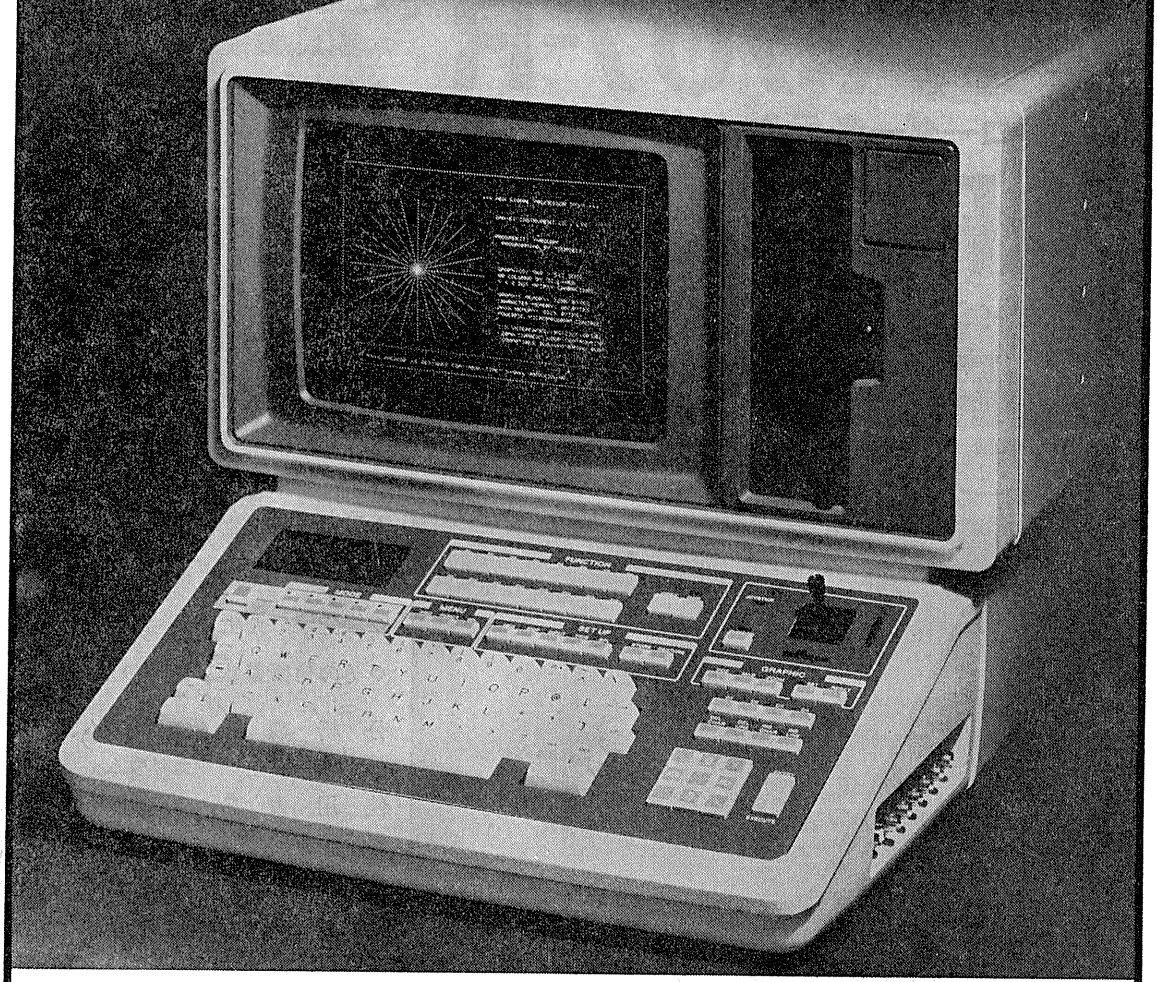
主な特長

1. 均一な照射が得られ、更に従来組織破壊の見られた視床下部もきれいに残ります。
2. アプリケータ内のラットの脳波を記録することができます。
3. サイズの異なる実験動物を使用する場合、ホルダーを交換するだけで済むので手間がかかりません。
4. マイクロ波エネルギーは0~5,000Wまで連続可変、照射時間は0.1~9.9秒まで0.1秒単位で設定できます。
5. 電源部・アプリケータ部・出力部が1つにまとまっているので設置に要するスペースが少なくて済みます。

日本総代理店

室町機械株式会社 〒103 東京都中央区日本橋室町4－3(大辻ビル)
電話 (03)241-2444(代表)

最大20チャネルの高速処理
卓越したグラフィック機能。



■多チャネルリアルタイム処理 ■12インチの大形ブラウン管を彩用 ■フロッピーディスク(8インチ)搭載
■BASIC言語でプログラム作成可能 ■多彩なインターフェイス ■サーマルプリンタを標準付属

シグナルプロセッサ
NEW 7T17

シグナルプロセッサ7Tシリーズの最高機種として7T17を新開発しました。最大20chの生体データを高速処理できるため、能率的な多チャネル解析をおこなえます。

明日の健康と福祉を守る
AAA San-ei 三栄測器
東京都新宿区大久保1-12-1 〒160 ☎03(209)0811(代)

超微量炎光々度計

$10^{-9}\ell$～$10^{-6}\ell$!!

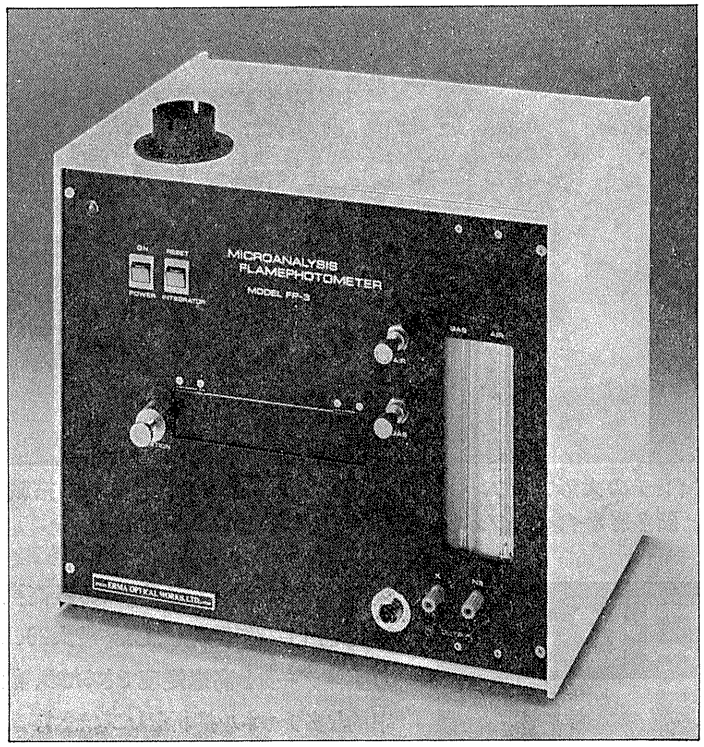
AFA-707-R
（△レコーダー表示タイプ）

AFA-707-D
（△ディジタル表示タイプ）

AFA-707-M
（△メーター表示タイプ）

★超微量炎光々度計の原理
本器は、超微量サンプルを白金線に添付、フレーム中での炎光反応によりNa，Kの濃度を測定します。

Na，K元素特有の光は、フィルターを通して受光体で検知され、さらに増幅、積分された後、メーターまたはデジタルパネルメーターに表示されます。



◇昆虫等微小生物の体液の分析
◇動物臓器の分泌物の分析
◇細胞液の分析
◇その他微量サンプルの分析

分析項目	ナトリウム、カリウム
使用ガス	プロパンガス
最小サンプル量	$10^{-9}\ell$
濃度	Na 0～350mEg/ℓ
	K 0～30mEg/ℓ
分析所用時間	4秒以内

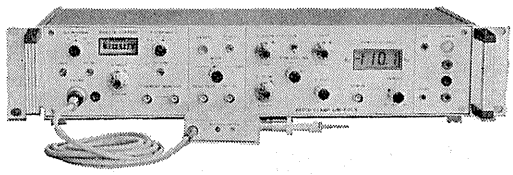
研究室で御使用になられる器機器具・測定器等の特別註文を受けたまわります。

株式会社 アペレ
〒333 埼玉県川口市根岸1155 0482-69-3040

EXTRACELLUAR PATCH CLAMP SYSTEM

"L/M-EPC-5"

西独リスト社 パッチクランプシステム



F. J. Sigworth と E. Neher 考案によるオリジナルパッチクランプシステム L/M-EPC-5型

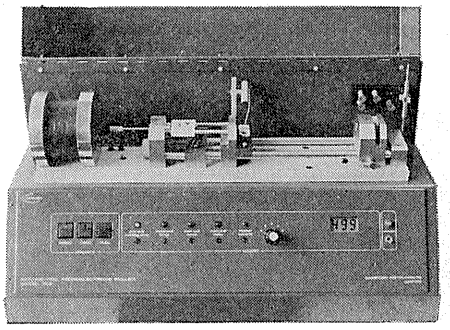
LOW NOISE：
0.1 PA RMS at 1 KHZ bandwidth

※詳しくはカタログをご請求下さい。

英国キャンプデン社 マイクロピペット プーラー

- シャンクが非常に短かく、抵抗の少ない微小電極が簡単に作成できる。
- 光学スイッチ切換えの弱・強2段式プルフォース
- 5つのパラメーターがデジタル表示され、極めて高い再現性
- ヒーター温度は絶えず赤外ディテクターでモニターされ、フィードバック回路にて保持

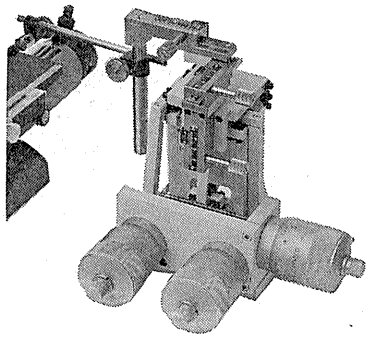
※詳しくはカタログをご請求下さい。



HUXLEY-GOODFELLOW MICROMANIPULATOR

Andrew Huxley 卿考案の英国グッドフェローメタル社製マイクロマニプレーター。

ミクロ実験に最適……最小可動単位 0.2ミクロン
信じられない様な精度……繰返し精度 0.1ミクロン
使用目的により2機種……{ HG-1000型 / HG-3000型



※詳しくはカタログをご請求下さい。

日本総代理店
株式会社 商 新

〒170 東京都豊島区南大塚2-40-1(大塚中央ビル)
TEL (03) 947-6461
〒462 名古屋市北区憧旛町1-6(志賀コーポ101)
TEL (052)915-9686

2点間の電位差を
ダイレクトに捉える

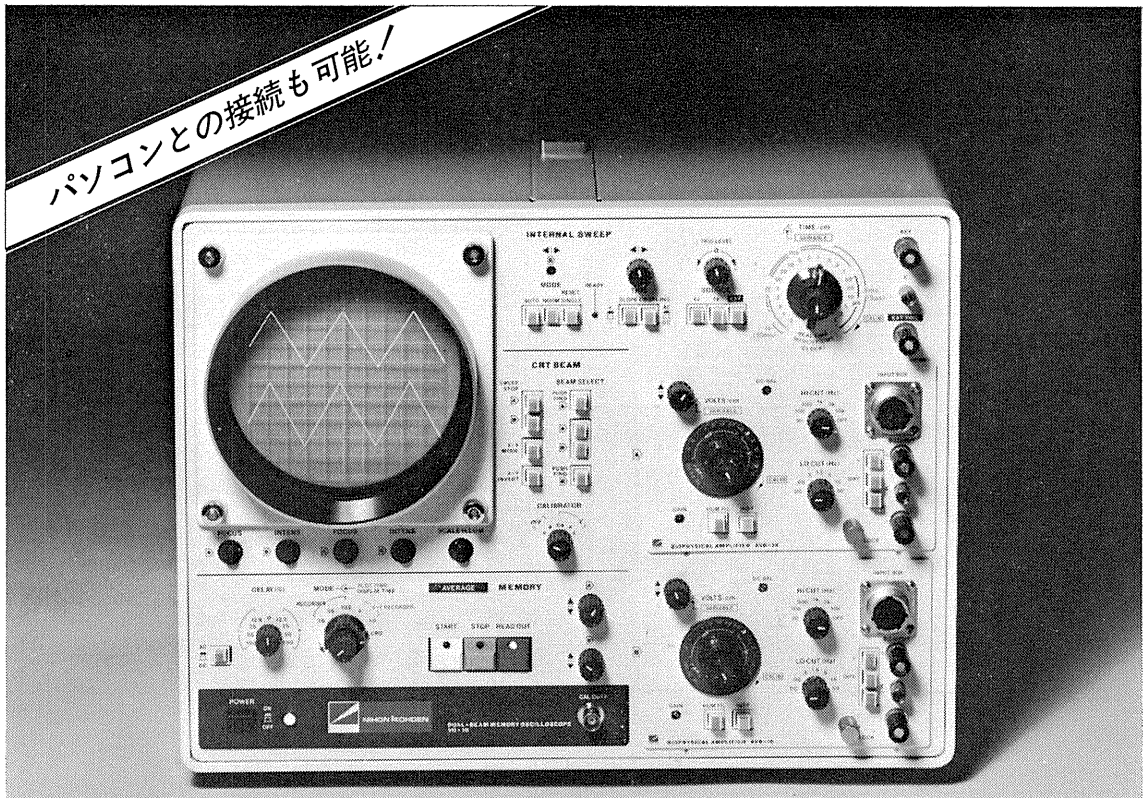
ME-3241は2つの独立したプリアンプを備えた差動型の微小電極用増幅器です。各プリアンプから直接の出力で異なる2点の同時観測もできるなど、豊富な機能が注目されています。

●操作しやすい小型化プローブ
微細な操作のしやすい小型化プローブを採用
●刺激通電をしながら観測可能
チャンネル別に外部装置を使った通電が可能
●電極抵抗値をデジタル直読
ブリッジのバランスで電極抵抗が読み取れます
●差動増幅器をビルトイン
2つの電極の電位差が1台の装置で検出可能

差動型微小電極用増幅器
ME-3241

株式会社
エム・イー・コマーシャル

本社　〒166 東京都杉並区和田3-54-11 ☎(03)317-1451(代)
大阪営業所☎(06)380-2601　名古屋営業所☎(052)451-3255　広島営業所☎(0822)92-3581　福岡営業所☎(092)863-2757



アベレージ・ヒスト…
拡張性を秘めたVC−10。

〔2-4現象 メモリオシロスコープ VC-10〕

- VC−9の使い易すさをそのままにメモリオシロにグレードアップしたVC−10
- 2チャネルメモリを内蔵、アベレージャヒストグラムユニットの追加が可能
- それに加え，これらの出力をパソコンへ接続するためのインターフェイスを内蔵等，大きな拡張性を秘めています。

〈オプション〉

■アベレージャ（DAT−1100）
チャネル：2チャネル
A/D変換：8ビット
メ モ リ：16ビット×1024ワード×2ch
出　　力：CRT用，直記式レコーダ用，XYレコーダ用，パソコン用（インターフェイス内蔵）

■ヒストグラムユニット（DAB−1100）
チャネル：1チャネル
処理プログラム：INTERVAL
DWELL TIME
LATENCY
PULSE COUNT
PULSE HEIGHT
（SEQUENTIAL及び NON SEQUENTIAL）
スライサ：ウインド型スライサ内蔵
出　　力：アベレージャに同じ

エレクトロニクスで病魔に挑戦する
NIHON KOHDEN
日本光電
〒161 東京都新宿区西落合1-31-4
☎03(953)1181

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印刷者　三浦経夫　山形県鶴岡市山王町一四―二四
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電話　振替東京三―八六四三〇
定価　三千八一五―一六二四円

KN-210
ラット尾動脈圧測定装置

非観血的にラットの尾動脈圧を測定するデジタル血圧計です。

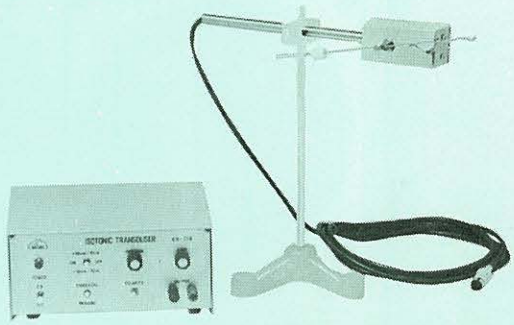
新製品

構成

① 血圧計・脈拍計本体
　測定部
　操作部
② ラット固定器
③ 予熱箱
④ デジタル プリンター

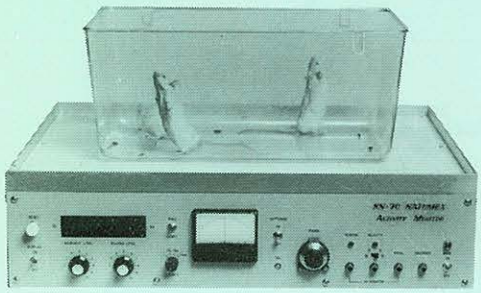
KN-259
生体用変位計

トランスジューサーと増幅器からなる，微少変位測定装置です。これまでキモグラフィオン・ヘーベルを用いて行なっていた測定を電気的測定におきかえることにより，取扱いの簡便さ，再現性および信頼性を高めました。



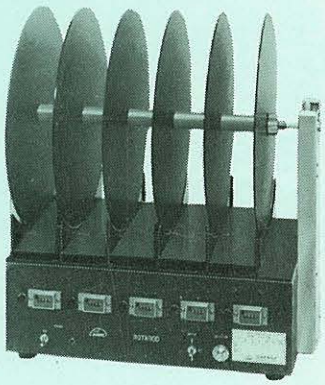
測定範囲	0～50mm（±25mm） （中心軸より100mmの時）
分解能	無限大
最大摩擦トルク	50mg・cm以下
直線性	±3％
出力インピーダンス	5KΩ以下
校正器	10mm 極性切換スイッチ付

KN-70
NATUMEX(誘導電波感応方式)
動物自発運動量測定装置



〔特徴〕
①ラット・マウスの移動がなくても姿勢を変えることにより，カウントがとれます。
②AUTO TUNING 回路がついており糞尿温度等による変化は自動的に補正されるので，長時間の測定もできます。
③ラット・マウスの立ちあがりの回数をはかれます。
④感度を揃えることが簡単に出来ます。

KN-75
KN式ロタロッド



マウス又はラットの自発運動活性を落下時間法で測定するための装置です。秒積算カウンターはそれぞれ独立した始動ボタンを備えていますので，5匹の動物を同時に試験できます。

回転数	3，5，8，10，15，20/min
回転軸径	φ30(マウス) φ90(ラット)
積算カウンター	1秒毎自動積算方式（4桁） 復帰ボタン付 自動停止装置付
寸法	W580×L330×H520mm

理化学器械・基礎医学器械・実験動物飼育機械器具・薬学研究器械・医科器械一般

NATUME 株式会社 夏目製作所　〒113 東京都文京区湯島2丁目18番6号　電話 03（813）3251（代表）