ANNUAL RESEARCH MEETING

FOR

GRADUATE STUDENTS

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Abstracts

FOLLOW-UP STUDY ON HISTOGENESIS OF MICROCEPHALY ASSOCIATED WITH ECTOPIC GRAY MATTER INDUCED BY PRENATAL γ-IRRADIATION IN THE MOUSE

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Brain malformation with ectopic gray matter was visualized with magnetic resonance imaging in small-sized heads of prenatally exposed atomic-bomb survivors. The identical brain malformation was reproduced in mice and its histogenesis was studies in the present experiment. Pregnant mice were exposed to ^{60}Co γ-irradiation at a single does of 1.5 Gy on embryonic day 13 (E13), and then injected intraperitioneally with 30 mg/kg BrdU on E15. The extensive dead cells appeared throughout the brain mantle at 6 hrs after exposure. On E16 cell aggregations formed rosettes. On E18 a high proportion of BrdU-labeled cells reached the superficial layers of the cortical plate with the remaining cells located in the ectopic neuronal masses. The quantitative study showed that labeled-cells in layers II-III were fewer and those in layers IV-VI more numerous in the adult mice prenatally irradiated than controls. The anti-GFAP immunostaining revealed that the glial fibers in the irradiated mice were preserved, but disorganized. These findings suggested that the majority of migrating neurons were able to arrive at their normal layers, but some neurons remained due to the interrupted migratory pathway and eventually formed ectopic neuronal masses beneath the subcortical white matter.

BRAIN DISTRIBUTION CHARACTERISTICS OF XANTHINE DERIVATIVES AND RELATION TO THEIR LOCOMOTOR ACTIVITY IN MICE

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The relationship between the brain distribution and motor activity in mice of the xanthines, theophylline, enprofylline, 1-methyl-3-propylxanthine (MPX) and oxpentifylline was investigated. Their plasma protein binding and hydrophobicity were also examined. When these xanthines were administered orally, enprofylline and oxpentifylline had no effect on motor activity. While theophylline increased motor activity over 10 mg kg^{-1}, MPX caused a decrease in such activity over 10 mg kg^{-1}. The protein-binding behaviour varied among these xanthines and was closely related to their hydrophobicity, which is represented as a logarithimic partition coefficient (log PC). MPX had the highest hydrophobicity, while oxpentifylline had the lowest. Brain distribution characteristics varied among these xanthines, with the rank order of their brain penetration ratio, calculated as the ratio of brain to unbound plasma concentrations, being theophylline > oxpentifylline > MPX > enprofylline. The inhibition constants (K_i) for adenosine

A_1 receptors and cyclic 3′5′-adenosine monophosphate (cAMP)-phosphodiesterase (PDE) of these xanthines were 44.6 and 134, > 1000 and 112, 26.4 and 49, and > 1000 and 111 μM for theophylline, enprofylline, MPX and oxpentifylline, respectively. These findings suggest that the lack of effects of enprofylline, and oxpentifylline on motor activity is probably due to their low brain penetration ratio or low adenosine A_1 affinity in comparison with theophylline. The decrease in the motor activity by MPX may be, in part, mediated by cAMP or adenosine.

CENTRAL ADMINISTRATION OF A NITRIC OXIDE SYNTHASE INHIBITOR IMPAIRS SPATIAL MEMORY IN SPONTANEOUS HYPERTENSIVE RATS

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Nitric oxide is widely recognized as putative retrograde messenger in the brain. We infused N^G-monomethyl-L-arginine (L-NMMA 25 mg/kg), an inhibitor of nitric oxide synthase (NOS), continuously for a week into the dorsal third ventricle (D3V) of spontaneous hypertensive rats (SHR) by an osmotic infusion pump. Rats administered with L-NMMA showed impaired performance of a radial arm maze task compared with control rats administered with saline. We observed significant reductions of the NOx level in the cerebrospinal fluid (CSF) of the rats administered with L-NMMA, but not in the control rats. Both groups showed no change in systolic blood pressure or serum NOx level. The results provide evidence of a more specific effect of NOS inhibition to the brain independent of alterations in the systemic haemodynamics.

EFFECTS OF SUPRACHISMATIC LESIONS ON CIRCADIAN RHYTHMS OF BLOOD PRESSURE, HEART RATE AND LOCOMOTOR ACTIVITY IN THE RAT

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To determine whether the circadian rhythms in blood pressure (BP), heart rate (HR) and locomotor activity are controlled by an internal biological clock located in the suprachiasmatic nucleus (SCN), we continuously measured these parameters in SCN-lesioned rats using a newly developed implantable radiotelemetry device and a computerized data collecting system. Although SCN-lesioned rats showed a weak but significant 24-hr periodicity in BP and HR under light-dark (LD) cycles, they became completely aperiodic in BP, HR and locomotor activity under constant dark (DD) conditions. The amount of locomotor activity was significantly

reduced in SCN-lesioned rats when compared to intact rats. BP tended to be higher in SCN-lesioned rats, but the differences were significant only in the comparison of systolic blood pressure (SBP) under LD and DD ($p < 0.05$) and mean blood pressure (MBP) under LD ($p < 0.05$). HR of the SCN-lesioned rats was significantly lower under LD ($p < 0.05$) but not under DD. The standard deviation (SD) and the variation coefficient (VC) of MBP as an index of short-term variability of this parameter were significantly larger in SCN-lesioned rats than intact rats, but those of HR and locomotor activity did not differ significantly between SCN-lesioned and intact rats. These results indicated that the SCN is important not only for generating circadian rhythms of BP, HR and locomotor activity but also for buffering the short-term variability of BP in rats.

EFFECT OF METOCLOPRAMIDE ON MUSCLE SYMPATHETIC NERVE ACTIVITY IN HUMANS

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The aim of this study is to determine the effect of metoclopramide on the sympathetic nervous system. The subjects were nine healthy men, with mean age of 32 ± 3.4 years (28–37). We measured muscle sympathetic nerve activity (MSNA), together with heart rate and blood pressure before and after an intravenous injection of metoclopramid or saline. We also determined the plasma concentration of norepinephrine and vasopressin. Immediately after metoclopramide injection, short-term increases in MSNA and heart rate followed a transient decrease in blood pressure. This early increase in MSNA may result from the unloading of arterial baroreceptors. MSNA and heart rate again began to rise approximately 4 min after injection. Approximately 7 min after injection, there were second peaks of MSNA and heart rate, followed by an increase in blood pressure. This late increase in MSNA may result from central activation of the sympathetic nervous system, but not from the unloading of arterial baroreceptors. The plasma levels of norepinephrine and vasopressin were significantly elevated 15 min after metoclopramide injection and the plasma level of vasopressin was also significantly elevated 30 min after metoclopramide injection. These results suggest that metoclopramide activates MSNA depending on two different mechanisms.

ROLES OF CL^- CHANNELS AND CA^{2+} MOBLIZATION IN STRETCH-INDUCED INCREASE OF SA NODE PACEMAKER ACTIVITY

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Ionic mechanisms underlying the enhancement of cardiac pacemaking activity by mechanical stretch were investigated in the isolated rabbit sinoatrial (SA) node. A 5-sec stretch of 0.2—2.0 g was applied to small tissue strips (1.5 × 3.0 mm) or the SA node using a mechanical stimulator. Cycle length of spontaneous excitation (SPCL) was monitored by recording endocardial surface potential through modified bipolar electrodes with high-gain amplification. Influence of neurotransmitters released from nerve terminals was eliminated by atropine and propranolol. A stretch > 0.2 g caused a significant shortening of SPCL; there was a positive correlation between the force and the maximum shortening of SPCL. Treatment of the preparation with gadolinium (10 μM) or glibenclamide (1 μM) did not affect the force-response relationship. The positive chronotropic response to the mechanical stretch > 0.5 g was reduced significanlty by treatment with DNDS (5 mM), SITS (1 mM), or DIDS (50 μM). The positive chronotropic response was also reduced in a low Ca^{2+} (0.36 mM) medium, and by bath application of ryanodine (0.1 μM) or thapsigargin (2 μM). These findings suggest the possible involvement of mechanosensitive Cl^- channels and intracellular Ca^{2+} mobilization in the stretch-induced enhancement of pacemaking activity in the mammalian SA node, though other conceivable mechanisms cannot be ruled out.

EFFECT OF CARBACHOL ON IONIC CHANNEL ACTIVITIES IN ISOLATED BOVINE CILIARY MUSCLE CELLS, STUDIED BY PATCH-CLAMP METHOD

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In smooth muscle cells isolated from the bovine ciliary body, effects of a muscarinergic agonist, carbachol (CCh), on ionic channel activities were examined by the patch-clamp method.

Smooth muscle cells were dispersed from the ciliary body of bovine eyes which were obtained from a local slaughter-house. In the current-clamp experiments, pipettes were filled with the solution containing 100 mM-K^+ and the free Ca^{2+} concentration was adjusted at 70—700 nM ([EGTA]=5 mM, pH 7.0, at 30°C). In the voltage-clamp experiments, K^+ in this solution was replaced with equimolar Cs^+. In both cases the extracelluar side of the membrane was bathed with a normal saline solution containing 5.9 mM-K^+ and 2.4 mM-Ca^{2+}.

Under whole-cell configuration, membrane potential (E_m) was measured by clamping the

total current at 0 pA. The resting level of E_m was -50 ± 4mV (n=50). Extracellular application of 2 μM-CCh depolarized the cells to -15 ± 5 mV (n=10) with an apparent increase in membrance conductance. At the onset of this response, a fast repetitive fluctuation of E_m in the negative direction was often observed. Under voltage-clamp conditions (E_m=−40 mV), extracellular application of CCh (2 μM) evoked an inward current with an accompaniment of a marked increase in current noise, indicating opening of some cation channels. The reversal potential of this current was about 0 mV. Removal of either Na^+ or Ca^{2+} from the bathing solution attenuated but failed to abolish the current and voltage responses. The effects of CCh were reversibly inhibited by 4-Diphenylaminopiperidine (10 nM) whereas they were not significantly affected by pirenzepine or AF-DX116.

Voltage-clamp experiments under inside-out configuration revealed a very dense distribution (10^6–10^8 mm^{-2}) of typical Ca^{2+}-dependent K^+ channels (K_{Ca}) with a large unitary permeability ($3–5 \times 10^{-13}$ cm^3 s^{-1}) in the ciliary smooth muscle cells. When the pH of the intracellular side was 7.0, the concentration of Ca^{2+} required for half-maximal activation was 2.5 μM and the Hill coefficient for the Ca^{2+}-channel activity relationship approached 10. The activity of K_{Ca} gradually faded out when pipettes back-filled with 50 nM-iberiotoxin were used. Under cell-attach configuration extracellular application of CCh (1–10 μM) increased the open-state probability in a dose-dependent manner. This effect of CCh was inhibited either by extracellular application of 4-Diphenylaminopiperidine (10 nM) or by removal of extracellular Ca^{2+}, whereas it was little affected by verapamil (1 μM).

These results suggest that CCh activates non-selective cation channels, but not voltage-sensitive Ca^{2+} channels, by stimulating an M3-type cholinergic receptor in bovine ciliary muscle. In this smooth muscle tissue the non-selective cation channels may play an important role as a major pathway for Ca^{2+} influx from the extracellular space to initiate contraction.

TOXIC EFFECTS OF HEXANE DERIVATIVES ON CULTURED RAT SCHEWANN CELLS

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The cytotoxic effects of the following five hexane-related compounds were examined on the DNA synthesis in Schwann cell: 2,5-hexanedione (2,5-HD), 2-hexanol (2-OH), 2-hexanone (MnBK), 2,5-dimethylfuran (DF), and γ-valerolactone (VL). Schwann cells were isolated from the sciatic nerves of neonatal Sprague-Dawley rats and cultured. [^{3}H]-Thymidine incorporation into nuclei of Schwann cells was measured by scintillation spectrometry and autoradiography when hexane derivatives were added to the culture medium. All of the hexane-related compounds suppressed the [^{3}H]-thymidine incorporation in a concentration-dependent manner. DF was the most cytotoxic among the compounds. The finding suggests that DF-mediated cytotoxicity should be taken into account as a possible additional mechanism of hexane intoxication, especially in the impairment of mitotic cells.

TUMOR CYTOTOXICITY OF NITRIC OXIDE PRODUCED FROM ALVEOLAR MACROPHAGES, WHICH WERE DIRECTLY STIMULATED WITH TUMOR CELLS

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Macrophages activated by lipopolysaccharide or interferon-γ have been shown to be cytotoxic to tumor cells by releasing nitric oxide. Here, we report that unstimulated rat alveolar macrophages cultures with some kinds of tumor cells produce nitric oxide and are cytotoxic to these tumor cells. Alveolar macrophages were taken from BUF/Mna rats, which are known to produce spontaneous thymoma, and cultured with syngeneic BUF/Mna-derived thymoma cells. They were strongly killed by syngeneic or allogeneic alveolar macrophages and this killing was partially abolished by addition of ^{N}G-monomethyl-L-arginine. X-ray irradiated, mitomycin C-treated or membranous fragment of BUF/Mna-derived thymoma cells directly stimulated rat alveolar macrophages to produce nitric oxide.

POTENTIATION OF HEXANE NEUROTOXICITY AND CHANGES IN URINARY METABOLITES OF HEXANE BY METHYL ETHYL KETONE

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MEK (methyl ethyl ketone) is known to potentiate hexane neurotoxicity. A urinary metabolite of hexane, 2,5-hexanedione is a biomarker to predict the risk of neurotoxicity. In previous studies, however, co-exposure to hexane and MEK decreased urinary metabolites of hexane. We aimed to clarify the relationship between urinary metabolites of hexane and neurotoxicity in co-exposure to hexane and MEK. Thirty-two Wister male rats were divided into four groups of eight. Each group was separately exposed to 2,000 ppm hexane, 2,000 ppm hexane plus 200 ppm MEK, 2,000 ppm hexane plus 2,000 ppm MEK, or fresh air, for 12 hours a day for 20 weeks. The value of MCV (moter nerve conduction velocity) was significantly lower in the hexane plus 2,000 ppm MEK co-exposed group than that in the hexane-only group at 16 and 20 weeks. The amount of urinary 2,5-hexanedione and 2-hexanol were significantly greater in the hexane plus 2,000 ppm MEK co-exposed group than those in the hexane-only group from 4 to 16 weeks. Thus, co-exposure to hexane and MEK potentiates the neurotoxicity of hexane. Enhancement of the first oxidation of hexane might be responsible for the potentiation of hexane neurotoxicity.

CA^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE KINASE CASCADE

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Recently we obtained evidence for the existence of Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK), which was partially purified in the process of the purification of Ca^{2+}/calmodulin-dependent protein kinase V (CaMKV). This enzyme promoted phosphorylation of the autophosphorylation site on CaMKV and activated the activity. Present study revealed that CaMKK also phosphorylated Ca^{2+}/calmodulin-dependent protein kinase IV (CaMKIV) in association with the activation of its activity. Phosphorylation of CaMKIV by CaMKK occurred on multiple sites. Furthermore, CaMKIV and CaMKV phosphorylated each other, resulting in their activation. The phosphorylation site of CaMKV by CaMKIV was the same as the autophosphorylation site. We also found that the phosphorylated sites of CaMKV by CaMKK were the threonines of cycle 23 of the peptide 1 (DSKIMISDFGLSKMEDPGSVLSTAXGTPGYVAP) and cycle 18 of the peptide 2 (QIKKNFAKSKWKQAFNRTAVV). Our study suggests the existence of a CaM kinase cascade consisting of a sequential activation of each CaM kinase.

A NEW AND POTENT CALMODULIN ANTAGONIST, HF-2035, WHICH INHIBITS VASCULAR RELAXATION INDUCED BY NITRIC OXIDE SYNTHASE

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HF-2035, 2-[N-(2-aminoethyl)-N-(2,4,5-trichlorobenzenesulfonyl)] amino-N-(4-chlorocinnamyl)-N-methylbenzylamine, was synthesized and its effects on calmodulin-dependent enzymes were investigated. HF-2035 inhibited calmodulin kinase I, calmodulin kinase II and myosin light-chain kinase with IC_{50} of 1.3 μM, 1.6 μM and 68 μM, respectively. HF-2035 also inhibited the activity of recombinant rat neuronal nitric oxide synthase, one of the calmodulin-dependent enzymes, with a K_i of 0.78 μM. Partially purified nitric oxide synthase of rat brain was also inhibited by HF-2035 with an IC_{50} of 3.2 μM. Kinetic analysis indicated that this inhibitory effect of HF-2035 was competitive with respect to calmodulin. We examined the effects of HF-2035 on constitutive nitric oxide synthase in a bioassay using vascular strips of rabbit carotid artery with and without endothelium. HF-2035 inhibited acetylcholine- and calcium ionophore, A23187-induced relaxation of endothelium-intact strips with an ED_{50} of 1.5 ± 0.5 μM and 2.8 ± 1 μM, respectively. This compound, however, did not inhibit N-nitroso-N-morpholinoaminoacetonitrile (SIN-1A), an exogenous nitric oxide donor-induced relaxation of endothelium-denuded strips. W-7 (N-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide)

inhibited acetylcholine-induced relaxation with an ED_{50} of 46 ± 7 μM, which was 30-fold less potent than HF-2035, HF-2035 was unable to inhibit the activity of the inducible form of nitric oxide synthase in isolated thoracic aorta of rat treated with E. coli lipopolysaccharide. These findings suggest that HF-2035 is a new and potent calmodulin antagonist, and may be used as a mother compound to develop more selective inhibitors of constitutive nitric oxide synthase.

RELATIVE IMMUNOGENICITY OF HEPATITIS B VIRUS-ENCODED ANTIGENS AS TARGETS FOR CYTOTOXIC T-CELL RESPONSE

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To analyse the immunological mechanism of hepatocellular injury in hepatitis B virus (HBV) infection, the immunoreactivity of HBV-encoded antigens as a target for cytotoxic T lymphocyte (CTL) response was examined using recombinant vaccinia virus (RVV) expressing surface protein (S), precore/core protein (PC), and core protein (C) of HBV. C3H/He mice ($H\text{-}2^k$) were inoculated with each RVV. Their spleen cells were then harvested and stimulated in vitro with the histocompatible transfectant, which stably expressed hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), and hepatitis B core antigen (HBcAg), and used as effectors. As the targets, L cells ($H\text{-}2^k$) infected with individual RVV were used. Cytotoxic test was performed with various combinations and ratios of effectors and targets. The reactivity of PC-primed effectors against PC-expressing targets was greatest with 71.4% specific lysis on average at an effector/target ratio of 12.5: 1 among all the combinations. C-primed effectors against C-expressing targets also revealed rather high cytotoxicity (specific lysis, 40.6% at an E/T ratio of 12.5: 1). Furthermore, PC-primed and C-primed effectors showed a cross-reactivity to the targets expressing other nucleocapsid antigen, respectively. S-primed effectors showed less lytic activity against S-expressing targets (specific lysis, 18.4% at an E/T ratio of 12.5: 1). The CTL response were blocked by anti-CD8 and anti-major histocompatibility complex (MHC) class I antibodies, but not by anti-CD4 or anti-MHC class II. These findings suggest that endogenously synthesized nucleocapsid antigen, especially PC, is a dominant target for the MHC class I-restricted CTL in $H\text{-}2^k$ mice and that this system work as an efficient model to study immunopathogenesis of HBV infection.

A NOVEL Vβ 2-SPECIFIC ENDOGENOUS MOUSE MAMMARY TUMOR VIRUS WHICH IS CAPABLE OF PRODUCING A MILK-BORNE EXOGENOUS VIRUS

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We have previously reported a new *Mtv* loci, *Mtv-48* and *-51* in the Japanese laboratory mouse strains, CS and NC. Here we show by backcross analysis that both *Mtv-48* and *-51* cosegregate with very slow deletion of T cells bearing Vβ 2. The nucleotide sequences of the open reading frames in the 3′ long terminal repeats of *Mtv-48* and *-51* were very similar to those of *Mtv-DDO*, mouse mammary tumor virus C4 [MMTV (C4)], and MMTV (BALB/cV) which encode Vβ 2-specific superantigens. Furthermore, backcross female mice carrying *Mtv-48* but not *Mtv-51* were found to be able to produce a milk-borne MMTV (CS), which can vigorously stimulate Vβ 2-expressing T cells after local injection *in vivo* in an I-E-dependent manner. On the other hand, those carrying *Mtv-51* but not *Mtv-48* could not produce such a MMTV in milk. The nucleotide sequences of MMTV (CS) open reading frame were completely matched with those of *MTv-48*. These results indicate that the provirus *Mtv-48* but not *Mtv-51* is capable of producing a milk-borne virus of which superantigen stimulates Vβ 2-expressing T cells.

IL-15 IS A NOVEL GROWTH FACTOR FOR MURINE γδ T CELLS INDUCED BY *SALMONELLA* INFECTION

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We have previously shown evidence for the early recruitment of γδ T cells during the disease course of primary infections with *Listeria monocytogenes* or *Salmonella choleraesuis* in mice. Since γδ T cells at this stage of the disease do not produce IL-2, the growth factor for the γδ T cells remains unknown. IL-15 is a novel cytokine that uses β- and γ-chain of IL-2R for signal transduction, and is produced by activated monocytes/macrophages. In this study, we investigated the proliferative activity of IL-15 for γδ T cells appearing after primary infection with *S. choleraesuis* 31N-1. The γδ T cells, which expressed β- and γ-chains of IL-2R, proliferated in the presence of recombinant IL-15 and produced appreciable levels of γ-IFN and IL-4. Addition of anti-IL-2Rβ mAb significantly inhibited the IL-15-induced proliferation of the γδ T cells. Furthermore, the γδ T cells produced γ-IFN in response to monocytes/macrophage cell line, J774A.1 infected with *S. choleraesuis*, which expressed an abundant level of IL-15 mRNA. This cytokine production was inhibited significantly by anti-IL-15 antibody. Taken together, these results suggest that IL-15 derived from infected macrophages may contribute to the early activation of γδ T cells during salmonellosis.

A PROTECTIVE ROLE OF γδ T CELLS IN PRIMARY INFECTION WITH *LISTERIA MONOCYTOGENES* IN AUTOIMMUNE NON-OBESE DIABETIC (NOD) MICE

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We investigated the host defence mechanism in primary infection with *Listeria monocytogenes* in non-obese diabetic (NOD) mice at pre-diabetic stage showing an impaired responsiveness of the αβ T cells to T-cell receptor (TCR) triggering. The NOD mice showed a deteriorated resistance at the late stage after an intraperitoneal infection with *L. monocytogenes* compared with BALB/c and C57BL/6 mice as assessed by bacterial growth in organs. Consistent with our previous findings, a prominent increase in number of γδ T cells was evident at the early stage after infection, while generation of *Listeria*-specific αβ T cells was impaired in these mice. *In vivo* administration of anti-TCR-γδ monoclonal antibody (mAb) allowed *L. monocytogenes* to grow exaggeratedly in the NOD mice. These results imply that γδ T cells may be mainly involved in protection against primary infection with *L. monocytogenes* in NOD mice.

CLINICAL FEATURES OF ANTI-CHROMO ANTIBODIES ASSOCIATED WITH ANIT-CENTROMERE ANTIBODIES

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Anti-chromo antibodies (AChA) are autoantibodies accompanying anti-centromere antibodies (ACA). We determined the frequency and clinical significance of AChA in autoimmune rheumatic diseases. Serum samples from 252 Japanese patients with rheumatic diseases were examined by immunoblotting with HeLa nuclear extracts and recombinant N-terminus of 25-kD chromo protein (p25). AChA were detected from 28 (36%) of 77 sera with anticentromere antibodies (ACA). This prevalence was higher than in previous reports of 10–15%. AChA were found only in ACA-positive sera. Twenty-two (79%) of 28 recognized a recombinant N-terminal portion of p25 including the chromo domain which is conserved among species. Although the chromo domain is one of the major epitopes of AChA, the term "anti-chromo antibodies" might not be suitable for all AChA. AChA were related to leucopenia, thrombocytopenia, elevated erythrocyte sedimentation rate, and existence of Sjogren's syndrome (SS). In ACA-positive patients, AChA might be a serologic indicator of systemic sclerosis (SSc) having features of systemic lupus erythematosus and/or SS or diseases other than SSc.

KINETICS OF MONOCYTOID B-LYMPHOCYTES IN LOCALIZED INFLAMMATORY LESIONS INDUCED BY LIPOPOLYSACCHARIDE IN MICE

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We studies immunohistochemically kinetics of B-lymphocytes (B-lys) in primary inflammatory responses induced in localized regions of SMA mice by subcutaneous injections of lipopolysaccharide (LPS). In the inguinal regions, medium-sized B-lys formed early inflammatory lesions with neutrophils and activated macrophages on days 1 and 2. The B-lys were morphologically similar to monocytes, but were not stained with Mac1 antibody. It was a remarkable evidence that the B-lys consisted of unique populations and showed the phenotypes of B220+, IgM+, IgD (little to negative), Ly-1-, CD23- by double immunohistochemic staining. The B-lys were also positive for alkaline phosphatase (ALP). Consequently the B-lys in the lesions could be identified with monocytoid B-lys as well as marginal zone B-lys (MZBs). Plasma cells were also observed, but no lymphoid follicles developed during the primary inflammatory responses. In the inguinal lymphnodes (LNs), the same B-lys' responses were mainly induced in the paracortical lesions (T-cell areas). These findings suggested that the B-lys, which were induced by injections of LPS, evolved into plasma cells in the localized inflammatory lesions independent of GCs, and that they were responsible for first in vivo defense mechanisms.

SUPPRESSION OF CD44 EXPRESSION DECREASES MIGRATION AND INVASION OF HUMAN GLIOMA CELLS

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Recently we reported a high expression of CD44H in human glioma cells. To investigate the role of CD44H in the invasion of human glioma, we established a CD44-antisense gene expression glioma cells line named U-251A1. The expression of CD44H in the G-418-selected U-251A1 cells was reduced to 20% of that in the parental U-251SP cells determined by flow cytometry analysis. At first, we examined the migratory responses of U-251A1 cells *in vitro* by time-lapse video-microscopic sparse cell migration assay on hyaluronic acid or on chondroitin 6 sulfate. U-251A1 cells did not show significant differences in motility on any substrate, while U-251SP and other CD44H-positive glioma cells showed dose-dependent increase of migration specifically on hyaluronic acid. To examine the physiologic function of CD44H in gliomas *in vivo*, U-251A1 and its control cells, U-251S1, which retain CD44 sense expression vector were injected stereotactically into the brains of nude mice. U-251A1 cells were localized in the region of the injection site, with relatively well demarcated borders between tumour and brain

tissue, while the control cells demonstrated a cell-infiltration pattern. These data suggest that CD44H may be required for infiltration of glioma cells through its interaction with hyaluronic acid, a major component of the brain extracellular matrix.

CELL CYCLE-DEPENDENT LOCALIZATION OF TISSUE INHIBITOR OF METALLOPROTEINASES-1 IMMUNOREACTIVITY IN CULTURED HUMAN GINGIVAL FIBROBLASTS

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Localization of the tissue inhibitor of metalloproteinases-1 (TIMP-1) was investigated in cultured human gingival fibroblasts by immunohistochemistry and western-blotting. TIMP-1 immunostaining was observed in the cytoplasm of a majority of cells, and in the nucleus of some cells. Depletion of fetal calf serum (FCS) from the culture medium reduced the density of immunoreactive TIMP-1 in the nucleus more remarkably than in the cytoplasm, and the following FCS-induced cell growth was accompanied by a recovery of immunoreactive TIMP-1 in both the nucleus and the cytoplasm. The frequency of the nuclear TIMP-1-positive cells changed in line with that of PCNA-positive cells, and was always much higher than that of S-phase cells, which were estimated by a pulse labelled 5-Bromo-2′-deoxyuridine (BrdU). These results suggest a localization of TIMP-1 or a related substance in the nucleus of proliferating human fibroblasts, and its depletion from the nucleus due to an arrest of cell growth.

THE BASIC STUDY OF NORMAL CULTURED SALIVARY GLAND

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To establish a selected salivary gland cell culture and determine the effect of neuropeptides, we cultured monolyer cells using 3T3 cells as a feeder layer. To confirm the origin of these cultured cells, we examined amylase production, observed electron microscopically, and carried out periodic acid Schiff-staining as well as immunocytochemical analysis of myosin, anti-cytokeratin (CK-1, CK 10/13, CK MNF116, CK LMW, CK HMW and CK-19) and amylase antibody. The cultured cells demonstrated secretion granules containing amylase. Until passage two, the cultured salivary gland cells retained the features characteristic of acinar cells. By using a feeder layer in conjunction with a newly formulated culture medium, we improved the selectability of these cells. We also examined changes in proliferation of cultured salivary gland cells in the presence of selected neurotransmitters. Isoproterenol enhanced cellular proliferation. On the other hand, vasoactive intestinal polypeptide (VIP) and substance P (SP), which increase

salivary gland weight *in vivo*, showed no significant enhancement of proliferation.

BIGUANIDES MAY PRODUCE HYPOGLYCEMIC ACTION IN ISOLATED RAT HEPATOCYTES THROUGH THEIR EFFECTS ON NA^+/L-ALANINE TRANSPORT SYSTEM

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The mechanism of the effects of the biguanides, namely, metformin and buformin, on hepatic gluconeogenesis in hepatocytes isolated from normal fasted rats, was studied. Both 10 nM glucagon and 50 mM dibutyryl cyclic AMP increased [^{3}H]alanine uptake in isolated hepatocytes of normal rats by about 150% and 55%, respectively, compared with the effect of 5 mM alanine alone. 3mM metformin reduced glucagon-stimulated alanine uptake to the level seen with alanine alone, while 3 mM buformin inhibited glucagon-stimulated [^{3}H]alanine uptake by about 69%. The effects of biguanides on dibutyryl cyclic AMP-stimulated [^{3}H]alanine uptake were similar, but of similar magnitude as those observed in the presence of glucagon. 3 mM ouabain had a stronger inhibitory effect on [^{3}H]alanine uptake than the biguanides. However, 3 mM tolbutamide failed to suppress [^{3}H]alanine uptake in the presence or absence of glucagon or dibutyryl cyclic AMP. These results suggest that the inhibition of alanine uptake, related to a reduction in the Na^+-dependent L-alanine transport system A, is a possible mechanism of biguanide-related inhibition of hepatic gluconeogenesis.

AN EVALUATION OF THE BIOLOGICAL EFFECTS OF THREE DIFFERENT MODES OF MAGNETIC FIELDS ON CULTURED MAMMALIAN CELLS

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The biological effects of static magnetic fields, and their combined effects with ionizing radiation, were studied using a cultured mammalian cell line (FM3A), The three different modes of magnetic fields evaluated in this report were the 0.3 Tesla (T) field with a gradient of 0.3T/m, the 0.7T field with a gradient of 0.7T/m and the 6.34T field with no gradient. Exposure to the 0.3T and 0.7T fields had no effect on cell survival. Exposure to the 6.34T field decreased cell survival. Survival curves showing the combined effect of the 0.3T and 0.7T fields with radiation had a smaller mean lethal dose (D37) value. The survival curve of the 6.34T field was influenced by the interval between magnetic exposure and ionizing irradiation. When the interval

was 6 or 12 h, the survival curve showing the combined effect of the 6.34T field had smaller D37 and quasithreshold dose (Dq) values, indicating the potentiation of the radiation effect. Flow cytometric analysis indicated that exposure to the 0.3T and 0.7T fields showed no change, whereas the 6.34T field showed an increase in the percentage of G1 phase cells. Our conclusions were as follows: 1) magnetic fields decreased the colony-forming abilities of cultured mammalian cells; 2) magnetic fields can affect the cell cycle; 3) a stronger magnetic field strength does not always have stronger biological effects, and 4) the gradient of a magnetic field may be an important factor when combined with ionizing radiation. Despite the foregoing analysis, the biological effects of magnetic fields on mammalian cells remain a complex phenomena.

THE ASSOCIATION OF PHYSICAL ACTIVITY LEVEL CHARACTERISTICS AND OTHER LIFE STYLES WITH OBESITY AMONG NAGOYA UNIVERSITY ALUMNI, JAPAN

TERUAKI FUJII

1st Division of Health Promotion Science

It is thought that life style is a useful modification for obesity. Students of Nagoya University, who were diagnosed as either obese or not obese by an obesity check-up at that university from 1974 to 1978, were followed after their graduation for an average period of 18 years, to analyze the relationship between life styles and post-graduation weight changes. The subjects were divided into three groups: Group A (51 individuals obese both during school and at present). Group B (22 individuals obese during school but not at present) and Group C (67 individuals not obese both during school and at present). (1) Group B often took care of eating and maintained a moderate eating pattern. The time spent eating also tended to be longer in Group B than in Group A or Group C. (2) The percentage of subjects whose physical activity level in daily life after graduation was moderate (level II) or higher was significantly higher in Group B than in Group A and C. (3) The percentage of nonsmokers in Group B was significantly higher than in Group A or C. These results suggest that life style modification is useful for reduction of obesity.

LIFE-STYLE DETERMINANTS FOR SOCIAL ACTIVITY LEVELS AMONG THE JAPANESE ELDERLY

RIE AOKI

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We conducted a self-administered questionnaire survey of a total of 5239 elderly persons in four areas in Japan in 1993, covering past life-styles and present social activities. Based on the survey data, we first developed social activity measures, and then examined associations of the present total social activity measure with past life-styles and physical conditions. The life-styles significantly associated with high social activity after 65 years of age were "high educational attainment," having been "healthy," "plump," "physically active," and "having had hobbies" at about 50 years of age; and having "frequent intake of many kinds of foods" from 30–50 years of age. Intake during 30–50 years of age of Japanese-style foods (rice, soybean paste soup, bean curd, pickles), noodles, beans, plant roots, and potatoes was not significantly linked with the social activity levels at old age in either males or females. The same was true for smoking and drinking habits at about 50 years of age. Our findings essentially suggest the importance of a positive attitude at middle age to maintain and promote health status and improve life-styles in order to attain a high level of social activity in old age.

SCANNING ELECTRON MICROSCOPIC STUDY OF INNER EAR BAROTRAUMA: IN THE GUINEA PIG UNDER HYPOBARIC PRESSURE

TSUTOMU TANABE

Department of Otorhinolaryngology

In order to investigate the mechanism of inner ear barotrauma, guinea pigs, with bilateral eustachian tube occlusion, were subjected to decompression and compression between 760 and 460 mmHg in a hypobaric pressure chamber. We divided the guinea pigs into two groups of A and B. Group A showed normal eustachian tubes, and group B showed bilateral occlude eustachian tubes. Group B were arranged into three types of B_1, B_2 and B_3, according to the rates of compression and decompression.

After pressure loading, morphological changes in the hair cells of the organ of Corti were studied by means of a scanning electron microscope.

There was no damage to hair cells in the setting of normal eustachian tube function group of A. On the other hand, mild to severe hair cell damage was observed in the rapid decompression groups. This consequence suggests that relative positive pressure in the middle ear cavity is an important factor in inner ear barotrauma.

The mechanism of hair cell damage due to inner ear barotrauma is presumed to be distortion

of the organ of Corti caused by a difference in pressure between perilymph and endolymph resulting in an injury to the stereocilica.

ELECTROPHYSIOLOGICAL STUDY OF GUINEA PIG COCHLEA AFTER THE EXPOSURE TO LOW PRESSURE ENVIRONMENT

YASUHIKO YAMAMOTO

Department of Otorhinolaryngology

It is well known that high/low pressure environment may cause severe to mild sensorineural hearing loss (aural barotrauma). In our department it has been reported that after the exposure to high pressure environment mainly the stereocilias of OHCs and IHCs are damaged. We examined CAP (compound action potential), CM (cochlear microphonics) and TEOAE (transiently evoked otoacoustic emission) of low pressure exposed guinea pigs cochleas, and after the measurement several cochleas were examined by SEM in order to examine the morphological studies so far. The elevation of CAP thresholds were observed in 19 cochleas out of 25 cochleas, which also shows the elevation of CM threshold, CAP and CM amplitudes reduction and TEOAE threshold elevation. From SEM study there were some damaged cochleas which showed disordered electrophysiological results, and the others morphologically normal cochleas which showed similar electrophysiological disorders. These results may indicate that low pressure barotrauma may cause not only OHCs damage but also afferrent nerve endings etc.

EFFECT OF INCREASED MIDDLE EAR PRESSURE ON BLOOD FLOW TO THE MIDDLE EAR, INNER EAR AND FACIAL NERVE IN GUINEA PIGS

HIROYUKI NAGAI

Department of Otorhinolaryngology

Aural barotrauma is caused by the difference between the pressure in the middle ear and that in the external or inner ear. In barotrauma of the middle ear, vascular engorgement, bleeding, and formation of exudate in the middle ear space are occasionally seen. Accordingly, impairment of blood flow in the ear is considered associated with aural barotrauma. We investigated the effect of increased middle ear pressure on blood flow to the inner ear, middle ear, and facial nerve in guinea pigs using a nonradioactive microsphere technique. The elevation of middle ear pressure significantly reduced blood flow to the middle ear. Blood flow to the facial nerve also decreased due to the elevation of the middle ear pressure but the reduction was not significant. Blood flow to the inner ear did not change even after the middle ear pressure was increased.

From these findings, we conclude that blood flow to the middle ear is lowered but blood flow to the inner ear is maintained during increased middle ear pressure. Blood flow to the facial nerve can decrease if increased middle ear pressure is transmitted through dehiscence of the facial canal.

ONSET OF ESOTROPIA AND MOTION PERCEPTION ASYMMETRY

SACHIKO UNO

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In early infancy, horizontal smooth pursuit is asymmetric and optpkinetic nystagmus (OKN) is evoked by a target that moves nasally during monocular viewing. By the 4—5 month of age the persuit becomes symmetric in normal infants, but it still remaines asymmetric in those who have infantile esotropia and adults with a history of infantile esotropia. The motion perception asymmetry (MPA) in order infants and adults suggests a delay in binocular development specific to visual motion pathway. We studied the correlation between MPA and the onset of infantile esotropia in 63 patients (1—18 yo) with a well documented history of the onset. Our patients were classified into 4 groups by the onset of esotropia; Group A: before the 6th month, Group B: from the 6th to 12th month, Group C: from the 12th to the 24 month, Group D: after 24th month. Using the Reversing Grating Test designed by Ai-Hou Wang et al., the motion perception was tested as follows. First, the true OKN stripes (sinusoidal grating) of 1 cycle per degree (cpd) and a velocity of 15 degrees per second (d/s) was presented on a CRT monitor of personal IBM/2 for observing true nystagmus. Secondly, reversing gratings of 1cpd at 15 Hz with a constant speed of apparent drift at 15 d/s were shown on the monitor, and the occlusion was switched from one eye to the other. Normal subjects perceived flicker-in-situ whereas motion-asymmetric patients perceived naselward drifting; temporalward beating was also induced. If the temporalward beating nystagmus was observed and changed direction immediately after switching the occlusion, the patients was judged to be MPA (+). The data were evaluated using the Mann-Whitney test, and we found a statistically significant correlation between the detection rate of MPA and the onset of esotropia. Our results suggest that detection of MPA can be a useful tool for estimating the onset of infantile esotropia.

EUSTACHIAN TUBE FUNCTION AND MIDDLE EAR BAROTRAUMA ASSOCIATED WITH EXTREMES IN ATMOSPHERIC PRESSURE

TAKASHI MIYAZAWA

Department of Otorhinolaryngology

Eustachian tube (ET) function was studied using sonotubometry and tubo-tympano-aerodynamography (TTAG) prior to and following exposure to hypobaric or hyperbaric conditions. Forty normal adults were subjected to hypobaric pressure. Fifty adults who underwent hyperbaric oxygen (HBO) therapy also were studied. Following hypobaric exposure, 14 of 80 ears (17.5%) exhibited middle ear barotrauma. Following hyperbaric exposure, 34 of 100 ears (34%) exhibited middle ear barotrauma. ET dysfunction, characterized by altered active and passive opening capacity, was more prevalent following exposure to extremes in atmospheric pressure compared to baseline. ET function, which was impaired after the first HBO treatment, improved gradually over the next 2 hours. Overall, however, ET function was worse after the 7th treatment. The patients who developed barotrauma exhibited worse ET function prior to hypobaric or hyperbaric exposure. Thus abnormal ET function can be used to predict middle ear barotrauma prior to exposure to hypobaric or hyperbaric atmospheric pressure.

THE EFFECT OF AGE ON SHORT WAVELENGTH SENSITIVE CONE ELECTRORETINOGRAM (S-CONE ERG) AND LONG AND MIDDLE WAVELENGTH SENSITIVE (LM) CONE ERG

SATOSHI SUZUKI

Department of Ophthalmology

Short wavelength sensitive cone electroretinogram (S-cone ERG) and long and middle wavelength sensitive (LM) cone ERG can be recorded with LED built-in contact lens electrode (Horiguchi et al., IOVS 1995). Age dependency of rod ERG and LM-cone ERG has been reported by several authors, but it was difficult to study the effect of age on S-cone ERG because of yellowing in human crystalline lens. Therefore, we recorded S-cone and LM-cone ERG from 34 pseudophakic subjects without fundus abnormality (25—91y.o.) and performed linear regression analysis against age on those responses. Significant age dependency was found for S-cone ERG b-wave amplitude, LM-cone ERG a-wave and d-wave amplitude, LM-cone ERG b-wave inplicit time and for LM-cone ERG b/a ratio. No significant age correlation was found for S-cone ERG b-wave implicit time, LM-cone ERG a-wave and d-wave implicit time and for LM-cone ERG b-wave amplitude. Our results indicate that there is significant effect of age on S-cone and LM-cone systems in human retina.

EVENT-RELATED POTENTIALS AND SKIN SYMPATHETIC NERVE ACTIVITY DURING ODDBALL PARADIGMS

HIROKI ITO

Department of Neurology

Skin sympathetic nerve activity (SSNA) from the tibial nerve and event-related brain potentials (ERPs) were recorded simultaneously during auditory oddball paradigms with a counting task on 10 healthy subjects to elucidate the relationships between the autonomic nervous system and the cognitive process. After the target tones, SSNA bursts were observed more frequently than after the non-target tones. However, when subjects ignored the series of tones, there was no significant difference between the incidence of SSNA bursts after rare tones and frequent tones. The P300 latencies for the target trials with SSNA bursts were shorter than those for the target trials without SSNA bursts. The averaged ERP wave forms for the target trials with SSNA bursts showed larger positive deflection in the early part of the P300 component than those for the target trials without SSNA bursts. We conclude that SSNA is generated in relation to the conscious cognitive process, as well as to the automatic process activated by chages in repeating stimuli. The early part of the P300 component, possibly P3a, may be related to the mechanisms that generate SSNA.

CHANGES IN COCHLEAR FUNCTION AFTER DOUBLE-MEMBRANE RUPTURE IN THE GUINEA PIG

MICHIKO SAITOH

Department of Otorhinolaryngology

We measured the transiently evoked otoacoustic emissions (TEOAEs), compound action potential (CAP) and cochlear microphonic (CM) in guinea pigs after rupture of only the round window membrane (n=5) or rupture of both Reissner's membrane and the round window membrane (n=10). We determined the time course of changes in the total echo power (TEP) in TEOAEs and the minimal detectable levels of CAP and CM. Endocochlear potential (EP) was measured in ruptured Reissner's membrane. There were no changes in TEOAEs, CAP or CM in animals in which only the round window membrane was ruptured. The minimal detectable levels of CAP and CM were increased in all animals in which TEOAEs were absent after rupture of the double membranes. Our results suggest that double-membrane rupture produces acute sensorineural hearing loss. Hearing loss appears to be related mainly to damage to hair cells, and to some degree, to damage to the cochlear nerve, which appeared to be induced by the influx of potassium-rich endolymph into the perilymph and by morphological damage of the scala media.

EFFECT OF PROSTAGLANDIN $F_{2\alpha}$ ON CA^{2+} INFLUX IN OSTEOBLAST-LIKE CELLS: FUNCTION OF TYROSINE KINASE

ATSUSIH SUZUKI

1st Department of Internal Medicine

We investigated the mechanism of prostaglandin $F_{2\alpha}$($PGH_{2\alpha}$)-induced Ca^{2+} influx in MC3T3-E1 cells. $PGF_{2\alpha}$-induced formation of total inositol phosphates (IPs) was markedly reduced by the depletion of extracellualr Ca^{2+} with EGTA. On the other hand, the depletion of extracellular Ca^{2+} had little effect on $PGF_{2\alpha}$-induced inositol 1,4,5-trisphosphate formation. $PGF_{2\alpha}$ stimulated $^{45}Ca^{2+}$ influx dose-dependently, attaining a maximum effect at 10 nM. Does of $PGF_{2\alpha}$ above 10 nM caused less than maximal stimulation. Genistein, an inhibitor of protein tyrosine kinase, which by itself had little effect on $^{45}Ca^{2+}$ influx, significantly suppressed the $PGF_{2\alpha}$-induced $^{45}Ca^{2+}$ influx in a dose-dependent manner in the range between 1 μg/ml and 0.1 mg/ml. Sodium orthovanadate, an inhibitor of protein tyrosine phosphatases, enhanced the $PGF_{2\alpha}$-induced $^{45}Ca^{2+}$ infux. Genistein also suppressed the $PGF_{2\alpha}$-induced total IPs formation dose-dependently in the range between 1 μg/ml and 0.1 mg/ml. However, it had little effect on the $PGF_{2\alpha}$-induced inositol 1,4,5-trisphosphate formation. The pretreatment with pertussis toxin, which has been reported to suppress $PGF_{2\alpha}$-induced IPs formation in MC3T3-E1 cells, had little effect on the $PGF_{2\alpha}$-induced $^{45}Ca^{2+}$ influx. These results strongly suggest that $PGF_{2\alpha}$ stimulates Ca^{2+} mobilization from extracellular space and phosphoinositide hydrolysis via independent pathyways in osteoblast-like cells, and the $PGF_{2\alpha}$-induced Ca^{2+} influx is regulated by protein tryosine kinase, resulting in the promotion of phosphoinositide hydrolysis.

EXCITATION AND SENSITIZATION OF THE HEAT RESPONSE INDUCED BY A PHORBOL ESTER IN CANINE VISCERAL POLYMODAL RECEPTORS STUDIED IN VITRO

SI-HONG LENG

1st Division Regulation of Organ Function

Bradykinin and histamine excite polymodal receptors and facilitate their heat response through the B_2 and H_1 receptors, respectively, which are known to induce activation of protein kinase C in cultured cells. In order to clarify the possible involvement of protein kinase C activation in polymodal receptor activities, the effects of phorbol 12,13-dibutyrate (PDBs) on the heat response of testicular polymodal receptors were studied in vitro using canine testis-spermatic nerve preparations. Different concentrations of PDBu were applied for 5 min, and effects were evaluated by the use of an inhibitor of protein kinases, staurosporine. Heat stimulation was carried out by applying prewarmed Krebs solution at 41—48°C for 30 s. PDBu (10^{-7}, 10^{-6}, and 10^{-5} M) evoked a significant increase in the on-going activities of the polymodal receptors

within 15 min. PDBu ($10^{-8} - 10^{-5}$ M) significantly augmented the subsequent heat responses of the polymodal receptors. Staurosporine (10^{-6} M) was applied for 13 min before PDBu and during 5 min application of PDBu (10^{-7} M), attenuated the sensitizing effect of PDBu on the subsequent heat response. These results suggest that activation of protein kinase C contributes to the activities of polymodal receptors.

MONOCLONAL ANTIBODY AGAINST THE FUSION JUNCTION OF DELETION-MUTANT EPIDERMAL GROWTH FACTOR RECEPTOR

SHO OKAMOTO

Department of Neurosurgery

A mouse monoclonal antibody (IgG2b), 3C10, was produced against the truncated epidermal growth factor receptor (EGFR), encoded by the (type III) in-frame deletion mutation of 801 nucleotides of *EGFR* affecting the external domain, known to be expressed in some human glioblastoma. Since this mutation newly generates a glycine residue at the fusion point, a 14-amino acid peptide around the fusion junction including this glycine was chemically synthesized, and used for immunization of (B6 × DBA/2) F1 mice. Flow cytometric analysis showed 3C10 antibody staining of a mouse NIH/3T3 transfectant (ERM5) with the type III *EGFR* deletion-mutant gene, but not one with wild type *EGFR*. The antibody immunoprecipitated the truncated EGFR protein with a molecular mass of approximately 140 kDa from ERM5 cells. Immunostaining of glioblastomas with the antibody was positive in the cases with the type III *EGFR* mutation, the five other specimens without the mutation being negative despite overexpression of *EGFR* in some cases.

EVIDENCE OF REDOX-LINKED SIGNALING FOR PRODUCING A GIANT SIGNAL COMPLEX

YOSHIAKI KATANO

2nd Department of Internal Medicine

Previously we showed that a thiol-reactive heavy metal, HgCl2, crosslinked multiple cell surface receptors through a ligand independent pathway, which produced massive aggregates of phosphotyrosine (PTYR)-containing proteins beneath the plasma membrane (Nakashima et al., 1994). In this study we characterized these unique aggregates at the molecular level. The lysates from Brij 96 of thymocytes treated with HgCl2 were separated into the supernatant and pellet fractions by simple centrifugation. Selected PTYR-containing proteins and $p56^{lck}$ appeared in the pellet fraction as quickly as 5 sec after exposure to HgCl2, and were further increased in amount by 5 min. Although the mechanism of triggering these events was redox-linked, the

majority of proteins in the Brij 96-insoluble aggregates were dissociated in SDS-PAGE under non-reducing condition. This suggested that PTYR-containing proteins and $p56^{lck}$ themselves do not form dimer or polymer directly by thiol-mediated bond. The pellet fraction was further found to include some other signal delivery elements such as GTPase activating protein, phosphatidylinositol 3 kinase, and mitogen-activated protein kinase. Finally, all of these signal elements and selected PTYR-containing proteins were collected in the same fraction by the sucrose density gradient centrifugation. These results suggest a unique redox-linked pathway of formation of a giant signal complex.

ELUCIDATION OF THE PROTEIN KINASE C-DEPENDENT APOPTOSIS PATHWAY IN DISTINCT SUBSETS OF T LYMPHOCYTES IN MRL-*lpr/lpr* MICE

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Department of Immunology

MRL-*lpr* mice are severely impaired in the Fas pathway of apoptosis induction. We here evaluate another pathway of apoptosis induction in MRL-*lpr* mice which is protein kinase C (PKC) dependent. Despite the defect of the Fas pathway, apoptosis developed during culture *in vitro* in splenic T lymphocytes from MRL-*lpr* mice more extensively than in T lymphocytes from MRL-+/+. Apoptosis induction in the former cells was then found to be greatly promoted by PKC inhibitor H-7, and partially prevented by PKC activator phorbol 12-myristate 13-acetate (PMA). High sensitivity to H-7, but not to PKA inhibitor HA1004 of these cells for apoptosis induction was confirmed by detailed time course and dose-dependency experiments of the drug effect. Population analysis showed that both $CD4^+$T lymphocytes and $CD8^+$T lymphocytes from MRL-*lpr* mice were highly sensitive to H-7, whereas $CD8^+$T lymphocytes but not $CD4^+$T lymphocytes, from MRL-+/+ mice were susceptible to the reagent. Interestingly, $B220^+Thy\text{-}1^+CD4^-CD8^-$T lymphocytes from MRL-*lpr* mice were most sensitive to H-7 for apoptosis induction. Correspondingly, the membrane-translocated activated PKC-α level in splenic T lymphocytes from MRL-*lpr* was more extensively up-regulated by PMA than that in splenic T lymphocytes from MRL-+/+. These results suggest that some signal consistently activated PKC in MRL-*lpr* T lymphocytes, and this event is needed for survival of these lymphocytes. On the other hand, $CD4^+CD8^+$thymocytes were deleted by apoptosis in culture with PMA, whether these thymocytes were from MRL-*lpr* mice of MRL-+/+ mice. This finding suggested that the apoptosis induction pathway linked to PKC activation is intact in $CD4^+CD8^+$thymocytes from the Fas-defective MRL-*lpr*. We concluded from these results that the PKC-dependent signal pathways for either cell death or cell activation are intact or even accelerated in *lpr* mice, which could both compensate for the loss of the Fas pathway and promote the generation of autoreactive T lymphocytes.

INTERACTION BETWEEN HUMAN INTESTINAL FIBROBLASTS AND MUCOSAL T-CELLS IN INFLAMMATORY BOWEL DISEASE

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The abnormality of mucosal immune system may contribute to the pathogenesis of inflammatory bowel disease. Recently mesenchymal cells have been reported to be actively interacted with immune cells. Thus we investigated whether the interaction between human intestinal fibroblasts (HIF) and mucosal T-cells might be different among Crohn's disease (CD), ulcerative colitis (UC), and normal control.

T-cell lines were established from mucosal biopsies. Apoptosis of T-cells was assessed by fluorescence staining with acridine orange plus ethidium bromide, and DNA fragmentation gel analysis. HIF were grown from gut mucosal explants. The surface markers of T-cells and HIF were examined using FACS analysis. T-cells were co-cultured with HIF, and adhesion, proliferation, and interaction assay were performed.

CD mucosal T-cells grew more and remained significantly more viable and less apoptotic than control and UC cells. HIF expressed HLA-DR, ICAM-1, and LFA-3 and UC HIF displayed an activated phenotype. Adhesion assay showed that large numbers of T-cells were retained by UC than control or CD HIF. This enhanced adherence was consistent with the results of differential ICAM-1 expression on HIF. T-cells cultured with HIF displayed a remarkably lower degree of apoptosis. Electron microscopy demonstrated that HIF had ingested apoptotic T-cells.

In summary, CD mucosal T-cells display a unique response to IL-2 exposure and deprivation, which might explain the excessive T-cell activation in this condition. Considering expression of adhesion molecules and adhesiveness to T-cells by HIF and their preventive function on T-cell apoptosis, HIF may contribute to maintenance of T-cell memory in the mucosal immunity.

COCAINE-INDUCED CREB PHOSPHORYLATION AND C-FOS EXPRESSION ARE SUPPRESSED IN PARKINSONISM MODEL MICE

TAKAHISA KANO

Department of Neurosurgery

Cocaine exerts multiple neurochemical effects in the central nervous system through inhibition of the dopamine transporter at the synapse. Here we report that systemic administration of the drug induces rapid phosphorylation of CREB in the mouse striatum where expression of the nuclear proto-oncogene c-fos is observed. In MPTP treated mice whose dopaminergic neurons are degenerated showing Parkinsonism-like behavior, however, CREB phosphorylation is not induced by cocaine exposure and c-fos expression is significantly depressed in comparison with the control mouse case. These data suggest that CREB may play a major role in the dopaminer-

gic activation of c-fos in the striatum and that the lack of a CREB-induced transcription cascade may have a critical relevance for long-lasting psychomotor disorders in Parkinsonism.

A COMPLETE cDNA SEQUENCE FOR CORE I PROTEIN SUBUNIT OF HUMAN UBIQUINOL-CYTOCHROME *C* REDUCTASE

MOHAMMED MONIRUL ISLAM

2nd Department of Biochemistry

Ubiquinol-cytochrome *c* reductase (cytochrome bc_1 complex) is the central part of mitochondrial respiratory chain catalyzing the transfer of electrons from ubiquinol to cytochrome *c* with concomitant proton translocation across the mitochondrial inner membrane. Cytochrome bc_1 complex is a multisubunit complex containing 10—11 subunits in mammals. Our group has so far isolated and analyzed the human cDNA or genomic DNA clones for cytochrome c_1, ubiquinone-binding protein, Rieske iron-sulfur protein, and core protein II to elucidate the molecular basis for the electron transport and energy transduction in human mitochondrial bc_1 complex. As the continuation of our project with human cytochrome bc_1 complex, I have isolated a complete cDNA clone for human core I protein from human fibroblast cDNA library by colony hybridization technique. The probe was prepared by a PCR from a bovine cDNA library using a pair of primers which were designed from the reported bovine cDNA. Isolated human core I protein cDNA was found to consist of 1575 nucleotides. Nucleotide sequence comparison showed that the human core I protein cDNA is 85% homologous with the reported bovine counterpart but with a large difference in the length of coding region which codes for 480 amino acids in human and 362 amino acids in bovine. Human core I protein is presumed to contain a presequence of 34 amino acids. Amino acid sequence alignment showed that the predicted human core I protein has a significant homology with other members of the matrix processing peptidase (MPP) and processing enhancing protein (PEP) family.

PROTEIN C NAGOYA, AN ELONGATED MUTANT OF PROTEIN C, IS RETAINED WITHIN THE ENDOPLASMIC RETICULUM AND IS ASSOCIATED WITH GRP78 AND GRP94

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1st Department of Internal Medicine

Protein C Nagoya, an elongated variant of the human protein C, is retained and degraded within the cells in which it is produced (Yamamoto K *et al.*, J Clin Invest 90: 2439, 1992). To determine the subcellular localization of the protein C Nagoya, the recombinant protein C

bearing this mutation was expressed in Chinese hamster ovary (CHO) cells. The mutant protein C was not secreted from the cells, and remained susceptible to endo-β-*N*-acetylglucosaminidase H (endo H). Immunoelectron microscopy indicated that protein C Nagoya was retained in the ER, whereas wild type protein C was observed in both the ER and the Golgi apparatus. Metabolic radiolabeling with [^{35}S] methionine in combination with chemical cross-linking revealed that the protein C Nagoya existed in the ER as an complex with 78-kDa glucose-regulated protein (GRP78) and 94-kDa glucose-regulated protein (GRP94). Because both GRP78 and GRP94 associate far lesser degree with wild type protein C than with protein C Nagoya, our data suggest that both stress proteins function as molecular chaperones and work in concert with the folding and assembly of protein C. These findings extend our understanding of the molecular pathogenesis of protein C deficiency.

DIFFERENT EXPRESSION OF SYNDECAN-1, 3, 4 IN RAT EMBRYONIC DEVELOPMENT

TORU NAKANISHI

Department of Obstetrics and Gynecology

Syndecan family is one of transmembrane proteoglycans that possess highly conserved cytoplasmic and transmembrane domains and may function as extracellular matrix receptors and/or low affinity receptors for signaling molecules. To examine an interaction between syndecan family and midkine (MK) that is a heparin-binding growth/differentiation factor, we studied the expression of syndecan family during rat embrionic development. In Northern blot analyses of heads of rat embryo from 10 to 16 day and 2-day-old neonate, expression of syndecan-1 and MK decreased as the embrionic development proceeds, whereas the expression of syndecan-3 and heparin-binding growth-associated molecule (HB-GAM) increased until 16 day and then decreased at neonate. Expression of syndecan-4 increased throughout fetal growth and neonate. Then we produced anti-syndecan-1 fusion protein (EP) antibody and anti-syndecan-3 antibody immuno-purifed from polyclonal rabbit antisera generated against bacterial EP. In immunohistochemistry using these antibodies, syndecan-1 were observed in developing central nervous systems of 10—12 day-embryo although not in 14—16 day-embryo. Syndecan-3 were observed in central and peripheral nervous systems of 10—16 day-embryo. These results indicate syndecan-1 may function in the 10—12 day-embrionic development of central nervous systems, and may interact with MK.