ANNUAL RESEARCH MEETING

FOR

GRADUATE STUDENTS

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Abstracts

EFFECTS OF STATIC LATERAL TILT ON OPTOKINETIC NYSTAGMUS, AFTER-NYSTAGMUS AND EYE-TRACKING IN HUMANS

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The influence of otolith input influence on both horizontal optokinetic nystagmus (OKN) and subsequent optokinetic after-nystagmus (OKAN) was examined by using a large-field optokinetic stimulation with constant acceleration combined with a static change of position using a tilting-bed. Eight subjects faced a f1.5m dome-screen at a distance of 1.15m, and were tilted on their sides from their upright posture to 45 and 90°. OK stimuli (4°/s^2 uniform, for 0 to 80°/s over 20s, or to 160°/s) were projected onto the screen 4 times at each tilt position. The stimulus profile was immediately followed by 60s of darkness for OKAN recording. Pursuit tracking was examined by means of a spot oscillating in a 20° sinusoidal wave at frequencies between 0.2 to 1.0 Hz. The amplitude gains were almost equal between the 5 positions. On the other hand, the slow phase optokinetic break-off point was highest in the upright position and was decreased in all the other tilt positions. Both the OKAN duration and the time-constant of its slow phase decay decreased with tilt positions. We thus conclude that otolith inputs influence OKN generation, and affect retinal nystagmus to a much greater extent than foveal nystagmus.

STUDIES ON THE EFFECT OF A CHECKERBOARD PATTERN ON SENSORY AND MOTOR FUSION

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We have investigated fundamental effects of a checkerboard pattern on sensory and motor fusion to clarify the effectiveness of the Checkerboard Pattern Stimulator (CPS), which has clinically improved anti-suppression and bifoveal fusion in the treatment of manifest strabismus. Studies were made on a size of the checkerboard pattern which could generate motor fusion to eliminate artificial deviations induced by various powers of prism by Aulhorn's Phase Difference Haploscope (PDH), with 20 normal subjects aged from 26 to 30 years old. Seven kinds of check sizes from 0.125 to 5 cycles/degree (c/d) were presented, and brightness was varied into 7 grades from 0.07 to 8.77 cd/m^2 by ND filters. We found that a check size of 0.5 c/d or more (narrower check size) had no effects on horizontal motor fusion and that of 1 c/d or more did no effects on vertical motor fusion. It was also found that brightness had no influence on motor fusion in cases kept on 0.36 cd/m^2 or over. Assuming that the lower limit of visual acuity for CPS should be considered as 0.05, the check size needs to be maintained approximately 1.5 c/d. Our results have suggested that a check size of 0.5 c/d applied for CPS is appropriate to promoting sensory bifoveal fusion and stereopsis.

SLEEP-RELATED CHANGES IN HUMAN MUSCLE AND SKIN SYMPATHETIC NERVE ACTIVITIES

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To characterize the features of sympathetic outflow during non-REM sleep, we measured muscle and skin sympathetic nerve activities (MSNA and SSNA) by double recording of microneurography in eight healthy volunteers during non-REM sleep. We monitored EEG, EOG, EMG and ECG, and gave acoustic stimuli during sleep stage 2. Both MSNA and SSNA were significantly reduced during light sleep stages. During slow wave sleep the MSNA rate further decreased, while minor changes were seen in SSNA, with large variability observed between subjects. Spontaneous and acoustic-stimuli-induced K-complex were frequently accompanied by either MSNA or SSNA, or both. The burst evolution time (interval between initiation of the burst and its peak) of MSNA became elongated with advancing sleep stages, whereas that of SSNA remained constant. The baroreflex latency of MSNA was constant at approximately 1.20 sec during non-REM sleep. However, the some MSNA after the acoustic stimuli often became out of range after acoustic stimuli. In conclusion, sympathetic nerve activities were centrally suppressed, and burst properties of MSNA became similar to those of SSNA during light sleep, indicating a suppressive effect on the inhibitory pathway in the baroreflex loop.

EFFECT OF OPTOKINETIC STIMULATION ON HUMAN BALANCE RECOVERY IN UNEXPECTED FORWARD FALL

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We examined whether optokinetic stimulation (OKS) affects balance recovery in an unexpected forward fall. Ten healthy male subjects participated in the study. Each was held in an initial, leaning-forward position supported by a cable connected to a strong magnet which enabled unexpected release. Instruction was given to move against forward fall by taking steps. To evaluate the relation between OKS velocities and balance recovery, ten stages of OKS velocities ranging from −100 (upward) degree/sec to +100 (downward) degree/sec were presented randomly. Balance recovery against forward fall was characterized by the reaction time, heel off, maximum vertical push, and heel contact. The latencies of these events decreased as the downward velocities of OKS increased; the latencies increased with the increment in the upward velocities of OKS. Changes in the latencies of the parameters took place in the early phase, in which the parameters of balance recovery depended little on the proprioceptive afferents from the lower limbs. This suggests that vection induced by the OKS affected the condition of the motor program which controls the initiation of the motor response. In other words, information from the visual system may modulate the condition of the motor program before the onset of movement.

PROTECTIVE EFFECTS OF CAROLINA RINSE SOLUTION AGAINST REPERFUSION INJURY IN CANINE RENAL AUTOTRANSPLANTATION

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2nd Department of Surgery

To assess the effect of Carolina rinse solution on the renal graft function after cold ischemic storage in the University of Wisconsin solution for 48 h, canine renal transplantation was performed. Survival rate of the kidneys rinsed with Carolina rinse solution (4 of 5, 80%) was higher than that of kidneys rinsed with lactate Ringer's solution (0 of 5, 0%). Postoperative serum creatinine level on day 2 was significantly lower in the Carolina group (3.48 ± 0.47 mg/dl) than that in the Ringer group (5.60 ± 0.68 mg/dl) ($p < 0.01$). All of the grafts in the Carolina group functioned immediately, whereas, in the Ringer group, none of the grafts functioned postoperatively. Histologically, severe renal damage was observed in the kidneys perfused with lactate Ringer's solution. On the other hand, the damage to the grafts was minimal in the Carolina group. With immunohistochemical staining using the antibody against factor VIII related antigen, the glomeruli were more severely damaged in the kidneys perfused with lactate Ringer's solution than with Carolina rinse solution. The present study showed that Carolina rinse solution was effective in the prevention of the kidney graft damage from reperfusion injury.

THE EFFECT OF CEREBRAL METABOLIC ACTIVATOR AND VASODILATOR ON COCHLEAR BLOOD FLOW

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We investigated the effects of intravenous injections of bifemelane hydrochloride, nicergoline and trapidil on cochlear blood flow (CoBF) and cerebral blood flow (CBF) using laser Doppler flowmetry, monitoring arterial blood pressure (BP) in 40 guinea pigs. Asphyxia was induced by stopping the respirator for 2 min before and after the injections. CoBF, CBF and BP rose after bifemelane hydrochloride administration. Nicergoline injection slightly decreased BP, but CoBF and CBF were unchanged. Trapidil injection slightly decreased BP and CBF, but CoBF was unchanged. The high concentration injections of all drugs resulted in increases in normalized cochlear blood flow (CoBF change / BP change; nCoBF). After asphyxia, CoBF, nCoBF, CBF and BP tended to reach high levels in guinea pigs pretreated with bifemelane hydrochloride, as compared with untreated animals. While administration of nicergoline and trapidil did not exert influence on the changes caused by asphyxia in CoBF, nCoBF, CBF and BP. In this experiment BP, CoBF and CBF responded in various ways to three drugs, but analysis of nCoBF indicated that these drugs had effect of vasodilation in the cochlea.

SURVIVAL OF PATIENTS WITH MULTIPLE SYSTEM ATROPHY

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We investigated the survival of patients with multiple system atrophy (MSA) in a follow-up study of patients admitted to Nagoya University Hospital between 1976 and 1991. There were 59 patients (43 males, 16 females) with a mean age at onset of 54 years. At the time of follow-up, 17 patients were alive and 42 had died. The 3-year survival rate from onset was 90%, and the 6-year survival rate was 54%. (The corresponding figures from diagnosis were 48% and 33%.) Comparison of the prognosis by survival curve showed no specific influences of age at onset or gender, whereas we found a poorer prognosis in patients with parkinsonism or autonomic failure at onset than in those with cerebellar symptoms at onset. Parkinsonian type at onset was more closely and more frequently associated with autonomic failure than cerebellar type. Thus, in MSA the earlier and more marked the degeneration of the autonomic nervous system, the poorer the prognosis tends to be.

COMPARISON OF CASUAL BLOOD PRESSURE AND 24-HOUR AMBULATORY BLOOD PRESSURE IN HIGH SCHOOL STUDENTS

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Twenty-four-hour ambulatory blood pressure measurements were obtained in 190 high school students, who were selected as showing casual blood pressure 145/85 mmHg or more at the mass-screening for hypertension (H group). They were divided into two subgroups; H1 group as consistently showing high blood pressure and H2 group as showing normal range in the subsequent measurement of casual blood pressure. For normal subjects, we obtained ambulatory blood pressure measurements of 62 normotensive volunteers, whose casual blood pressures were less than 145/85 mmHg consistently on more than two different occasions. Averaged SBP and DBP in H1 group were significantly higher than those in other groups. Those in H2 group showed a significantly higher level while awake, but not while asleep, compared with those of normal subjects. Of H group, those who showed high body mass index had a significantly higher level of SBP than others while awake, and those with family history of hypertension also showed a higher level of BP, but not significantly. Using the standard defined from the measurements of normal subjects while awake, 42% of H1 and 64% of H2 group were diagnosed as normal by ambulatory blood pressure measurements. In conclusion, high body mass index and a family history of hypertension have influences on high BP levels, and high casual blood pressure will be referential to high 24-hour ambulatory blood pressure. Even if casual blood pressure consistently shows a higher level, 24-hour ambulatory blood pressure will prove to be normal in about 40% of the subjects (white coat phenomenon).

RISK FACTORS FOR BREAST CANCER AMONG JAPANESE WOMEN: A HOSPITAL-BASED CASE-CONTROL STUDY

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A hospital-based case-control study was conducted in Tokyo, from 1990 to 1991. Information on potential risk factors for breast cancer was obtained by a self-administered questionnaire from 5,084 out- patients. Of the patients, 300 incident breast cancer cases were recruited, and 900 age-matched controls were randomly selected. The following emerged: (a) The more live births, the smaller the risk in premenopausal women; the relative risk (RR) for 3 or more births relative to none: 0.24; 95% confidence interval (CI): 0.08–0.65). (b) In premenopausal women, the regular menstrual cycle increased the risk (2.50; 1.16–5.38), and its increasing duration decreased the risk (p for trend <0.05). (c) Current smokers experienced an increased risk (1.63; 1.11–2.39). (d) Obese women were at a greater risk of postmenopausal breast cancer: RR for those weighing 70 kg or more relative to those weighing 50 kg or less being 4.82 (1.53–15.2). (e) The older the woman at first live birth, the higher the postmenopausal breast cancer risk (2.85; 1.16–6.99; 3.54; 1.03–12.2 for ages of 30–34 and 35 years and more, respectively).

NEUROCHEMICAL DIFFERENCES BETWEEN DOPA-RESPONSIVE AND NON-RESPONSIVE PARKINSONIAN PATIENTS

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The differences of I-dopa responses were studied in 12 patients with Parkinson's disease (PD), including 6 I-dopa responsive Parkinson's disease (PD-R) and 6 non-responsive Parkinson's disease (PD-NR), and 10 controls. The amounts of Goα and Gi_2 measured by enzyme immunoassay, and the activities of tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AADC) measured by HPLC-ECD methods, in the putamen and caudate nucleus of control, PD-R and PD-NR were compared in human post-mortern brains. The levels of the GTP binding proteins, Goα and Gi_2, were the same in PD and control brains. TH activity was remarkably reduced in parkinsonians brains, but there were no differences between PD-R and PD-NR. In contrast, AADC activity was generally reduced in PD brains, but more remarkably in PD-NR brain than in PD-R brains. These results indicated that GTP binding proteins, Goα and Gi_2, were not modified in PD, suggesting no disturbances in the post synaptic sites, and that the decreased AADC activity may determine the efficacy of I-dopa therapy, the ratio of the decreased enzyme activities between TH and AADC presumably causing the differences of I-dopa response in PD.

MECHANISMS OF ANTIBACTERIAL ACTION OF LIDOCAINE HYDROCHLORIDE AND ACETYLSALICYLATE

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Lidocaine hydrochloride (LH), a local anesthetic, and acetylsalicylate (AcSAL) were compared with respect to effects on the cytoplasmic membrane and antibacterial activity. Both LH and AcSAL inhibited the growth of various kinds of bacteria. Kinetic studies indicated that LH was bacteriostatic whereas AcSAL was bactericidal for gram-negative and gram-positive bacteria. At 1/4 MIC, both LH and AcSAL enhanced the sensitivity of *Escherichia coli, Salmonella typhimurium,* and *Pseudomonas aeruginosa* to novobiocin and nalidixic acid (The synergistic effect of AcSAL was higher than that of LH). Based on our findings and those of previous reports, this synergism was explained as permeabilization of the outer membranes of the bacteria and not as the alteration of biosynthesis of the porin proteins. As a number of studies have suggested, ionophore-forming antibiotics and some non-antibiotic drugs that depolarize membranes are bactericidal. We therefore determined the effects of LH and AcSAL on the membrane potential generated in the inverted membrane vesicles of bacteria. Consistent with the results of previous reports, AcSAL completely depolarized the membrane potential. LH also depolarized the membrane potential after the vesicles were energized with NADH. However, unlike AcSAL, pre-treatment of vesicles with LH had no effect on the generation of membrane potential. This difference in action on inverted membrane vesicles may be relevant to the bacteriostatic versus bactericidal activities of LH and AcSAL.

BACTERICIDAL ACTION OF TACHYPLESIN I AGAINST ORAL STREPTOCOCCI

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Tachyplesin I, a polycationic antimicrobial peptide isolated from hemocytes of horseshoe crabs, kills bacteria through disrupting the membrane potential of the cytoplasmic membrane. The present study shows that, among 36 oral streptococcal strains, 12 of 21 *Streptococcus sanguis, Streptococcus mutans,* 9 *Streptococcus salivarius* and 3 *Streptococcus milleri* were resistant. Interestingly, these resistant strains include the clinical isolates from both Kawasaki disease and Behcet patients. According to the time-kill study, tachyplesin I inhibited irreversibly the growth of *S. sanguis, S. mutans* and *S. salivarius* strains within 20 minutes and an *S. milleri* strain within 80 minutes. Although it has been suggested that *Escherichia coli* cultured in rich media were more susceptible to tachyplesin I, the present results show that only 3 *S. milleri* strains were more sensitized to tachyplesin I in a glucose-supplemented medium, and other tested strains

were not. Similarly, only 4 strains were more resistant to tachyplesin I in saline than these were in a rich medium.

DIFFERING EFFECTS OF VASOPRESSIN ON REGIONAL CEREBRAL BLOOD FLOW OF DOGS FOLLOWING INTRACISTERNAL VS. INTRAARTERIAL ADMINISTRATION

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We investigated the differential effect of the intracisternal and intraarterial administration of vasopressin on the regional cerebral blood flow (rCBF) in the parietal cortex of dogs. Regional CBF, velocity and blood volume were assayed by laser flowmetry. The intracisternal injection of 1 nmol vasopressin significantly increased the rCBF and velocity, without affecting blood volume. However, the intravertebral arterial injection of 1 nmol vasopressin significantly decreased the rCBF and velocity. This discrepancy can be explained by a difference in the affected vasculature; large blood vessels in the subarachnoid space vs. whole cerebral vascular system. The intracisternal and intraarterial injection of the nitric oxide inhibitor N^G-monomethyl-L-arginine reduced the rCBF from the base line, and significantly suppressed the rCBF elevation induced by vasopressin. The effect of vasopressin may be considered as the summation of the increased flow from the dilated large vessels via the release of nitric oxide from the endothelium, and of the decreased flow from the contracted small vessels.

THE CARDIOPROTECTIVE EFFECT OF γ-GLUTAMYLCYSTEINE ETHYL ESTER DURING CORONARY REPERFUSION IN CANINE HEARTS

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In the present study, we investigated the cardioprotective effect of reduced form of glutathione (GSH, one of the most important mitochondrial antioxidant defenses) or γ-glutamylcysteine ethyl ester (GCE, a prodrug of GSH biosynthesis) on reperfusion induced myocardial damages in canine hearts. Since mitochondria produce high energy phosphate exclusively and contain most of the intracellular GSH, we focused on mitochondrial function, mitochondrial GSH and GSSG concentrations, and mitochondrial GSH peroxidase and GSSG reductase activities. ECG and arterial blood pressure were also monitored throughout the experiments. Two hour of coronary occlusion induced mitochondrial dysfunction with depletion of mitochodrial GSH level. One hour of reperfusion following the 2h of ischemia induced further mitochondrial

dysfunction with a marked depletion of mitochodrial GSH level. Administering GCE immediately before coronary reperfusion reduced the mitochondrial dysfunction and the depletion of mitochondrial GSH level. In contrast, administering GSH showed no cardioprotective effect. Reperfusion arrhythmias were also prevented by GCE, while arterial blood pressure and heart rate were not affected by the administration of GCE or GSH. In conclusion, decrease in mitochondrial GSH level might be responsible for reperfusion injury, and clinical application of γ-glutamylcysteine ethyl ester might be expected.

COMPARISON OF CHARACTERISTICS OF BOVINE AROMATIC L-AMINO ACID DECARBOXYLASE WITH HUMAN ENZYME

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Aromatic L-amino acid decarboxylase (AADC; EC 4.1.1.28) catalyzes the decarboxylation of L-3,4-dihydroxyphenylalanine (L-DOPA) and L-5-hydroxytryptophan (L-5HTP) to dopamine and serotonin, respectively. Dopamine and serotonin are major mammalian neurotransmitters and AADC exists in the central and peripheral nervous systems localized in catecholamine- and serotonin-containing neurons besides adrenal gland and pineal gland.

In the present study, I purified AADC from bovine adrenal medulla, and compared the properties of this enzyme with those of AADC from human pheochromocytoma. The molecular weights of the subunits were identical between human and bovine enzymes and estimated to be 50,000 by SDS-polyacrylamide gel electrophoresis. An isoelectric point of the human enzyme was 5.7, while the bovine enzyme showed several distinct bands at the region of pH 4.9—5.3 in the absence of urea. Multiplicity of the isoelectric point of bovine AADC disappeared in the presence of urea. These results showed that there were some differences between the properties of human and bovine AADC in spite of the high homology (88%) in their primary structures.

EFFECTS OF THYROID AND GLUCOCORTICOID HORMONES ON THE LEVEL OF MESSENGER RIBONUCLEIC ACID FOR IODOTHYRONINE TYPE I 5'-DEIODINASE IN RAT PRIMARY HEPATOCYTE CULTURES GROWN AS SPHEROIDS

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Iodothyronine type I 5'-deiodinases (5'DI) is an enzyme to catalyze the conversion of thyroxine (T_4), to the biologically active 3,5,3'-triiodothyronine (T_3). 5'DI is present mainly in liver

and kidney and the activity of 5'DI is known to be regulated by thyroid hormone and glucocorticoid. However, the direct effects of these hormones on the level of 5'DI mRNA in hepatocytes are not known. In this study, the effects of T_3 and dexamethasone on 5'DI mRNA were investigated using primary cultures of rat hepatocytes.

Hepatocytes were isolated from rat liver by collagenase perfusion, and cultures as spheroids, which is known to maintain the differentiated functions of hepatocytes. After the hormonal treatments, cells were harvested for RNA extraction, and 5'DI mRNA was determined by Northern blot.

The present study demonstrated that T_3 increased 5'DI mRNA in a dose and time dependent manner. The T_3-induced increase in 5'DI mRNA was not inhibited by pretreatment with cycloheximide. Dexamethasone also increased 5'DI mRNA. Together with T_3, dexamethasone increased 5'DI mRNA synergistically. Dexamethasone-induced increase in 5'DI mRNA was inhibited by pretreatment with cycloheximide. It is indicated that T_3 increases 5'DI mRNA through a direct action on its gene whereas the effect of dexamethasone requires *de novo* protein synthesis.

PURIFICATION OF ERp61 FROM CULTURE MEDIUM OF MURINE COLON CARCINOMA CELLS AND ASSOCIATION OF ITS INCREASED EXPRESSION WITH IgG PRODUCTION IN HYBRIDOMA CELLS.

KEN-ICHI KOZAKI

Department of Oral Surgery

A protein of molecular weight 60 kDa was purified from the culture medium of a murine colon carcinoma line, colon26, and its partial amino-acid sequence determined. Extremely high homology was found with the deduced sequence from cDNA of rat ERp61, earlier found to be an endoplasmic reticulum (ER)-resident protein with redox activity and a similar structure to protein disulfide isomerase (PDI). Western blotting analysis showed that colon 26 cells secrete a significant amount of ERp61 into culture medium although most remains intracellular. The ER location of the protein in fibroblasts was immunocytochemically confirmed by double-staining for ERp61 and another ER-resident protein, PDI or HSP47. Immunohistochemical studies of murine tissues showed a ubiquitous distribution of ERp61 in a wide variety of cell types. However, it was particularly abundant in plasma cells, mucus secreting cells in various tissues, neuroendocrine cells including neurons, and follicular epithelia of thyroid gland that actively synthesize and secrete proteins containing cysteine residues. Furthermore, a high correlation was observed between intracellular amounts of ERp61 and immunoglobulin production by hybridoma cells. These results indicate that ERp61 may be involved in disulfide bond formation for such proteins.

ABUNDANT BUT INACTIVE-STATE gp140$^{proto\text{-}trk}$ IS EXPRESSED IN NEUROBLASTOMA OF GOOD PROGNOSIS

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The *trk* protooncogene encodes a transmembrane tyrosine kinase that works as a high-affinity receptor for NGF. To study the role of trk product in neuroblastoma cells, steady state levels of gp140$^{proto\text{-}trk}$ in cell lines and tumor tissues of neuroblastoma were examined by immunoblotting with anti- gp140$^{proto\text{-}trk}$ antibody. The level of gp140$^{proto\text{-}trk}$ varied but showed good correlation with the stage of the tumor and age of the patients at the time of diagnosis. Moreover, patients with higher expression of gp140$^{proto\text{-}trk}$ clearly had a far better survival rate than those with lower expression, suggesting that suppression of gp140$^{proto\text{-}trk}$ strongly correlates with the malignant conversion of the tumor. However, we found that neither autophosphorylation of gp140$^{proto\text{-}trk}$ nor tyrosine phosphorylation of cellular proteins was elevated in tumors of the higher expression group. These results suggest that gp140$^{proto\text{-}trk}$ dose not actively participate in the process of transformation or the suppression of malignant conversion. Rather, the higher level of gp140$^{proto\text{-}trk}$ may reflect the greater level of differentiation of tumor cells. Thus, our results suggest that gp140$^{proto\text{-}trk}$ relates the character of neuroblastoma and its expression level can be used as a marker to predict the prognosis of neuroblastoma patients.

PURIFICATION OF NUCLEAR PHOSPHOLIPASE C SPECIFIC FOR PHOSPHOINOSITIDES

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A phosphoinositide-specific phospholipase C (PLC) was solubilized from the isolated and highly purified nuclei of rat ascites hepatoma cells (AH 7974) by 2MKCl and ultrasonication. The extract was then subjected to 5 steps of column chromatographies in the order of Sephacryl S-300, phosphocellulose, Mono Q, Mono S and Superose 6. The four forms of PLC (tentatively designated N1, N2, N3 and N4) were purified 440 to 1400-fold. N1, N2, N3 and N4 showed similar apparent molecular masses of 85 kDa, 83kDa, 80 kDa and 88kDa respectively on SDS-polyacrylamide gel electrophoresis, and only N1 crossreacted with the antibody against δ1 isoform. They absolutely required Ca^{2+} with optimal concentration of 10^{-4}-10^{-3}M Ca^{2+} for both PIP and PIP_2. For PI, N1, N2 and N3 required more than 10^{-2}M Ca^{2+}, whereas N4 revealed significant activity even at 10^{-6}M Ca^{2+}. Comparison of the DNA-binding capacities of the four enzymes, using a double-stranded DNA cellulose column, showed N4 had the highest affinity. We could not detect PLC forms corresponding to N4 in either cytosol and plasma membrane of AH7974. Further, we found N4 in the regenerating liver nuclei (S phase), but not in the resting

liver nuclei. These results might indicate that N4 is unique to the growing liver nuclei and play an important role in the cell growth.

RFP IS A DNA BINDING PROTEIN ASSOCIATED WITH THE NUCLEAR MATRIX.

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We reported that the RFP (*ret* finger protein) gene encodes a protein with putative zinc finger domains and was involved in the activation of the *ret* proto-oncogene. In order to further characterize the RFP protein, we developed a polyclonal antibody against the product which was synthesized from a fragment of the RFP cDNA expressed in *Escherichia coli.* Immuno blot analysis showed that RFP was identified as a 58 kDa protein in cell lysates from several human and rodent cell lines and from mouse testis. In addition, a unique 68 kDa protein was detected in the testis. Using AH7974 (rat ascites hepatoma) and Raji (human Burkitt lymphoma) cells, we demonstrated strong association of RFP with the nuclear matrix. Furthermore, RFP solubilized from the nuclear matrix had DNA-binding activity although it appears to bind more preferentially to double-stranded DNA than to single-stranded DNA. These results suggest that RFP may play a role in molecular processes which occur in the nuclear matrix.

A NEWLY SYNTHESIZED BIFUNCTIONAL INHIBITOR, W-77, ENHANCES ADRIAMYCIN ACTIVITY AGAINST HUMAN OVARIAN CARCINOMA CELLS

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A newly synthesized calmodulin antagonist, (S)-P-(2-aminoethyloxy)-N-[2-(4-benzyloxy-carbonylpiperazinyl)-1-(P-methoxybenzyl)ethyl]-N-methylbenzenesulfonamide dihydrochloride (W-77), acts as a calcium-independent uncompetitive antagonist which binds to glutathione-S-transferase (GST). We purified GST from human placenta using drug affinity chromatography on a column of W-77 coupled with Sepharose 6B, and demonstrated that W-77 bound to GST. A spectrophotometric assay also showed that W-77 inhibited GST activity. We prepared Adriamycin-resistant and -sensitive cells from human ovarian serous cystadenocarcinomas. Immunoblot analysis revealed that GST expression was increased in the Adriamycin-resistant cells. We also purified GST from A driamycin-resistant cells, and found that W-77 bound to the GST obtained from these ovarian carcinoma cells. Adriamycin-resistance was partially overcome by the

addition of W-77 (10 μM) to the cultured cells. In addition, we investigated the effect of W-77 on P-glycoprotein. Northern blot analysis revealed MDR1 gene expression in Adriamycin-resistant cells. Although W-77 was less potent in increasing the intracellular Adriamycin content than Verapamil, it was more effective in overcoming Adriamycin resistance. These results suggest that W-77 enhances the antitumor activity of Adriamycin by inhibiting both GST and P-glycoprotein.

TRANSDUCTION OF A DRUG SENSITIVE TOXIC GENE INTO HUMAN LEUKEMIA CELL LINES WITH A NOVEL RETROVIRAL VECTOR.

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To investigate the possibility of killing tumor cells by the expression of an exogenously introduced toxic gene, we have constructed a novel retroviral vector (LTRNL) which has the Poly A signal deleted herpes simplex virus type 1 thymidine kinase (HSV1-tk) gene. The vector becomes toxic by treating cells expressing HSV1-tk with the antiherpetic drugs acyclovir (ACV) or ganciclovir (GCV). Cells of the human leukemia lines (K562, MEG- 01) were infected with this vector and two transduced cell lines (K562/LTRNL, MEG-01/LTRNL) were established. Southern blot analysis confirmed the integration of the HSV1-tk transgene in these cells and Northern blot analysis exhibited the expression of 4.8 kilobase viral mRNA containing the HSV1-tk gene. The MTT assay for the *in vitro* cytotoxic effects of GCV to these cells demonstrated that concentration of about 2.5 μM for K562/LTRNL and 1.25 μM for MEG-01/LTRNL cells resulted in 50% inhibition of cell growth after 72 hours. Subcutaneous tumors of MEG-01/LTRNL in KSN nude mice, but not those of uninfected MEG-01 cells showed durable regressions after exposure of the mice to 40mg/kg GCV given subcutaneously once a day for 15 days. This study indicates that the LTRNL-infected human leukemia cells exhibit inducible susceptibility to GCV.

CNS REGULATION OF BLOOD LACTATE CONCENTRATION IN ANESTHETIZED RATS

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This study evaluated the effect of stimulating the central nervous system (CNS) with neostigmine, an inhibitor of acetylcholinesterase, on the blood lactate concentration in fed rats and in rats fasted for 48 hours. After the rat was anesthetized with pentobarbital, neostigmine was stereotaxically injected into the third cerebral ventricle. In fed rats, the central injection of neos-

tigmine significantly increased the blood lactate level, with concomitantly increasing plasma glucagon, epinephrine and norepinephrine concentrations. Prior infusion of somatostatin, to inhibit glucagon secretion from the pancreas, did not affect alterations in blood lactate dependent on CNS stimulation. In adrenomedulloectomized rats, neostigmine continued to significantly increase norepinephrine. However, the alteration in blood lactate was only one-third of that in intact rats. Intraperioneal propranolol, but not phentolamine, prevented the rise in lactate. Neostigmine increased lactate in fasted rats as well as in fed rats. We conclude that in anesthetized rats, stimulation of the CNS by neostigmine increases blood lactate mainly through circulating epinephrine and partially through circulating norepinephrine or direct sympathetic nervous stimulation; glucagon does not appear to be involved in the increase in blood lactate.

EFFECTS OF DEXAMETHASONE ON MIGRATION OF HUMAN MONOCYTES IN RESPONSE TO OXIDIZED β-VERY LOW DENSITY LIPOPROTEIN

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Objective: In a previous study, we showed that dexamethasone inhibited the development of atherosclerosis in cholesterol-fed rabbits. The aim in this study was to investigate the mechanisms of dexamethasone on the inhibition of cholesterol-induced atherosclerosis. Methods: Human peripheral blood monocytes were isolated from the blood of a healthy donor. Cell suspensions were incubated with dexamethasone for 90 minutes at 37°C. The concentration of dexamethasone was 10^{-11}–10^{-4}M. Rabbit β-very low density lipoprotein (β-VLDL) was obtained from New Zealand White rabbits which were fed chow containing 1% cholesterol. Oxidative modification of β-VLDL was performed by auto-oxidation. Monocytes migration in response to native and oxidized β-VLDL was measured in a 48-well micro chemotaxis chamber. Results: Oxidized β-VLDL stimulated the migration of monocytes dose-dependently in the range between 0.5 and 2 nmol/mg protein. Dexamethasone inhibited monocyte chemotaxis to oxidized β-VLDL in a dose-dependent manner more than 10^{-9}M. Conclusions: Inhibition of the chemotaxis of monocytes in response to oxidized β-VLDL may be one of the anti- atherogenic mechanisms of dexamethasone in cholesterol-fed rabbits.

IMMUNOSUPPRESSIVE ACTIVITY INDUCED BY NITRIC OXIDE IN CULTURE SUPERNATANT OF ACTIVATED RAT ALVEOLAR MACROPHAGES

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We studied the mechanism and the implication of the nitric oxide synthetase pathway (NOSP) in alveolar macrophage (AM)-mediated suppression of Concanavalin A (Con A)-induced lymphocyte proliferation in rats. The culture supernatant (SN) from AM co-cultured with nonadherent spleen cells (SC) had the immunosuppressive activity to Con A-induced response of SC, but SN of AM alone did not have this activity. Con A-pulsed AM also liberated the immunosuppressive factor. When AM and autologous SC were cultured separately under the condition that medium could freely communicate, SN did not suppress the Con A-induced response of SC. This indicated that the immunosuppressive factor was liberated when AM was activated by cell-to-cell contact with SC. Further, we examined the immunosuppressive activity of SN of AM co-cultured with autologous SC to Con-A-induced responses of allogeneic SC and xenogeneic murine SC, and allogeneic mixed leukocyte reaction. We found that this SN could suppress all of these proliferative responses. Nitrite accumulation was markedly augmented in SN of Con A-pulsed AM or AM co-cultured with SC. N^G-monomethyl-L-arginine (MMA), a competitive inhibitor of NOSP, abrogated both nitrite accumulation by AM and AM-mediated immunosuppressive activity. These findings suggest that NOSP is important in AM-mediated suppression of Con A-induced lymphocyte proliferation.

PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) STIMULATES ARGININE VASOPRESSIN RELEASE IN CONSCIOUS RATS

TAKASHI MURASE

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The effect of pituitary adenylate cyclase activating polypeptide (PACAP) on arginine vasopressin (AVP) release was investigated in conscious rats. Intracerebroventricular (i.c.v.) administration of PACAP raised plasma AVP concentration in a dose-dependent manner (50–500 pmol/rat), and maximum effect was obtained at 5 min after the administration. This AVP-releasing effect was not accompanied by a fall of blood pressure or changes of plasma total protein and Na. Vasoactive intestinal peptide (VIP), which is homologous to PACAP, also raised plasma AVP concentration by i.c.v. injection. [Lys, Pro, Arg, Tyr]-VIP, an antagonist for VIP receptor, inhibited the VIP-induced increase of plasma AVP, but had little effect on PACAP-

induced increase of plasma AVP. These results suggest that PACAP stimulates AVP release, via specific receptors which are distinct from VIP receptors.

A HISTOMETRICAL STUDY ON THE GLOBUS PALLIDUS IN HUNTINGTON'S DISEASE

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Pathological change of the globus pallidus (GP) in 6 cases of Huntington's disease (HD) was examined histometrically by comparing with 10 normal control cases (NC). Five cases of HD were in late stages of the disease. Total neuronal count, area of GP, and neuronal cell density were measured in five selected regions of coronal sections taken along the antero-posterior axis. Contrary to the findings of previous reports, no neuronal depletion was recognized in HD in any region despite marked atrophy of tissue bulk. The atrophy was more severe in the external segment (GPe) than in the internal segment (GPi). Reactive astrocytosis and fibrillary gliosis were observed in the atrophic lesions. These results indicate that atrophy of the GP can be attributed to striato-pallidal fiber loss and not to neuronal depletion even in the late stages. These findings support the hypothesis that loss of striato-GPe fibers plays the most important role in choreic movements in HD. It remains to be determined whether the pallidal neurons are also preserved in the end stage of the disease.

COMPARISON OF ENVELOPE AND PRECORE/CORE VARIANTS OF HEPATITIS B VIRUS (HBV) DURING CHRONIC HBV INFECTION

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3rd department of Internal Medicine

We analyzed entire pre-C/C and pre-S/S coding genes of hepatitis B virus (HBV) in serial serum samples from 4 chronic HBV carriers with 4–5 years of follow-up. Two patients with chronic active hepatitis became seronegative for HB e antigen (HBeAg), while the other 2 were persistent asymptomatic carriers with normal aminotransferase values. DNAs amplified by PCR were cloned and sequenced. After HBeAg became negative, one patient had 96–183 bp deletions in 4/6 clones for C-gene. In addition, both patients had 129–183 bp deletions in 3/6 and 2/5 clones for pre-S1-gene. Divergence rate of deduced amino acid for both pre-C/C and pre-S/S regions from the adr subtype was significantly higher in patients with chronic hepatitis than in asymptomatic carriers. Furthermore, the divergence rate for pre-S/S region was usually

greater in asymptomatic carriers as well as chronic hepatitis patients compared with that for pre-C/C region. However, no significant difference was found in the rate of amino acid divergence for the entire HBV genes between the serial samples from both chronic HBV carriers. These results suggest that active hepatitis induces variation of HBV genes and that defective viruses are often selected along with disappearance of HBeAg. In addition, pre-S/S region as well as pre-C/C region is suggested to be a target of the host immune response during chronic HBV carrier state.

PURIFICATION AND BIOCHEMICAL CHARACTERIZATION OF A PROTEIN KINASE INDUCED BY HERPES SIMPLEX VIRUS TYPE 2

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We have previously reported that a US3 lacZ insertion mutant of HSV-2 has unique biological properties when compared with those of the parental virus. In this study we have purified the US3-encoded protein kinase (PK) of HSV-2 and characterized the biochemical properties. The enzyme was purified more than 1000-fold from the post-ribosomal supernatant, and the final preparation contained one major protein of apparent molecular weight 66 kilodalton (K), which was phosphorylated in the autophosphorylation reaction. Western blotting analysis showed that antibodies to a synthetic peptide corresponding to the 15 amino acids of the predicted HSV-2 US3 protein sequence strongly reacted with a 66K protein in the enzyme fractions. On Superose 12 HR chromatography, the protein kinase activity was eluted as a single major peak at a position corresponding to an apparent molecular mass of approximately 60 K. These results suggest that the 66 K protein is the protein kinase encoded by the US3 gene of HSV-2 and that it acts as a monomer. The HSV-2 protein kinase had basic pH optimum and relatively resistant to high concentrations of KCl. When the substrate specificity was investigated using synthetic oligopeptides, the peptides containing arginyl residues on the amino-terminal side of the target seryl residue were found to be the best substrates for the protein kinase. Quercetin, a bioflavonoid, inhibited the protein kinase and the inhibitory effect was competitive towards ATP (Ki=10 μM.)

IMPAIRED HUMORAL RESPONSES TO HEPATITIS C VIRUS AMONG RENAL TRANSPLANT RECIPIENTS

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Hepatitis C virus (HCV)-infected patients with immunosuppression are known to have low prevalences of antibodies to the virus. Humoral responsiveness to two kinds of antigens derived from HCV was examined among twelve renal transplant recipients (RT) who were under immunosuppressive treatment, and results were compared to those of eighteen immunocompetent patients who underwent open heart surgery (OHS). HCV infection was confirmed in all involved cases by means of polymerase chain reaction (PCR) which amplified 5'non-coding region of the virus. Only three out of 12 RT had positive anti-C100-3 by EIA, while all of the OHS showed positive results. The same tendency was found in the titers of antibody to p22, core protein of HCV, where titers of anti-p22 were significantly lower in RT than those in OHS.

To investigate the mechanisms of low responsiveness to HCV antigens among RT, further virological studies were performed. Antibody activities against mumps virus and cytomegalovirus (CMV) were measured by EIA using the same samples. Significantly higher antibody activities against CMV, and almost equal titers against mumps virus were found in RT when compared to OHS. Then, quantitation of HCV RNA in sera desclosed that there were $10^{2.0}$ times more HCV RNA in sera of RT than in those of OHS, showing that the low responsiveness could not be due to the lower concentration of the virus antigens. From these results, the low prevalences of anti-HCV in RT could imply weak immunogenicity of HCV for immune system.

(A) A PROTECTIVE ROLE OF EXTRATHYMIC α/β T CELLS IN MURINE SALMONELLOSIS. (B) COSTIMULATION WITH LFA-1 TRIGGERS APOPTOSIS IN γδ T CELLS ON TCR ENGAGEMENT

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The liver comprises unique T cells differentiating extrathymically and expressing an intermediate intensity of α/β T cell receptor (TCR) and a high intensity of leukocyte function-antigen-1 (LFA-1). To elucidate the functional roles of the intermediate α/β T cells in host defense against bacterial infection, we examined the effects of depletion of the intermediate α/β T cells by in vivo administration of mAbs to intercellular adhesion molecule-1 (ICAM-1)/ LFA-1 and α/β TCR on the bacterial growth in the liver after infection with *Salmonella choreaesuis* in mice. Our results suggest that intermediate α/β T cells may play important roles in protection at the early stage after *Salmonella* infection in liver and that the interaction of ICAM-1 / LFA-1 is critically involved in protective roles of extrathymic T cells bearing inter-

ediate α/β TCR in liver at the early stage after *Salmonella* infection. (A)

Stimulation of T cells through the T cell receptor (TCR) initiate activation pathways, and paradoxically can also result in activation-induced cell death. Many factors influence a stimulated cell's decision to manifest either. Here we show that costimulation with LFA-1 play a key role in the choice of the two fates, differentiating between αβ and γδ T cells. Peripheral γδ T cells but not αβ T cells underwent apoptosis by co-crosslinkage of TCR and LFA-1 in MRL lpr/lpr mice as well as +/+ mice. Our results suggest that apoptosis of γδ T cells is inducible by combined stimuli independently of Fas-mediated pathway. (B)

SELECTIVE T-CELL RECEPTOR GENE USAGE IN ALLORECOGNITION AND GRAFT-VERSUS-HOST DISEASE

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Immune recognition of foreign HLA molecules is initiated by T cell recognition mediated by alloreactive T cell receptor (TCR) molecules. This allorecognition process reflects the ability of T cells to discriminate "self" from "non-self". We analyzed the diversity of TCR expression in the clinical setting of allorecognition in a patient with acute graft-versus-host disease following bone marrow transplantation from an unrelated donor. Nearly 200 TCR transcripts from peripheral blood lymphocytes were cloned and sequenced at two time points during GVHD. HLA genes in the transplant donor and the recipient were mismatched for a very specific HLA-DR subtype: HLA-DRB1 genes in the donor (DR4/Dw4) and the recipient (DR4/Dw14) encode HLA molecules that differ at only two amino acids, providing a very restricted target for allorecognition. We also studied TCR genes from five T cell clones derived in vitro from mixed lymphocyte cultures between Dw4-positive responder and Dw14-positive stimulator cells. Comparisons of the derived TCR sequences implicate non random patterns of TCR selection both in vivo and in vitro.

DEVELOPMENT OF AN ISOLATED LIVER PERFUSION CIRCUIT WITH DOUBLE BYPASS USING AUTOMATIC BLOOD PUMPS

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Isolated perfusion of the liver is a useful and promising therapeutic method for various hepatic diseases. However, previous techniques and apparatus were designed exclusively for application to adult patients. They often used a roller pump, which requires a large priming volume and can not run at the small flow rate. These disadvantages did not allow their use in smaller

pediatric patients. The authors solved these problems successfully for the first time by using unique sac-type air-driven blood pumps with an oxygenator primed with blood of 65 ml in total circuit. They can be operated at the smaller perfusion flow, and keep the flow automatically without any danger of excessive negative pressure in the inflow route. The authors evaluated the usefulness of these blood pumps to liver perfusion in small animals and determined an optimal perfusion flow in experimental animals. A liver perfusion circuit was established between the portal vein and the inferior vena cava. Experimental animals weighing 3.5—6.0 kg were grouped according to the flow rate. The blood pumps worked without any trouble and stable flow could be maintained at each flow rate. No hepatocellular damage or anaerobiosis of the liver was observed in the 20 ml/min/kg group. The results indicated the preservation of the hepatic cells under aerobic metabolism. In conclusion, isolated liver perfusion using these blood pumps can be applied in infants and young children safely. Liver function was maintained most satisfactorily at the flow rate of 20 ml/min/kg.

EFFECTS OF CLASS-III ANTIARRHYTHMIC AGENTS ON THE VENTRICULAR REPOLARIZATION OF RABBIT HEARTS

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Effects of the Class-III antiarrhythmic drugs (amiodarone, sematilide, sotalol, E-4031, MS-551) on ventricular repolarization were investigated in Langendorff perfused rabbit hearts. Forty to fifty electrograms were recorded through modified bipolar electrodes (MBE) from anterior to lateral epicardial surface of both ventricles under His bundle pacing at 1.0 Hz. The interval from the initial sharp negative deflection to the apex of T wave (Q-aT) of the MBE signals reflects action potential duration (APD) at the recording site. In the hearts treated with 100 mg/kg/day oral amiodarone for 4 weeks, the epicardial activation sequence was almost identical to that of control, whereas Q-at was prolonged uniformly throughout the whole mapped area by 10—13%. Spatial inhomogeneity of ventricular repolarization was slightly decreased by the drug. Acute application of sotalol (30 μM), E-4031 (0.1 μM), MS-551 (1 μM) or sematilide (10 μM) also caused a prolongation of Q-aT without affecting the activation sequence. The Q-aT prolongations were, however, greater in the apex than in the base, resulting in a marked increase in the spatial inhomogeneity of ventricular repolarization. Such regional differences of Class-III drug action would be responsible for their potential proarrhythmic activity by setting the stage for re-entry.

EFFECTS OF CLASS-I ANTIARRHYTHMIC DRUGS ON THE CONDUCTIVITY OF ANISOTROPIC CARDIAC MUSCLE

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The anatomical architecture of cardiac muscle influences the conduction of excitation. Particularly the conduction velocity and the configuration of action potential upstroke are dependent on the direction of propagation in relation to the fiber orientation. In a computer simulation study to mimic the anisotropic conduction properties, Spach et al. (1987) demonstrated that the total open time of sodium channel at each excitation was longer for longitudinal propagation (L) than the transverse one (T). Therefore we speculate that class-I antiarrhythmic drugs, which block the sodium channel mainly during the activated state, would cause greater inhibition of conduction in the L propagation than the T propagation. To test this possibility, we examined the effects of flecainide (FI), quinidine (Quin), aprindine (Apr) and SD3212 (SD) on the conduction velocity (θ) in the epicardial ventricular muscle isolated from rabbit hearts. Extracellular potentials were recorded through modified bipolar electrodes to identify regional activation time. In control condition, θL was higher than θT ($\theta L/\theta T=2.5\pm0.1$), so the isochrone map of activation showed an elliptic pattern with its long axis parallel to the L direction. All the four drugs caused dose-and-frequency dependent reduction of θ. Fle and Quin (activated channel blockers) caused greater depression of θL than θT, resulting in an alteration of activation sequence to a circular pattern. In contrast, Apr and SD (inactivated channel blockers) caused a similar reduction of both θL and θT so that the elliptic pattern of activation sequence was well preserved. These findings suggest that the negative dromotropic effects of antiarrhythmic drugs on the conduction of anisotropic cardiac muscle are largely influenced by the state-dependence of their sodium channel block.

AUTOIMMUNNE MYOCARDITIS INDUCED BY CARDIAC C-PROTEIN: A MEMBER OF IMMUNOGLOBULIN SUPERFAMILY

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1st. Department of Internal Medicine

Autoimmune myocarditis is one of the possible causes of dilated cardiomyopathy (DCM). We have succeeded in making a new autoimmune myocarditis model induced by repetitive injection of 100μg of murine cardiac C-protein mixed with 100μg of *Klebsiella pneumoniae* O3 lipopolysaccharide (KO3 LPS) adjuvant in SMA mice. For examining the antigenic epitopes, we isolated and sequenced the cDNA clones encoding this protein. Sequence analysis revealed cardiac C-protein is a member of the intracellular immunoglobulin-superfamily containing fibronectin- like domains. Recombinant cardiac C-protein expressed in *E. coli,* effectively produced autoimmune myocarditis in some strains of mice including SMA, DBA/1J, SJL, and O20/A,

suggests that C-protein is one of the important antigens for developing autoimmune myocarditis.

We also found two DCM patients in 16 had specific autoantibodies against C-protein. These results suggested that some types of DCM may be caused by autoimmune mechanism triggered by cardiac C-protein.

CYTOKINE PRODUCTIONS IN INFLAMMATORY BOWEL DISEASE (IBD)

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Interleukin 2 (IL2) and its receptor (IL2R) were investigated from cellular and molecular levels using lamina propria mononuclear cells (LPMC) as well as peripheral blood mononuclear cells (PBMC) in IBD such as Crohn's disease (CD) and ulcerative colitis (UC). Cells were cultured with or without mitogens, and IL2 and soluble IL2Rα (sIL2Rα) values contained in the supernatants were measured using a bioassay or ELISA, respectively. Additionally, mRNA levels of these two molecules were examined by Northern blot analysis. IL2 activity in PHA-stimulated control cultures was significantly ($p < 0.01$) greater than that of IBD cultures although no IL 2 activity was detected in unstimulated cultures. In contrast, sIL2Rα was spontaneously secreted significantly ($p < 0.01$) more by CD LPMC than that of control, but not UC. Compared between PBMC and LPMC, LPMC could produce significantly ($p < 0.01$) these two mediators in a higher level than PBMC. Consistent with protein levels, both IL2Rα and β mRNA levels in CD LPMC were elevated compared to control cells regardless of the decreased levels of IL2mRNA in IBD. On the other hand, LPMC contained substantially more transcripts of IL2 and IL2 receptors than PBMC. These results indicated that LPMC could contribute to mucosal immunity with greater capacity to produce soluble factors. In addition, highly expressed IL2R might be involved in enhanced immunoreactivity in CD in spite of defect of IL2 activity.

ROLE OF INTESTINAL INTRAEPITHELIAL LYMPHOCYTES DURING ACUTE GRAFT-VERSUS-HOST DISEASE

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We examined the kinetics of intestinal intraepithelial lymphocytes (IEL) and incidence of apoptosis at villus or crypt sites during the development of unirradiated acute graft-versus-host disease (GVHD). The first IEL to increase were host-origin on day 3 and the donor-derived IEL appeared first on day 12 after GVHD induction. Unique $CD3^+CD4^+CD8\alpha/\alpha^+$ IEL were significantly increased on day 6 and an appreciable number of IEL bearing T cell receptor

(TcR) Vβ capable of recognizing self-superantigen were detected on day 9. The sudden appearance of apoptosis and reduction of mitotic activity occurred on day 12, accompanied by a dramatic decrease of $CD3^+CD4^-CD8\alpha/\alpha^+$ IEL of host origin. CD8α/α IEL of host origin, which expand and then decrease by apoptosis at the early stage of acute GVHD, may be associated with pathogenesis of the enteropathy occurring during acute GVHD.

HISTOLOGICAL STUDIES ON THE PALATE AND PHARYNX OF THE MOUSE: METHOLOGICAL IMPROVEMENTS OF WHOLE-MOUNT PREPARATIONS, AND FINE ARCHITECTURE OF NERVES AND VASCULATURES

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A dental impression technique was applied for preparing a histological whole-mount specimen of the oral cavity and pharynx of the mouse. Hydrophilic vinyl polysiloxane impression material mixed with a hardening accelerator was injected into the lumen under pressure. After sufficient hardening (5min. after the injection), mucosal tissue were removed together with surrounding glands and musculatures. The tissue blocks were dipped *en bloc* in the ZIO solution according to the method of Jabonero (1964) for 24 hrs at room temperature. Tissue sheets containing various layers of the oral cavity and pharynx were prepared using watchmaker's fauceps, mounted on the glass slide, and observed under the light microscopy. Three-dimensional interrelationships between the nerves and vasculatures were demonstrated: both the blood vascular network and the nerve network were continuous in the median plane of the soft palate. Some whole-mount preparations were served for the immunohistochemical observations after fixation in Bouin's fluid. By immunostaining using an S-100b protein antiserum, Schwann cell networks of the autonomic nerve terminal apparatus appeared within the smooth muscles and salivary glands in the soft palate and other organs.

Peculiar blood vessels existed in the periphery of the SCCVII tumors transplanted into the soft palate and pharynx; however neither lymphatic vessels nor nerve elements were seen in those tumors. Thus the improved whole-mount method of specimen preparation is useful for the histological investigation of the mechanisms of tumor invasion and metastasis in the field of oral surgery.

A CORRELATIVE IMMUNO-LIGHT AND ELECTRON MICROSCOPIC STUDY ON THE TYPE I COLLAGEN IN THE BONE MORPHOGENETIC PROTEIN-INDUCED CARTILAGE

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Bone morphogenetic protein (BMP) partially purified from bovine bone was implanted into the thigh muscles of AKR-strain mice to induce an ectopic endochondral ossification tissue. The extracellular matrix components around hypertrophic chondrocyte in the induced tissue were examined by electron microscopy and immunohistochemistry for type I collagen. At 14 days after implantation, chondrocytes became hypertrophic with calcifying matrix around them. Some hypertrophic chondrocytes showed no degenerative appearances. They were ringed with a pericellular matrix with no metachromasia which was distinct from the metachromatic cartilage matrix. The localization of type I collagen was demonstrated by immuno-staining in this matrix. Ultrastructurally, ring-shaped matrix consisted of interwoven collagen fibrils on which a D-periodic banding pattern could be discerned. Immunoelectron microscopy demonstrated immunoreactivity of type I collagen on these fibrils. These results suggest that some of the BMP-induced hypertrophic chondrocytes maintain their activity and produce type I collagen-rich matrix before their lacunae are eroded.

ASSEMBLY OF 100nm PERIODIC FIBRILS (TYPE VI COLLAGEN) IN THE HUMAN INFANT CORNEAL STROMA

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An experimental model for age-related changes in the extracellular matrix of the human cornea was developed. Long-spacing collagen with a periodicity of about 100 nm increases in amount with age in the extracellular matrix of human eye, and we earlier observed that fibrillar substances which resembled long-spacing collagen were experimentally formed in mice by adenosine 5'-triphosphate (APT) treatment. So we treated human infant cornea with ATP, in which no long-spacing collagen occurred naturally, in order to examine whether the ATP treatment offers an experimental model for age-related changes of human eye. Small fragments of corneas from eyeballs of two infant males, which were enucleated due to retinoblastoma, were used. They were incubated in phosphate buffered saline containing 20 mM ATP•2Na, pH 4.15, at 37°C for 2 or 24 hr and observed in a transmission electron microscope. Numerous periodic structures with about 100 nm periodicity (100 nm periodic fibrils) that resembled long-spacing collagen were formed by the treatment. These fibrils appeared in close contact with D-periodic collagen fibrils. As we confirmed that 100 nm periodic fibrils were composed of type VI

collagen, a possible connection between type VI collagen and collagen fibrils was suggested. By ruthenium red staining, the 100 nm periodic fibrils were positively stained, indicating that glycosaminoglycans or proteoglycans are involved in the fibrils.

IMMUNOHISTOCHEMICAL CHARACTERIZATION OF EXTRACELLULAR MATRIX COMPONENTS OF GRANULOSA CELL TUMOR OF OVARY

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In order to clarify the characteristics of granulosa cell tumors of the ovary, extracellular matrix components were investigated by immunohistochemical technique. Twenty-three granulosa cell tumors (GCTs) (8 juvenile and 15 adult type) were studied in comparison with non-neoplastic granulosa cells of human ovaries. In all 23 cases of GCTs, chondroitin 6-sulfate proteoglycan (PG) revealed with antibody 3B3 (Couchman et al. 1984) was characteristically observed in the extracellular matrix in the solid nest, as well as in microfollicles. In the cases of juvenile type, the extracellular matrix also contained large PG revealed with antibody 2B1 (Sobue et al. 1989). Macrofollicles as well as microfollicles contained PG having chondroitin 6-sulfate side chains with a significant amount of chondroitin 4-sulfate. By biochemical analysis using HPLC, it was also found that disaccharide composition of GAG fractions extracted from granulosa cell tumor tissues consisted mainly of 2-acetamide-2-deoxyl-3-O-(β-D-gluco-4-enepyranosyluronic acid)-6-O-sulfo-D-galactose (△Di-6S). The characteristic feature of granulosa cell tumors is the accumulations of chondroitin sulfate PGs, especially chondroitin 6-sulfate PG which may be synthesized by tumor cells themselves. Immunohistochemical characterization of the extracellular matrix components (collagen, laminin, heparan sulfate PG, chondroitin 4-sulfate PG) was also studied in relation to chondroitin 6-sulfate PG localization.

DETECTION OF O_2^- GENERATION AND NEUTROPHIL ACCUMULATION IN RAT LUNGS AFTER ACUTE NECROTIZING PANCREATITIS

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2nd Department of Surgery

We used chemiluminescence (CL) technique to measure superoxide anion (O_2^-) generation in situ from the surface of rat lungs in which necrotizing pancreatitis was induced by injection of trypsin and deoxycholic acid directly into the pancreatic duct. The animals had been infused

continuously with a highly luminescence reagent, 2-methyl-6-phenyl-3,7-dihydroimidazo[1,2-α]pyrazin-3-one (CLA) and CL was detected using a sensitive photon counter. Transient bursts of CL, which reflected O_2^- generation, were observed at the lung surface 3 ~ 5 hours after injection of trypsin and deoxycholic acid. No O_2^- dependent CL was detected at the lung surfaces of the neutropenic rat made by pretreatment with polyclonal antineutrophil antibody. Myeloperoxidase activity in the lung tissue was measured by a new chemiluminescence method to quantitate pulmonary neutrophil sequenstration. These data confirmed that activated neutrophils and neutrophil-derived superoxide anions were implicated in lung injury after acute severe pancreatitis.

ACTIVATION OF TRYPSINOGEN IN EXPERIMENTAL MODELS OF RAT ACUTE PANCREATITIS.

YASUYUKI NAKAE

2nd Department of Internal Medicine

The appearance of trypsinogen activation peptides (TAP) in serum or urine reflects premature activation of trypsinogen. Most of active trypsin in serum formed α2-macroglobulin-trypsin complex (α2M-T) and its residual activity is considered to play an important role in aggravating acute pancreatitis. Therefore, concurrent increases in TAP and α2M-T activity suggest reflect the massive activation of trypsinogen and subsequent exacerbation of pancreatitis. We measured TAP concentration (radioimmunoassay) and α2M-T activity (colorimetry) in two experimental models of rat acute pancreatitis to evaluate the significance of activation of trypsinogen in acute pancreatitis. Both TAP concentration and α2M-T activity in serum rose significantly high in trypsin-taurocholate-induced hemorrhagic acute pancreatitis while either of the two did not increase in cerulein-induced edematous acute pancreatitis in spite of similar increases of serum immunoreactive trypsin in the two models. When rats in trypsin-taurocholate-induced pancreatitis were treated with trypsin inhibitor (nafamostat mesilate: TI group), serum α2M-T activity (20.8 ± 1.5 U/I) was significantly ($p < 0.01$) lower than that in nontreated controls (79.1 ± 24.5 U/I). The survival rate at 24 h was significantly improved in the TI group when compared with that in controls (70% vs 43%, $p < 0.05$), although the increase of TAP concentration in the TI group was similar to that in controls. In conclusion, activation of trypsinogen and its residual enzyme activity play an important role in subsequent development of severe acute pancreatitis.

ULTRASOUND IMAGES OF THE RETROHEPATIC VENA CAVAL WALL BY PERCUTANEOUS ENDOCAVAL SONOGRAPHY (PECS)

AKIMICHI KUME

1st Department of Surgery

Small-aperture endoluminal ultrasound imaging devices, comprising a 7.5- or 20-MHz transducer percutaneously placed inside the inferior vena caval lumen, were evaluated in the imaging of the retrohepatic vena caval wall both in normal dogs and clinical patients with hepato-biliary carcinoma. Using 20-MHz systems, a three-layered appearance of the retrohepatic vena caval wall was delineated both in dogs and humans although the imaging field was limited. For dogs, the depth of the complex inner two layers measured sonographically coincided with the distance from the luminal surface to the outer surface of the muscular layer as determined from histologic sections, whereas the total depth of the three layers measured sonographically revealed to be larger than the distance from the luminal surface to the surface of the hepatic parenchyma determined histologically. Endocaval sonography could also establish a negative diagnosis of carcinomatous infiltration to the retrohepatic vena caval wall in clinical patients. Our results suggest that percutaneous endocaval sonography (PECS) might be useful in diagnosing carcinomatous infiltration to the retrohepatic vena caval wall.

FUNDAMENTAL AND CLINICAL EVALUATION OF CHEST CT IMAGING IN DETECTABILITY OF PULMONARY NODULE

NICOLAS MILLA

Department of Radiology

Fundamental and clinical evaluation of chest CT imaging in detectability of pulmonary nodules was carried out in comparison with projection chest radiography including conventional screen- film system, advanced multiple beam equalization radiography (AMBER System), and computed radiography with imaging plate (CR). Detectability of simulated nodules in the fundamental study and metastatic pulmonary nodules in ten patients in the clinical study was analysed by five radiologists. CT found the largest number of nodules and also showed the highest sensitivity to detect smaller nodules less than 5 mm in both fundamental and clinical studies. Its ability of computed tomography to visualize small pulmonary nodules may be possible substitute for chest radiography as the primary mass screening method, and survey of small metastatic nodules (lesser than 5 mm) in patients with extrathoracic malignancy.

ANALYSIS OF KNEE MOVEMENT WITH LOW FIELD MR EQUIPMENT — NORMAL VOLUNTEER —

YOKO ANDO

Department of Radiology

This study was performed for the purpose of making the normal standard by analyzing the normal knee movement in detail. An open-typed low field unit was used for 23 healthy knee joints. With three-dimensional Fourier transform (3DFT) gradient echo sequence, 50 sagittal slices of 4.5 mm thickness were obtained at four flexion angles; 0, 30, 60 and 90 degrees, in the lateral position.

Although the tension ratio of the anterior and posterior cruciate ligaments (ACL, PCL) increased during knee flexion, the change of tension ratio was different between ACL and PCL significantly. Femur-ACL angle and femur-PCL angle were parallel with knee flexion angle, but tibia-ACL angle and tibia-PCL angle changed complexly. The lateral and medial condyles rolled and slid during knee flexion, and the medial side moved more than the lateral did consistent with the internal rotation of the lower thigh. The difference of the sliding distance between both condyles of female was significantly larger than that of male. This might explain the reasons of the female dominance of knee osteoarthritis.

Although the lateral position is not completely physiological, we could show the initial kinematic data of knee flexion up to 90 degrees using the open-typed MRI, which is impossible with high and middle field machines.

SERIAL PET STUDY IN NEWLY DIAGNOSED WEST SYNDROME

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Department of Pediatrics

We serially performed positron emission tomography (PET) with ^{18}F-FDG in 12 patients with newly diagnosed West syndrome. MRI showed morphological abnormalities in only 5 patients while PET revealed diffuse or focal cortical hypometabolism during the course in 11 patients. In 8 of the 11 patients, the cortical hypometabolism improved with disappearance of seizures. PET at the disease onset showed cortical hypometabolism in 8 patients; diffuse cortical hypometabolism in 3 and focal in the other 5. The second PET showed normal findings in 6 of the 8 patients. On the other hand, in 3 of the 4 patients showing normal findings at the disease onset, the second PET showed focal cortical hypometabolism. In all 7 patients showing normal findings at the second PET, tonic spasms disappeared after initial treatment, and no attacks occurred thereafter. However, in all 5 patients showing cortical hypometabolism at the second PET, tonic spasms persisted or recurred, or partial seizures appeared. All patients with normal MRI and second PET findings showed normal development at the latest follow-up. Our results suggest that diffuse or focal cortical hypometabolism, which can not be detected by MRI or CT,

are frequently present in patients with West syndrome. In addition, the cortical hypometabolism revealed by PET are not permanent, but change with clinical symptoms. These functional abnormalities of cerebral cortex may be involved in the development of the West syndrome.