ANNUAL RESEARCH MEETING

FOR

GRADUATE STUDENTS

Nagoya, December 1998

Abstracts

LONG-TERM USE-DEPENDENT ENHANCEMENT OF IMPULSE-INDUCED EXOCYTOSIS BY ADRENALINE AT FROG MOTOR NERVE TERMINALS

SHU-MING HUANG

1st Department of Physiology

It has been known that catecholamines increase transmitter release from presynaptic nerve terminals. Previous studies suggested that noradrenaline acts on a process of exocytosis after an increase in intracellular Ca^{2+} ($[Ca^{2+}]_i$).

In frog motor nerve terminals I have further studied the mechanism underlying facilitatory actions of adrenaline by recording postsynaptic potentials intracellularly, a $[Ca^{2+}]_i$ in the terminals with Ca^{2+}-sensitive probes and the extent and rate of staining and de-staining of synaptic vesicles with FM1-43. Adrenaline produced the long-term enhancement of end-plate potentials (Epps) in a low Ca^{2+}, high Mg^{2+} solution with a decrease in the coefficient of variation of Epp amplitude. In a normal Ringer solution containing d-tubocurarine adrenaline also augmented Epps in a use-dependent manner. There were, however, no change in the amplitude and frequency of miniature end-plate potentials and impulse(s)-induced rises in $[Ca^{2+}]_i$. Adrenaline increased the extent of staining of synaptic vesicles with FM1-43 after tetanic stimuli and the rate of de-staining during tetanic stimuli. The results suggest that adrenaline enhances transmitter release by increasing the readily releasable pool of synaptic vesicles in frog motor nerve terminals.

SPATIOTEMPORAL PROPERTIES OF ACTIVITY PROPAGATION FROM THE SUBICULAR COMPLEX TO THE POSTERIOR CINGULATE CORTEX IN RAT BRAIN SLICES DETECTED BY THE OPTICAL RECORDING TECHNIQUE

JIAN WANG

2nd Department of Physiology

I investigated the functional neural circuits of the subicular-to-posterior cingulate cortex using an optical recording technique. Brain slices were prepared by cutting the brain in an oblique coronal plane so as to include the both areas, and were stained with the voltage-sensitive dye, RH-482. When the subicular complex was stimulated, an excitation wave propagated along the superficial layers of the posterior cingulate cortex. This propagation was clearly divided into three steps. The first step was a fast conduction process that might arise from propagation of action potentials along nerve fibers in the subiculum. The second one was a slow process, that the excitation wave propagated from the superficial layers to deep layers and back to the superficial layers around the subiculum. During this process a gradual, but a significant increase

in the amplidue of the excitation wave was observed along the pathway. The third step was a relatively slow propagating process along the superficial layers of posterior cingulate cortex where the enhanced excitation wave propagates in a long distance, sometimes to the visual cortex. Pharmacological treatments suggested that the signal enchancement in the second step seen in the subicular complex adjoining the posterior cingulate cortex was mediated by glutaminergic neurons and that the magnitude of enhanced signal was controlled by GABAergic inhibitory neurons.

MOLECULAR CLONING AND CHARACTERIZATION OF AN *N*-ACETYLGLUCOSAMINE-6-*O*-SULFOTRANSFERASE INVOLVED IN THE BIOSYNTHESIS OF 6-SULFO SIALYL LEWIS X

KENJI UCHIMURA

1st Department of Biochemistry

We isolated a cDNA clone encoding mouse *N*-acetylglucosamine-6-*O*-sulfotransferase based on sequence homology to previously cloned mouse chondoroitin 6-sulfotransferase. The cDNA clone contains an open reading frame that predicts a type II transmembrane protein composed of 483 amino acid residues. The expressed enzyme transferred sulfate to the 6 position of non-reducing GlcNAc in GlcNAcβ1-3Galβ1-4GlcNAc. Galβ1-4GlcNAcβ1-3Galβ1-4GlcNAc and various glycosaminoglycans did not serve as acceptors. Co-transfection of the enzyme cDNA and fucosyltransferase VII in COS-7 cells resulted in cell surface expression of 6-sulfo sialyl Lewis X. The sulfotransferase mRNA was strongly expressed in the cerebrum, cerebellum, eye, pancreas and lung of adult mice. *In situ* hybridization revealed that the mRNA was localized in high endothelial venules of mesenteric lymph nodes. It was strongly suggested that the sulfotransferase is involved in biosynthesis of an L-selectin ligand.

APPROPRIATELY SPACED NUCLEAR LOCALIZING SIGNALS ARE NECESSARY FOR NUCLEAR IMPORT OF NON-NUCLEAR PROTEINS

HARM-JAN BORGELD

2nd Department of Biochemistry

In order to deliver non-nuclear protein into the nucleus, we have examined the locations and number of nuclear localizing signals by use of simian virus 40 large T-antigen (SV40Ta) and yeast enchanced green fluorescent protein (yEGFP) in *Saccharomyces cerevisiae*. When only one SV40Ta was added to either the N- or C-terminus of yEGFP, the fluorescence of yEGFP was detected in both the nucleus and the cytoplasm. When two SV40Ta signals were added,

(SV40Ta-yEGFP-SV40Ta), the fluorescence of yEGFP was localized in only the nucleus. When the presequence of cytochrome oxidase subunit IV (pCOXIV) was inserted (SV40Ta-pCOXIV-yEGFP-SV40Ta), the fluorescence was located in both the nucleus and the cytoplasm, suggesting that the increased distance between the two SV40Ta signals decreased the efficiency of transport into the nucleus. When an additional SV40Ta signal was inserted (SV40Ta-pCOXIV-SV40Ta-yEGFP-SV40Ta), the fluorescence of yEGFP was re-localized within the nucleus. Even when the SV40Ta at the C-terminus was deleted (SV40Ta-pCOXIV-SV40Ta-yEGFP), the fluorescence was localized only in the nucleus. These results indicate that two SV40Ta signals spaced appropriately are essential for the efficient transport of non-nuclear proteins into the nucleus.

HMN-1180, A SMALL MOLECULE INHIBITOR OF NEURONAL NITRIC OXIDE SYNTHASE

MASAHIRO NISHIO

Department of Pharmacology

A newly synthesized isoquinolinesulfonamide, HMN-1180 (1-(5-isoquinolinyl-sulfonyl)-7-methylhomopiperazine), was shown to have selective inhibitory action against rat neuronal nitric oxide synthase (nNOS) with a Ki value of 5.4 μM. Kinetic analysis indicated that the inhibition was competitive with respect to L-arginine but not to calmodulin (CaM). However HMN-1180 exhibited no significant influence up to a concentration of 1 mM on activity of endothelial NOS (eNOS) and it was less active toward inducible NOS (iNOS) (IC50>100 μM). Moreover, nNOS bound to a HMN-1180-coupled Sepharose column, but eNOS snd iNOS did not. These results suggest that inhibition of nNOS activity is due to direct binding of the compound to the L-arginine binding site of the synthase. Several HMN-1180 derivatives were synthesized and analyzed for their inhibitory actions against nNOS, eNOS, and iNOS to cast light on its structure-activity relationships. The potency of inhibition proved dependent on the position of methyl group in the homopiperazine molecule. HMN-1180 was also found to inhibit glutamate stimulated NO production generated by nNOS in a human neuroblastoma cell line SK-N-MC, thus indicating that it is a useful tool for elucidating the physiological role of nNOS in neuronal function.

POINTING ARM-MOVEMENTS DURING Z-AXIS LINEAR ACCELERATION, AND ASSOCIATED ANTICIPATORY EYE MOVEMENTS

YANI CHEN

Research Institute of Environmental Medicine

Target-pointing arm movements with and without a small LED target in darkness were measured goniometrically around the shoulder joint, and accompanying eye movements with conventional EOG recording during a step-mode linear acceleration up to 0.5 G. No significant deviation was observed in pointing to a memorized target as long as the direction of resultant G-force vectors ranged from 27 degrees to true vertical. A slight but consistent shift in vertical eye movement was observed during G-loading in some subjects. When the LED target was visible, applications of wave than 0.2 G in the positive Gz direction skewed downward the pointing direction by several degrees. Anticipatory transient eye movements which were connected closely with pointing action were newly discovered. Based on the above finding that target pointing without visual feedback in a supine position was not affected by Gz-loading, we concluded that rich somatosensory inputs from the subject's back might have suppressed the otolith inputs for spatial orientation.

DIVERSE PROFILE OF T CELL SURFACE ATTACHMENT OF HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 NEF PROTEINS FROM PATIENTS

KAORI OTAKE

Department of Bacteriology

Human immunodeficiency virus (HIV) type 1 Nef plays an important role in down-regulation of CD4 and alteration of T cell activation as well as enhancement of virus infection through interactions with host cell factors. However, a consensus of *in vitro* Nef-functions linked to the cytopathogenicity of AIDS has not formed. As another possible function *in vitro*, the cell-free Nef protein of the HIV-1 laboratory strain has been shown to attach to the surface of T lymphocytes. To investigate characteristics of Nef derived from patients, we examined the ability of Nef attachment with flow cytometry. Six purified Nefs from patients showed attachment to the cell surface of Molt-4 T cells dose-dependently. Nefs from rapid progressors significantly required for one C-terminal epitope (position of amino acid 170-181) to attach to the T cell surface with more frequency than other Nefs from intermediate and slow progressors. Nef-attachment occurred at 4°C or room temperature. On the contrary, all patient Nefs showed internalization into T cells upon incubation at 37°C. Data show that these biological activities of Nef may be dependent not only on target cell type but also on the particular allele of *nef* being expressed in the infected individual.

MICROGLIAL NO INDUCES DELAYED NEURONAL DEATH FOLLOWING ACUTE INJURY IN THE STRIATUM

AKIHIDE TAKEUCHI

Department of Immunology

We established a novel CNS injury model by a stereotaxic ethanol injection into rat striatum to induce necrosis. With this model, we clarify a function of iNOS in a healing mechanism around the injury. A RT-PCR revealed the iNOS mRNA arose at 6h, peaked at 24h, and declined to a lower level at 48h after the injury. From in situ hybridization, this iNOS was expressed in the area surrounding the injury. By immunohistochemistry, mononuclear cells at this surrounding area were stained with anti-iNOS antibody on the 1st day. These cells turned out to be reactive microglia with positive staining of GSA-I-B4, ED-1 and OX-42. Hematoxylin-eosin staining showed that neurons in this boundary area gradually disappeared by 5 days with an increment of microglia. Nuclei of neurons in this area were stained positive with TUNEL assay. These TUNEL-positive neurons gradually disappeared toward the 3rd day while microglia increased. L-NAME, a competitive NOS inhibitor, administration diminished the elimination of neurons in this boundary area. Microglial NO may act as a neurotoxic agent to eliminate damaged neurons near the necrosis in the form of delayed neuronal death, and may reintegrate the neuronal circuits with functionally intact neurons.

CHARACTERIZATION OF THE PRODUCT OF THE UL55 GENE OF HERPES SIMPLEX VIRUS TYPE 2

HIROSHI YAMADA

Department of Virology

The herpes simplex virus type 2 (HSV-2) UL55 gene product was identified using a rabbit polyclonal antiserum raised against a recombinant 6xHis-UL55 fusion protein expressed in *Escherichia coli*. The antiserum reacted specifically with a 23-kDa protein in HSV-2 186-infected cell lysates. The protein was not detectable in the presence of the viral DNA synthesis inhibitor, phosphonoacetic acid. Indirect immunofluorescence studies localized the UL55 protein within and at the periphery of the nucleus as discrete granules at late times postinfection, and nuclear fractionation studies showed that the protein was associated with the nuclear matrix of infected cells. Moreover, these discrete regions containing the UL55 protein were found to be adjacent to compartments, designated assemblons, containing the capsid protein ICP35. However, the UL55 protein was not detected in purified virions. These results suggest that the UL55 protein of HSV-2 may play an accessory role in virion assembly or maturation.

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELING OF METABOLIC INTERACTIONS BETWEEN N-HEXANE AND TOLUENE IN HUMANS

XIAOZHONG YU

Department of Occupational and Environmental Health

In order to evaluate the effect of dose-dependent metabolic interaction between toluene and n-hexane, especially in occupationally relevant exposure conditions, a physiologically based pharmacokinetic (PBPK) model for co-exposure to n-hexane and toluene was developed. According to the model, 8 hours of constant exposure to 50 ppm n-hexane and 25, 50, 100 and 500 ppm toluene will cause about 7%, 18%, 62% and 96% decreases in the urinary excretion of 2,5-hexanedione (2,5-HD) and 4%, 10%, 25% and 30% increases in the n-hexane concentration in blood at the end of the fifth day of exposure. Simulations of co-exposure to 50 ppm n-hexane and 50 ppm toluene in a standard man who inhaled 50 ppm n-hexane with 0 or 50 ppm toluene for 8 hours at different work loads suggest that toluene causes a slight decrease in urinary 2,5-HD in the resting condition, a 17% decrease at 25 W, and a 41% decrease at 50 W work load. The simulations of co-exposure in different exposure patterns with the same time-weighted concentration of 50 ppm, i.e. 50 ppm for 8 hours, 100 ppm of 4 times for 1 hour and 200 ppm of twice for 1 hour, showed reductions in urinary 2,5-HD of 17%, 40% and 67%, respectively. These simulations suggest that co-exposure to n-hexane and toluene around 50 ppm (TWA) could affect urinary n-hexane metabolites to various degrees depending on the fluctuations in exposure concentrations and variety of work activities in the workplace.

THE EFFECT OF DAILY PHYSICAL ACTIVITY ON FAT DISTRIBUTION

TAEKO KAJIOKA

First Division of Health Promotion Science

To investigate the association between daily physical activity and fat distribution, we used the number of steps walked per day (s/d) as an index of physical activity. Ultrasonography has been employed to measure fat distribution. Subjects were 77 females, aged 31 to 72 years. They were divided into four groups by their average number of steps walked per day (Group A $<$ 7500, 7500 $\leq$ Group B $<$ 10000, 10000 $\leq$ Group C $<$ 12500, 12500 $\leq$ Group D). Abdominal fat distribution was assessed by Abdominal wall Fat Index (AFI). The minimum thickness of subcutaneous fat (Smin) and the maximum thickness of preperitoneal fat (Pmax) at subxiphoid process were measured. AFI was calculated as Pmax divided by Smin. Fat distribution was analyzed by ANCOVA, controlled for age, BMI, percent body fat and menopausal status. Energy and macro nutrient intake were obtained through nutritional survey. There were no significant differences in anthropometric variables, energy and macro nutrient intake, and subcutaneous fat thickness in trunk and limbs. Pmax of Group A was significantly higher than

Group D. Furthermore, Group A showed significantly higher AFI than the other three groups (B,C,D). The results of this study suggested that women who walked less than 7500 s/d tend to have significantly increased intra-abdominal far accumulation. In order to prevent intra-abdominal fat accumulation, it is suggested that women should walk a minimum of 7500 s/d.

RAT C-PEPTIDE I AND II STIMULATE GLUCOSE UTILIZATION IN STZ-INDUCED DIABETIC RATS

LING LI

First Division of Health Promotion Science

The effects of physiological concentrations of rat C-peptide I and II on whole body glucose utilization were studied in streptozotocin (STZ) diabetic and healthy rats. A sequential insulin clamp procedure was employed (insulin infusion rates 3.0 and 30.0 $mU \cdot kg^{-1} \cdot min^{-1}$) in awake animals. Blood glucose was clamped at 7.7 ± 0.3 mmol/l in the diabetic rats and at 3.9 ± 0.1 mmol/l in the healthy rats. In diabetic rats infused with C-peptide (0.05 $nmol \cdot kg^{-1} \cdot min^{-1}$) glucose utilization increased by 79–90% ($p<0.001$) compared to saline-infused diabetic animals. Raising the rate of C-peptide infusion 10 fold did not significantly increase glucose utilization further. C-peptide I and II elicited similar effects. The metabolic clearance rate for glucose in the C-peptide infused diabetic animals was not different from that in the healthy rats. During high-dose insulin infusion (30.0 $mU \cdot kg^{-1} \cdot min^{-1}$) glucose utilization increased markedly and no significant C-peptide effects were observed. About 85% of the C-peptide-induced rise in glucose utilization could be blocked by L-monomethylarginine (L-NMMA) administration. It is concluded that 1) physiological concentrations of homologous C-peptide stimulate whole body glucose utilization in the diabetic rats but not the healthy rats, 2) C-peptide I and II elicit similar effects and 3) the effect of C-peptide on glucose utilization may be nitric oxide (NO)-mediated.

TRUNCATED c-Myb EXPRESSION IN A HUMAN LEUKEMIA CELL LINE' TK-6

AKIHIRO TOMITA

1st Department of Internal Medicine

The c-*MYB* proto-oncogene encodes a transcription factor which plays an important role in hematopoiesis. I demonstrated that the expression of truncated c-*MYB* mRNA (2.0 kb) and c-Myb protein (55 kDa) in TK-6 cell line, which was established from an patient with chronic myelogenous leukemia in T cell blast crisis. Mutated c-*MYB* cDNA clone (WTK-1) was isolated from a TK-6 cell cDNA library and sequenced. The sequence of WTK-1 diverged from c-*MYB* at the 3' ends of exons 9. A termination codon was present as the second codon down-

stream from the point of divergence. The conceptual protein encoded by WTK-1 (Myb^{TK-6}) comprises 402 amino acids and lacks the negative regulatory domain of wild-type c-Myb. Luciferase reporter assay in NIH3T3 cells showed that the expression vector encoding Myb^{TK-6} stimulated Myb-regulated *mim-1* promotor more effectively than that encoding wild-type c-Myb, suggesting that Myb^{TK-6} is functional as a transcription factor. Southern blot and mutant allele specific polymerase chain reaction analyses showed that the same rearrangement of the c-*MYB* gene in TK-6 was present in late specimens obtained from the patient. These data suggest that the C-terminally truncated Myb protein expression may contribute to the patient's disease progression.

SUPPRESSION OF NEOINTIMA FORMATION IN MIDKINE-DEFICIENT MICE IN A RESTENOSIS MODEL

MITSURU HORIBA

1st Department of Internal Medicine

Growth factors are involved in the pathogenesis of atherosclerosis and restenosis after balloon angioplasty. Midkine (MK), a heparin binding-growth factor, was induced in neointima of the carotid arteries in both rat and mouse models. Neointima formation was almost completely suppressed in MK-deficient mice. Moreover, continuous administration of MK protein to MK-deficient mice resumed neointima formation. CD45-positive leukocyte recruitment to the vascular wall markedly decreased in MK-deficient mice. These results indicate that MK is a novel growth factor crucial for neointima formation.

COMPARISON OF CONTRACTIONS PRODUCED BY CARBACHOL, THAPSIGARGIN AND CYCLOPIAZONIC ACID IN THE GUINEA-PIG TRACHEAL MUSCLE

MASAOKI TAKEMOTO

2nd Department of Internal Medicine

Thapsigarin (TPG, 3 μM) and cyclopiazonic acid (CPA, 10 μM) slowly increased muscle tone in the guinea-pig tracheal muscle. A large sustained contraction was produced by a 5 min treatment with Ca^{2+}-free solution. This contraction was partially inhibited by nifedipine (3 μM) and highly dependent on external Ca^{2+}. The TPG- and CPA-induced contractions were 75% and 67% of the carbachol (Cch, 1 μM)-induced contraction in the presence of nifedipine. The contractions produced by Cch, TPG, CPA were all inhibited by isoprenaline (ISO) and sodium nitroprusside (SNP). In the presence of nifedipine, the IC_{50} of ISO was 11, 17, 23 nM and that

of SNP was 0.5, 1, 0.8 μM for Cch-, TPG-, CPA-induced contractions, respectively. The contraction produced by 60 mMK^+ was only weakly inhibited by ISO and SNP. These contractions were also similarly inhibited by SKF96365 (100 μM) and cadmium (Cd^{2+}, 100 μM). It was concluded that TPG and CPA increased Ca^{2+} influx probably *via* a mechanism activated by Ca^{2+} depletion of the sarcoplasmic reticulum. The susceptibility of the contractions produced by TPG, CPA and Cch to inhibition by ISO, SNP, SKF96365 and Cd^{2+} suggests that these contractions share a common pathway for increasing intracellular Ca^{2+}, and that a different Ca^{2+} pathway plays a dominant role in the high-K^+-induced contraction.

PROSTACYCLIN SYNTHASE GENE TRANSFER ACCELERATES REENDOTHELIALIZATION AND INHIBITS NEOINTIMAL FORMATION IN RAT CAROTID ARTERIES AFTER BALLOON INJURY

YASUSHI NUMAGUCHI

2nd Department of Internal Medicine

The protective barrier function of endothelial cells is disrupted after angioplasty. Loss of this function may induce neointimal formation and result in restenosis. Prostacyclin (PGI_2) has vasculoprotective effects of vasodilation, anti-platelet aggregation and inhibition of smooth muscle cell proliferation. To test the hypothesis that overexpression of endogenous PGI_2 may accelerate the recovery from endothelial damage and inhibit neointimal formation in the injured artery, we investigated in vivo transfer of PGI_2 synthase (PCS) gene into rat balloon injured carotid arteries by the liposome-mediated transfection. At 7 days after transfection, the recovery of endothelium was observed in arteries transferred PCS gene (pPCS) but little in those of the control lacZ gene (placZ). Moreover, at 14 days. PCS gene transfer resulted in significant inhibition of neointimal formation (Intima/Media areas ratio; 0.16 ± 0.05 and 1.31 ± 0.13 for pPCS and placZ, respectively, $p < 0.001$) without affecting medial area. Arterial segments transferred pPCS produced higher level of 6-keto-$PGF_{1\alpha}$, the main metabolite of PGI_2, than those of placZ. In conclusion, in vivo PCS gene transfer accelerated reendothelialization, restored PGI_2 production, and markedly inhibited neointimal formation in rat carotid arteries after balloon injury. PCS may be a promising candidate for gene therapy against restenosis after angioplasty.

DECREASED EXPRESSION OF SHPS-1 CORRELATES WITH TRANSFORMING ACTIVITY OF v-*src* IN RAT 3Y1 CELLS

KAZUYA MACHIDA

2nd Department of Internal Medicine

SHPS-1 is a transmembrane glycoprotein possessing three immunoglobulin-like domains in its putative extracellular region as well as four potential tyrosine phosphorylation sites in the cytoplasmic region. It is indicated that SHPS-1 may be a direct substrate for activated protein tyrosine kinases and non-transmembrane protein tyrosine phosphatases to regulate some physiological and pathological signals. In the process of characterizing the function of SHPS-1, we found that the expression of SHPS-1 was down regulated by activated tyrosine kinases such as v-Src in rat fibroblast. From the result of Northern blot and RT-PCR analysis, SHPS-1 mRNA levels are specifically suppressed in the transformed cells, suggesting that SHPS-1 expression was transcriptionally down regulated. However, the protein half-life of SHPS-1 was not changed in the transformed cells compared to that in the non-transformed counterpart cells as it was examined by using a temperature mutant of v-Src. The expression of SHPS-1 was transcriptionally silenced in the cells transformed with different type of oncogenes such as v-fps, v-crk, and H-ras, suggesting that a functional oncogenic cascade in malignant tumors can override the biological consequences of the expressed SHPS-1.

ANALYSIS OF ALIMENTARY FUNCTION AND STRUCTURE BY FAST MRI

YASUNAGA SEKI

2nd Department of Internal Medicine

Advances in fast magnetic resonance imaging (MRI) techniques enable us to visualize dynamic movements of the gastrointestinal tract and its fine structure. ^{1}H imaging of the esophagus was carried out using a 4.7 T magnetic resonance spectrometer (Biospec, Bruker) with a bird-cage RF-coil. Median sagittal images were taken by a fast gradient-echo imaging (snapshot) at 3 images/sec in conscious rabbits. A rapid movement of the balloon inflated with 0.6 ml of 0.3% ferric ammonium citrate by the peristaltic movement of the esophagus was clearly visualized. The primary peristalsis that followed the voluntary act of swallowing was also visualized. Peristaltic movement visualized by MRI was in good agreement with the pressure wave changes measured by the simultaneous manometry. Under light anesthesia without artificial ventilation edematous changes of the rat pancreas and ascites formation 24 h after the induction of trypsin-taurocholate pancreatitis were clearly visualized by the snapshot technique. Development of the pancreatic pseudocyst following pancreatitis was also observed in the same animal for over 30 days. Fast MRI is a promising technique for dynamic analysis of the motor function of the gastrointestinal tract and structural changes after pancreatitis.

c-RAS AND THE FOCAL ADHESION KINASE (FAK) ARE REQUIRED FOR THE ACTIVATION OF MAP KINASE BY HYALURONAN IN 3Y1 CELLS

MIHAELA SERBULEA

3rd Department of Internal Medicine

Hyaluronan (HA) is a non-sulfated high molecular mass glycosaminoglycan, a component of the extracellular matrix which appears to be implicated in a wide array of cellular functions including homeostasis of water and plasma proteins in the intracellular matrix, cell migration and proliferation. Evidence has been accumulated indicating that HA plays an important role in tumor invasion and metastasis. Despite its importance, however, the intracellular signaling pathway critical for the pathophysiological functions triggered by HA remains largely unknown. We found that HA treatment of 3Y1 cells substantially increased tyrosine phosphorylation of cellular proteins and activated MAP kinase in a time-and dose-dependent manner. This HA activity was resistant to boiling at 100°C for 10 minutes but completely abolished by treatment with hyaluronidase, suggesting that HA itself and not any HA-associated proteins has the effect. Subsequently we examined the effect of HA on cell growth and observed it could stimulate cell growth in the presence of serum. We transfected the S17N *ras* mutated gene, ligated into the expression vector pMAM2-BSD which has an MMTV promotor, into 3Y1 cells. After selecting Blasticidin-resistant colonies we induced dominant negative *ras* expression by treatment with Dexamethason and found that HA-dependent activation of MAP kinase was strongly suppressed by the expression of dominant negative *ras* (S17N *ras*). Moreover we investigated the effect of FAK on MAPK activation by HA. Oligonucleotides directed against the portion of the FAK gene encoding aminoacids 1-8 were electroporated into cells using 150V, 1,2 F current. By immunoblotting with anti-Phospho-MAPK antibody, we observed that antisense FAK strongly blocked MAPK. These results suggest that Ras-MAP kinase pathway is activated by HA and that FAK is required, at least in part, for the activation of MAPK by HA.

EFFECT OF HIGH GLUCOSE AND AN ALDOSE REDUCTASE INHIBITOR, EPALRESTAT, ON PDGF-INDUCED PROLIFERATION OF CULTURED RAT AORTIC SMOOTH MUSCLE CELLS

YASUHIDE KASUYA

3rd Department of Internal Medicine

Platelet-derived growth factor (PDGF) has been proposed to play an important role in the hyperglycemia-induced proliferation of smooth muscle cells (SMCs), a characteristic feature of diabetic macroangiopathy. Polyol pathway hyperactivity has been investigated as the pathogenesis of diabetic microangiopathy, but not as that of macroangiopathy. This study was conducted

to examine the role of PDGF and the polyol pathway in the growth activity of SMCs. [^{3}H]-Thymidine incorporation, [^{125}I]-PDGF-BB binding and expression of PDGF-β receptor protein were measured in rat aortic SMCs cultured with 5.5 or 20 mM glucose with or without anti-PDGF antibody or an aldose reductase inhibitor, epalrestat, for 4 weeks. SMCs cultured with 20 mM glucose demonstrated an accelerated [^{3}H]-thymidine incorporation compared with that with 5.5 mM glucose, which was prevented by anti-PDGF antibody. This acceleration of growth activity by 20 mM glucose was accompanied by an increase in [^{125}I]-PDGF-BB binding, which was due to the increased number of PDGF-β receptors and the overexpression of PDGF-β receptor protein. Epalrestat significantly prevented all these abnormalities. These observations suggest that polyol pathway hyperactivity plays an important role in the proliferation of SMCs and the further development of diabetic macroangiopathy, which may be mediated through the accelerated expression of PDGF-β receptor protein.

THE ROLE OF POLYMORPHONUCLEAR LEUKOCYTE INFILTRATION IN HERPES SIMPLEX VIRUS INFECTION OF MURINE SKIN

DAISUKE WATANABE

Department of Dermatology

We undertook the present study to investigate the role of polymorphonuclear leukocytes (PMN) in defending skin against a herpes simplex virus (HSV) infection. For this purpose, we established a mouse model of cutaneous HSV infection. The hind limb footpad skin of 4-week-old ICR mice was abraded linearly once with a feather edge file and infected with various strains of HSV with different virulence. In uninfected control mice, PMN appeared at the abraded skin lesion within 24 h, and were eliminated from the epidermis after 3 days. Mice infected with a highly virulent strain of HSV demonstrated wide and severe erythematous lesions of footpad skin and histologically, virus antigen-positive ballooning degenerated keratinocytes were observed. However, in infections with attenuated strains of HSV, the epidermis was regenerated and a viral antigen was discharged within 5 days, together with any infiltrated PMN. Macrophages and NK cells numbered less than PMN. In mice treated with anti-PMN antiserum before HSV infection, PMN infiltration was significantly suppressed 1 day after infection, and these animals developed a severe cutaneous disease even if infected with an attenuated virus. These results indicate the importance of PMN in the control of HSV cutaneous infections, especially in the primary infectious phase.

THE EFFECTS OF STATIC MAGNETIC FIELDS AND X-RAYS ON INSTABILITY OF MICROSATELLITE REPETITIVE SEQUENCES

TAKAHITO OKUDA

Department of Radiology

To determine the genetic effect of static magnetic fields (SMF), which are not supposed to produce any significant DNA damage, we took advantage of DNA mismatch repair (MMR) deficient cells, in which all the errors produced during DNA replication are left uncorrected. We first established a simple and less labor-intensive method to analyze genetic changes in microsatellite repetitive sequences in the MMR-deficient cells. After exposure to a strong SMF (6.34T) for 24h, both MMR deficient HCT116 cells and proficient HeLa S3 cells did not exhibit any significant effect on microsatellite changes. Moreover, when HCT116 cells were synchronized at the G1/S boundary by aphidicolin and exposed to SMF during the whole S-phase, no increase in microsatellite changes was either observed. In contrast, irradiation by a low dose X-ray (2Gy) significantly increased microsatellite changes in HCT116 cells. This suggested that exposure to strong SMF may not induce any significant level of genetic changes in microsatellite sequences.

EXTRASTRIATAL MEAN REGIONAL UPTAKE OF FLUORINE-18-FDOPA IN THE NORMAL AGED BRAIN – AN APPROACH USING MRI-AIDED SPATIAL NORMALIZATION

ATSUKO NAGANO

Department of Radiology

Studies of 6-[^{18}F]fluoro-L dopa (FDOPA) with positron emission tomography (PET), provide a method for assessing *in vivo* the integrity of presynaptic dopaminergic terminal function, but the accuracy of the anatomical localization through the entire brain in an FDOPA PET image is limited due to the low geometrical resolution of the FDOPA PET image and the uneven distribution of FDOPA uptake.

In this study, we created spatially-normalized images of FDOPA uptake, with the aid of individual, co-registered MR images, using the normalization program in SPM95. The examination of the accuracy of this procedure in 11 normal aged subjects, and 24 subjects with a movement disorder, revealed the accuracy was superior to that using only FDOPA images. After evaluating the accuracy of MRI-aided spatial normalization of the FDOPA Ki image, automatic region of interest (ROI) analysis of the 11 normal subjects was performed, to define normal FDOPA uptake in the striatal and extrastriatal regions. Automatic ROI analysis of the spatially-normalized FDOPA Ki images of the normal subjects, showed high Ki values in the

midbrain regions, amygdala, hippocampus, and medial prefrontal cortex, in addition to the caudate nucleus, and putamen. These regions correspond to the dopaminergic projections in the brain.

PERFORMANCE OF DIGITAL FLUOROSCOPIC IMAGE CAPTURE IN SELECTIVE FALLOPIAN TUBE CATHETERIZATION; LABORATORY AND CLINICAL EVALUATION

EIKO MURASE

Department of Radiology

PURPOSE: To reduce patient radiation dose from digital radiography during selective fallopian tube catheterization, diagnostic performance of fluoroscopic last-image hold was evaluated.

MATERIALS AND METHODS: The phantom studies were performed with polyethylene tubes of 16-G, 18-G, 20-G, 22-G, and 24-G. Tubes with/without stenosis were randomly selected and were recorded by digital radiography and fluoroscopic last-image hold. Five radiologists interpreted these images regarding the presence or absence of stenosis, and the results were analyzed with the receiver operating characteristic (ROC) method. Observation studies were performed in 26 tubes regarding the tubal visualization as well as the presence or absence of steno-occlusive disease. These results were analyzed using a two-way analysis of variance for non-repeated measures.

RESULTS: The phantom studies indicated that, for tubes greater than 22-G (inner diameter, 0.57 mm), no statistically significant difference was present between digital radiography and fluoroscopic last-image hold unlike for 24-G tubes (inner diameter, 0.45 mm). In clinical cases, fluoroscopic last-image hold offered significantly inferior tubal visualization and diagnostic performance.

CONCLUSION: Although diagnostic capability in detecting steno-occlusive lesions by fluoroscopic last-image is not satisfactory, the patient radiation dose can be reduced by partly replacing digital radiography by fluoroscopic last-image hold, especially for documentation of tubal patency.

DETECTION OF SUBTLE PULMONARY DISEASE ON CR CHEST IMAGES: MONOCHROMATIC CRT MONITOR VS. COLOR CRT MONITOR

SHINGO IWANO

Department of Radiology

We examined the diagnostic efficacy of color soft-copy computed radiographic (CR) images of the chest in the detection of subtle pulmonary abnormalities. Twenty observers compared 87 soft-copy CR images on the four types of CRT monitor (nonmagnified monochromatic CRT, magnified monochromatic CRT, nonmagnified color CRT, magnified color CRT). Of 87 test images, 45 (including two identical sets of 12 images to test intraobserver variability) were abnormal and 42 (including two identical sets of 12 images) were normal. Of the 45 abnormal images, 15 showed subtle abnormalities, 15 showed mild abnormalities, and 15 showed obvious abnormalities. Observers could use three kinds of processed-images in the interpretation only on the monochromatic monitor. There were no statistically significant differences among the four types of CRT display formats even in the detection of subtle abnormalities. Although the sensitivity of chest radiologists showed statistically significant inferiority in color-monitor display formats, in the ROC analyses there were no statistically significant differences both in the group of chest radiologists and nonchest radiologists. Color CRT monitors may be available in place of monochromatic CRT monitors in PACS and teleradiology environment even in the task of image interpretation of subtle pulmonary diseases.

OUTCOMES OF LIVING WILLS COMPLETED BY PATIENTS IN JAPAN: A SURVEY OF THE DECEDENTS' FAMILIES

YUICHIRO MASUDA

Department of Geriatrics

In recent decades, increasing attention has focused on the expression and enhancement of individual autonomy in making medical decisions at the end of life in Japan. However, there is no legislation that recognizes expression of patient autonomy when an individual is no longer competent in Japan. In such circumstances, some people make their living wills and many of them have executed them. The purpose of this research was to investigate the outcomes of preparing written living wills among people who registered with The Japan Society for Dying with Dignity and subsequently died. Specially, we sought to determine the extent these people actually implemented their living wills, and the factors that would predict implementation of their written living wills. In the present survey, we found that 36.0 percent of the decedents who had written living wills did not implement them when they died (586/1,626). The decedents who died of cardiovasculor disease were less likely to use their written living wills than those who died of neoplasms and the decedents who died in hospitals were more likely to implement

them than those who died in their homes. Age did not influence implementation of their written living wills.

CENTRALLY ADMINISTERED TRH INDUCED INSULIN SECRETION IS IMPAIRED IN THE OTSUKA-LONG-EVANS-TOKUSHIMA FATTY RATS, A MODEL OF SPONTANEOUS NON-INSULIN-DEPENDENT DIABETES MELLITUS

YI CHEN

Department of Geriatrics

To investigate whether insulin secretion induced by stimulation of the vagus nerve is preserved or impaired in Otsuka Long-Evans-Tokushima-Fatty (OLETF) rats, we injected 10^{-8} mol of thyrotropinreleasing hormone (TRH) into the third cerebral ventricle and determined the serum level of insulin in the unanesthetized, unrestrained rats. Intracerebroventricular (ICV) injection increased the serum levels of glucose and insulin in both OLETF and Long-Evans-Tokushima-Otsuka (LETO) rats, a nondiabetic control strain, at 8–12 weeks of age. At 24–28 weeks of age, the increased level of glucose in OLETF rats was comparable to LETO rats but that of insulin was lower than control after the ICV injection of TRH. Pretreatment with intravenous (IV) atropine had no significant effect on such hyperglycemia. However, the increases in the serum levels of insulin were suppressed in both OLETF and LETO rats. The plasma levels of epinephrine, norepinephrine, and glucagon rose significantly after TRH. There was no significant difference in the levels of any hormones between the two groups. In OLETF rats at 24–28 weeks of age, IV glucose load induced significantly higher serum levels of glucose and insulin than LETO rats. The results suggest that the vagus nerve-mediated insulin secretion is impaired in OLETF rats in the early stage of diabetes, which may be an autonomic diabetic neuropathy. This impairment may play some role in deteriorating glucose tolerance in this spontaneously developed diabetes model.

VULVAR SQUAMOUS CELL CARCINOMA IN NAGOYA UNIVERSITY HOSPITAL FOR 34 YEARS (1965–1998)

KOMEI AKASHI

Department of Laboratory Medicine

Five hundred and twelve cases of vulvar lesion were recovered from our files at Nagoya University Hospital for 34 years. Sixty-seven of them had primary malignant neoplasm, which consisted of thirty-six cases of squamous cell carcinoma (SCC), and 23 cases of Paget's dis-

ease. SCCs were reviewed clinicopathologically. Age distribution ranged from 35 to 87 years old (average 65 years old), and had a peak in the 60s and 70s. Histologically, 31 cases were classified as common-type SCC, two were basaloid type, one warty type and two verrucous type, respectively. In FIGO staging the Ist and IInd stages together accounted for 60%. One of 26 cases examined with ISH method was HPV positive. Coexistent and/or adjacent lesions with SCC were lichen sclerosis in 3 and squamous hyperplasia in 3 cases, respectively, and no condyloma acuminatum was found. One patient had a history of cervical SCC and another immune deficiency disease. Smoking habit was found in 5 of 25 cases. Recurrence of vulvar SCC was found in 4 cases, and 3 cases died from the tumor. Relative frequency of Paget's disease in this hospital was much higher than other institutions in Japan and in Western countries.

EVALUATION OF A PC-BASED VIDEO-TELECONFERENCE SYSTEM ON CHEST RADIOGRAPHS

ZHI-XING XU

Department of Medical Information & Medical Records

Chest radiographs of 50 patients, including 25 with lung cancer were digitized at 100 dpi resolution and saved in the JPEG format at a low compression rate. Four respiratory specialists observed these images on a video-teleconference system display with 800 × 600 pixels resolution. After one month, they observed original chest radiographs. ROC analysis was performed of the answers based on a 5-point confidence scale. The observer-specific Az index values for the video-teleconference system ranged from 0.803 to 0.944, and the corresponding Az values for the conventional radiographs from 0.926 to 0.957. No differences were found between the video-teleconference system image and original images, showing that a video-teleconference system will be useful as a supplement to a diagnosis of chest X-ray films.

IMMUNOHISTOCHEMICAL ANALYSIS OF ARTERIAL WALL CELLULAR INFILTRATION IN BUERGER'S DISEASE (ENDARTERITIS OBLITERANS)

MASAYOSHI KOBAYASHI

1st Department of Surgery

The diagnosis of Buerger's disease depends on clinical symptoms and angiographic findings, whereas pathologic findings are considered of secondary importance. The aim of this study is to histologically analyze artery from patients with Buerger's tissue, including immunophenotyping of the infiltrating cells in order to elucidate the nature of Buerger's disease as a vasculitis. Thirty three speciments from 9 patients between 1980 and 1995 at Nagoya University Hospital were studied. Immunohistochemical studies were performed on paraffin-embedded

tissue using a labeled streptoavidin-biotin method. The general architecture of vessel walls was well preserved regardless of its stage and cell infiltration was observed mainly in the thrombus and the intima. CD3+ T cells greatly outnumbered CD20+ B cells. CD68+ macrophages or S-100+ dendritic cells were detected in the intima during acute and subacute stages. All cases except one showed infiltration by HLA-DR antigen bearing macrophages and dendritic cells in the intima. Immunoglobulins and complement factors were deposited along the internal elastic lamina. We concluded that Buerger's disease is strictly an endarteritis, which is introduced by T-cell mediated cellular immunity as well as by B-cell mediated humoral immunity associated with activation of macrophages or dendritic cells in the intima.

EFFECT OF SKIN SYMPATHETIC RESPONSE TO LOCAL OR SYSTEMIC COLD EXPOSURE ON THERMOREGULATORY FUNCTIONS IN HUMANS

NAOKI SAWASAKI

1st Department of Surgery

This study was conducted to clarify how sympathetic response to cold exposure determines the individual thermoregulatory function. Three female and seven male volunteers aged 23.2 ± 1.9 years old were exposed to acute local cooling and gradual systemic cooling with simultaneous recordings of microneurographic skin sympathetic nerve activity (SSNA), skin temperatures, tympanic temperature (Tty), laser Doppler skin blood flow, and sweat rate by a ventilated capsule method. Acute local cooling induced an abrupt vasoconstrictive SSNA increase and Tty rise, and there was a significant correlation between these parameters. On the other hand, systemic cooling of 0.2°C/min caused an SSNA enhancement and a gradual Tty fall, with a significant correlation between them. The cross correlogram showed that there was a 10-min delay for the Tty rise after SSNA enhancement in local cooling, whereas the delay for the SSNA increase after the Tty fall was within 1 min in systemic cooling. These experiments suggested that 1) good SSNA-responders are capable of Tty Maintenance, 2) 10 min is necessary to raise the Tty by the sheltering effect of systemic vasoconstriction, 3) SSNA responds linearly to the Tty fall, and 4) this response occurs with a delay of < 1 min.

ACTIVE FORM OF HUMAN HEPATOCYTE GROWTH FACTOR IS EXCRETED INTO BILE AFTER HEPATOBILIARY RESECTIONS

YASUHIRO KURUMIYA

1st Department of Surgery

Previously we showed that Hepatocyte growth factor (HGF) is excreted into bile after hepatectomies in patients with biliary tract carcinomas. However, it has not been certain whether

HGF in bile is an active molecule with the intact molecular weight or not. Bile samples were obtained from patients after hepatobiliary resections. HGF was purified using a heparin-Sepharose column and was subjected to the Western blotting. Under non-reducing conditions, purified bile HGF migrated to the molecular weight at 85 kDa, concordant with the native weight for a heterodimer. Under reducing conditions, monoclonal antibodies reacted with polypeptides at 69 kDa and 34 kDa, corresponding to the α- and β-subunits of HGF, respectively. The bile HGF stimulated [^{3}H]thymidine incorporation in primary cultured hepatocytes with a specific activity comparable to the recombinant human HGF. These results indicate that human bile obtained after hepatobiliary resections contains the intact and active form of HGF that can stimulate hepatocyte growth.

Bile samples from 50 patients who underwent various types of hepatobiliary resections were examined with respect to the HGF content by an enzyme-linked immunosorbent assay (ELISA). Biliary HGF excretion increased not only after hepatectomies but after bile duct resections. These results suggest that the biliary tract system plays an important role in the production of bile HGF.

ANALYSIS OF DOPPLER WAVEFORM OF HEPATIC VEINS FOR ASSESSMENT OF LIVER FUNCTIONAL RESERVE

KENJI OSHIMA

2nd Department of Surgery

Doppler waveform of the hepatic veins in healthy humans is a triphasic waveform (two negative waves and one positive). Recent studies emphasize the role of Doppler ultrasonography in the evaluation of liver cirrhosis. The analysis of the doppler waveform of hepatic veins might be a useful and non-invasive method to assess hepatic functional reserve and the amount of the liver to be excised. In 30 patients scheduled for hepatectomy, we analyzed the doppler waveform of hepatic veins and correlated Pulsatility Index (PI) and Resistance Index (RI) with ICG clearance values and histologic findings of resected liver specimen in order to ascertain the clinical use of this method. The values of PI and RI diminished according to the severity of cirrhosis and correlated with ICG clearance values. Furthermore, we found a statistically significant correlation between PI, RI and periportal and bridging necrosis ($r = -0.596$, $p = 0.0013$; $r = -0.608$, $p = 0.0011$) and intralobular degeneration and focal necrosis ($r = -0.548$, $p = 0.0031$; $r = -0.571$, $p = 0.0021$) and fibrosis ($r = -0.611$, $p = 0.0010$; $r = -0.647$, $p = 0.0005$). Portal inflammation and steatosis showed no significant correlation with PI and RI. These results suggest the usefulness of the doppler waveform of hepatic veins as an easy and reliable method for determining hepatic functional reserve and the amount of the liver to be excised.

α-GALACTOSYL OLIGOSACCHARIDES CONJUGATED WITH POLYETHYLENE GLYCOL AS POTENTIAL INHIBITORS OF HYPERACUTE REJECTION UPON XENOTRANSPLANTATION

TAKAHARU NAGASAKA

2nd Department of Surgery

Antibodies to an α-galactosyl saccharide structure are mainly responsible for hyperacute rejection after pig-to-primate xenotransplantation. The beneficial effect of α-galactosyl oligosaccharides has been shown on the inhibition of anti-pig natural antibodies. We synthesized polyethylene glycol (PEG)-conjugates of α-galactosyl disaccharide (Di) and trisaccharide (Tri) as potential inhibitors of the rejection reaction. The half lives of Di, Tri, PEG-conjugated Di (Di-PEG) and PEG-conjugated Tri (Tri-PEG) were 18.1 ± 2.3 min, 20.2 ± 0.9 min, 38.7 ± 2.8 min and 35.8 ± 1.6 min, respectively. Furthermore, Di-PEG and Tri-PEG showed biphasic clearance, and their half lives at the second phase were longer than 10 hours. Di-PEG and Tri-PEG markedly inhibited cytotoxic action of human sera to pig kidney cell line (PK15) compared to Di and Tri. At 5 mM concentration, Di-PEG and Tri-PEG caused 95 and 85% inhibition, respectively, while Di and Tri showed weaker effects. The binding of IgM antibodies to PK15 cells, however, was more strongly blocked by Di and Tri than Di-PEG and Tri-PEG. This phenomenon can be explained by the finding that PEG has anti-complement activity and masks antigenic sites of oligosaccharides. In conclusion, conjugation of PEG to oligosaccharides provided two beneficial effects; prolonged intravascular retention time and anti-complement activity, upon systemic application of the oligosaccharides.

EXPRESSION OF NM23-H1 IN PLACENTA AND TROPHOBLASTIC DISEASE

KATSUTOSHI IWASE

Department of Obstetrics and Gynecology

The nm23 gene is a potential metastasis suppressor gene identified using a murine melanoma cell line. The nm23 proteins are nucleoside diphosphate (NDP) kinases; and there are two human isotypes. The reduced expression of nm23 has been associated with increased metastasis and decreased survival in a variety of malignancies. In the present study, the expression of nm23-H1 was examined by Northern blot and immunohistochemical analyses in placenta and trophoblastic disease; the latter of which has a high potential of metastasis. Immunohistochemical staining of nm23-H1 was found in all specimens and localized at the cytoplasmic region of the cytotrophoblast of placenta. There was higher immunoreactivity in the first-trimester placenta compared with the second or third-trimester placenta. Among the hydatidiform mole specimens, the expression of nm23-H1 mRNA was significantly higher in the invasive mole than in the non-invasive mole. Similar levels of nm23-H1 gene expression were observed

between normal placenta and invasive mole samples. In conclusion, our data suggest that the increased expression of nm23-H1 in hydatidiform mole may be related to the development of invasive mole with lung metastasis.

DEVELOPMENTAL CHANGES IN PERICILIARY ARCHITECTURE IN RAT PHOTORECEPTOR CELLS. A FREEZE-ETCH STUDY

NAOTAKA WATANABE

Department of Ophthalmology

The purpose of this study is to find morphological machinery for selective transport of proteins required in the outer segments of the rat photoreceptor cells. At the first step, the three dimensional architecture of periciliary regions and their developmental changes were examined. Freeze-deep-etching and freeze-substitution methods combined with rapid freezing technique were used for morphological analysis of periciliary regions. Myosin S1 decoration method were employed for preservation of actin filaments and detection of their direction. Apical surface of the inner segment swelled and enclosed partially the base of the connecting cilium in postnatal early stages, so that the basal region of the connecting cilium was inevitably surrounded by a groove. However, no specialized periciliary ridge complex seen in frog photoreceptor cells was observed in rat photoreceptor cells. The plasma membrane in the vicinity and at the base of the connecting cilium was undercoated with the loose mesh work of fine filaments. However, it was unlikely to be a possible structural candidate for selective transport of membrane proteins. This study also revealed the interior structure of the connecting cilium. Actin filaments in the distal axoneme formed complicated mesh work together with unknown substance. Since S1 decorated filaments was not detected in the middle region of the connecting cilium, actin filaments in the base of outer segment seem to be independently polymerized locally from G-actin transported from the inner segment.

STRUCTURAL CHANGE OF THE α AND β SUBUNITS OF BOVINE RETINAL cGMP PHOSPHODIESTERASE (PDE) BY RELEASE OF γ SUBUNIT: DIRECT IMAGING BY IMPROVED LOW ANGLE ROTARY SHADOWING

SHU KACHI

Department of Ophthalmology

Cyclic GMP phosphodiesterase (PDE), a key enzyme for phototransduction, contains α (Pα), β (Pβ) and two γ (Pγ) subunits. Improved low angle rotary shadowing was used to describe the

three-dimensional structure of bovine PDE (Pαβγγ) and its changes by Pγ release. Images of Pαβγγ consisted of two strands, one with a mirror image of elongated S shape ("mirror S") and another with a mirror image of elongated F shape ("mirror F"). These two strands faced each other to make a ring shape that is bent slightly at the center line. In Pαβγ, the "mirror F shape" strand was changed to a "C" shape, but the other strand was not changed so drastically. These two strands make a ring structure that is bent much more. In Pαβ, both strands were changed to "C" shapes to make a twisted quasi ring shape. These observations suggest that each subunit of Pα and Pβ appears to be complexed with Pγ in Pαβγγ. The shape of each complex is drastically changed by Pγ release, and Pγ-free Pαβ forms a pseudo ring shape. Based on these data, we discuss relationship between conformational changes of Pαβ subunits by Pγ release and regulation of Pαβ functions.

BINOCULAR DEPTH-FROM-MOTION IN INFANTILE AND LATE-ONSET ESOTROPIA PATIENTS WITH POOR STEREOPSIS

MANAMI MAEDA

Department of Ophthalmology

Binocular depth perception can be separated into depth perception from disparity differences (stereopsis) and depth perception from the direction of motion (binocular depth-from-motion, BDFM). To evaluate BDFM in esotropic patients who have poor or no stereopsis, and to compare the results with conventional static binocular function tests, we examined forty-one infantile and twenty-eight late-onset esotropia patients with poor stereopsis. Dynamic stereopsis and BDFM were tested with computer-generated random dot stereograms and kinematograms. We investigated the correlation between BDFM and other factors. Fifteen (36.5%) of the infantile esotropia patients and 16 (57.1%) of the late-onset esotropia patients (total = 31, 44.9%) passed the BDFM test, and none of them passed the random dot stereo test under static or dynamic conditions. Fusion with Worth four dot test at near was associated with BDFM. The angle of strabismus was significantly smaller in the positive BDFM group for both the infantile and the late-onset esotropia groups. (Chi-square, $p < 0.05$). Testing BDFM in the clinical setting gives us a better understanding of binocularity in strabismic patients under dynamic conditions. Stereopsis under static conditions is difficult to obtain in patients with infantile esotropia, and better eye alignment is associated with BDFM.

THE STUDY OF REGENERATED CARTILAGE USING BIODEGRADABLE POLYMER SCAFFOLDS AND CULTURED CHONDROCYTES

MASAKI HONDA

Department of Oral Surgery

We have developed new biodegradable polymer for tissue engineered cartilage. The new polymer was prepared from a 50 : 50 (w/w) mixture of L-lactide-caprolactone. This study analyzed ectopic formation of cartilage from composite grafts of living cultured chondrocytes and new polymer. The costal cartilage of 6 week-old Rewis rats was digested by collagenase. After washing with PBS, the cartilage was put on a Petri dish and cultured primarily in DMEM supplemented with 10% FCS. After 3-week-culture, the migrated cells were collected by trypsin-EDTA treatment and inoculated into the polymer. The cells in the co-polymer were further incubated for 24 hours and were implanted in the subcutaneous dorsum. After 4 weeks, we killed the mice and removed composites. The specimens were examined by the histollogically, immunohistochemistry and mRNA of aggrecan. Hematoxylin-eosin staining showed hyaline cartilage-like tissue formation in all of the experimental groups. The extracellular matrix of the cartilage-like tissues was strongly stained with alcian blue and with anti-aggrecan antibody. The aggrecan gene expression was confirmed in regenerated tissue by Northern Blot Analysis. These results indicated that chondrocytes seeded on L-lactide-caprolactone polymer formed new cartilage tissue.

TREATMENT OF AUTOIMMUNE DISEASE DEVELOPED IN NEONATALLY THYMECTOMIZED MICE. DEPLETION OF EFFECTOR CD4$^+$ CELLS BY MONOCLONAL ANTIBODY AND RECONSTITUTION OF IMMUNE SYSTEM BY CD4$^+$ CONCANAVALIN A BLASTS

KAORI MAKI

Department of Oral Surgery

Multiple organ-localized autoimmune diseases, such as oophoritis and lacrimal gland adenitis were spontaneously developed in neonatally thymectomized (nTx) mice. These lesions could be cured by injection of monoclonal antibodies (mAb) against CD4$^+$ cells. Next we try to reconstitute CD4$^+$ cells in the immune system of the mice for acquire functional immunity. Ly 5 congeneic mice were used for the analysis. Injection of untreated CD4$^+$ cells from normal mice to the mAb treated mice did not work. Injection of CD4$^+$ concanavalin A (Con A) blast cells to the mice could work, and the reconstituted immune system of the mice did not act autoimmune reaction and could act function response against allogeneic lymphocyte antigens and

exogenous antigens such as OVA and SRBC. This immune reconstitution method may provide possible treatment of human autoimmune disease.

THE GENE STRUCTURE AND PROMOTER SEQUENCE OF MOUSE HYALURONAN SYNTHASE 1 (mHAS1)

YOICHI YAMADA

Department of Oral Surgery

The structure and organization of mouse hyaluronan synthase 1 gene, *HAS*1, were determined by direct sequencing of 1 phage clones carrying the entire gene and by application of the long and accurate (LA)-PCR method to amplify regions encompassing the exon/intron boundaries and all of the exons. This gene spans about 11 kb of genomic DNA and consists of 5 exons and 4 introns. The similarity in the exon-intron organization was found between the genes of mouse HAS1 and *Xenopus lavies* DG42 which was recently identified as *Xenopus* hyaluronan synthase. The transcription initiation site was determined by rapid amplification of the cDNA (5'-RACE). The position +1 is located 55 nucleotides upstream of the ATG initiation codon. The promoter region of the HAS1 gene has no typical TATA box, but contains CCAAT box located 190 upstream of the transcription initiation site. Further analysis of 1.4 kb of the 5' flanking region revealed several potential binding motifs for transcription factors. The information of the gene structure may be useful for proceeding studies on the promoter activity.

MOUSE MONOCLONAL ANTIBODY, 3C10 AGAINST TYPE III MUTANT EPIDERMAL GROWTH FACTOR RECEPTOR: SCINTIGRAPHIC DIAGNOSIS AND scFv (single chain Fv) ANTIBODY PRODUCTION

NORIHISA NAKAYASHIKI

Neurosurgery

Mouse monoclonal antibody, 3C10, was produced against the truncated epidermal growth factor receptor (EGFR), encoded by the in-frame type III deletion mutation of EGFR, which creates a unique sequence with a glycine residue at the fusion junction. Since the sequence around the fusion junction is expressed only a proportion of human glioblastomas, it is a potential target for diagnostic and therapeutic approaches. We labelled 3C10 antibody with ^{99m}Tc and estimated in vivo distribution after intravenous administration into mice bearing ERM5 tumor transfected with the type III EGFR deletion-mutant gene. At 24hr, tumor/blood accumulation ratio was over 25. To achieve higher tissue penetration efficiency, faster blood clearance

rate and lower imunogenicity of antibody, we initiated producing scFv of 3C10. Amino acid sequence of 3C10 antibody was determined, and then adequate primers for VH and VL genes were prepared to clone these genes by RT-PCR. The genes cloned were ligated into bacterial and eukaryotic expression vectors to express 3C10 scFv. 3C10 scFv with a good binding activity is yielded not only by eukaryotic, but also by bacterial expression systems. This 3C10 scFv may be an useful reagent for diagnosis and therapy of glioblastoma patients.

ELEVATION OF NITRIC OXIDE METABOLITES IN THE CEREBROSPINAL FLUID OF PATIENTS WITH MOYAMOYA DISEASE

ATSUSHI NODA

Department of Neurosurgery

OBJECTIVE: To investigate whether nitric oxide (NO) contributes to the formation of abnormal collateral circulation in the patients with moyamoya disease.

METHODS: Cerebrospinal fluid (CSF) samples were obtained from the subarachnoid space of the sylvian fissure during combined bypass surgery for moyamoya disease and kept frozen until NO metabolites, nitrate and nitrite, were measured using a Griess method.

RESULTS: Compared with control speciments of CSF obtained from 16 patients with hemifacial spasm (n = 8), trigeminal neuralgia (n = 2), unruptured aneurysm (n = 5), or tremor (n = 1), concentrations of NO metabolites in the 23 CSF samples of 16 patients with moyamoya disease were significantly higher (18.6 ± 1.1 vs. 11.0 ± 1.0 μM, $P < .01$). NO metabolites concentrations (21.6 ± 2.3 μM) in CSF obtained during initial surgery in 7 resampled patients decreased to 16.9 ± 1.5 μM ($P < .05$) in CSF obtained during a second, contralateral procedure. Angiographically moyamoya disease with greater development of moyamoya vessels at stages 3 and 4 tended to show higher concentrations of NO metabolites than cases at early and late stages.

CONCLUSION: NO concentrations in CSF were chronically elevated in moyamoya disease, probably reflecting development of collateral circulation. Vascular bypass surgery can reduce NO metabolites together with abnormal collateral circulation.

EXPRESSION OF MIDKINE IN NORMAL AND BURN SITES OF RAT SKIN

NOBUHIKO IWASHITA

Department of Plastic and Reconstructive Surgery

Expression of midkine (MK), a retinoic acid-inducible heparin binding cytokine, was examined immunohistochemically in normal and burn sites of rat skin. In the normal skin, MK was localized in the epidermis and dermal appendages such as hair follicles and sebaceous glands. Mast cells in the subdermal connective tissue also accumulated MK. After burn injury, MK-positive cells began to infiltrate into subdermal connective tissue and the number of MK-positive cells in the region increased to a maximum at postburn day 2 and then decreased gradually. Western blotting analysis of both normal and postburn skin revealed a 30 kDa band reactive with anti-MK antibody: This band was concluded to be a dimer of MK. These findings were evaluated from the viewpoint of the possible role of MK in wound healing.