P4AM-24-4

EFFECTS OF A TIME-VARYING MAGNETIC FIELD ON VOLUME REGULATION OF BOVINE ADRENAL CHROMAFFIN CELLS

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We tested the effects of exposure to a switched 1.5 T magnetic field on functions of cytoskeletal protein in bovine adrenal chromaffin cells. It is well known that intracellular actin fiber is closely related to mitochondrial movement, Ca^{2+} release from endoplasmic reticulum and cell volume regulation during anisosmotic medium. The exposure also changed morphology of actin fiber and reduced it's content. Control cells showed a regulatory volume decrease (RVD) after replacement to hypotonic medium. But, in exposed cells, the cell volume was increased at a slower rate and reached a peak value of higher than control, and recovery to the original value was also delayed. This volume regulation of exposed cells was similar to the regulation observed in cytochalasin D-treated cells. After replacement to the hypotonic medium, intracellular F-actin content was initially decreased and then increased in control cells, but it's content continued to be decreased in both exposed cells and cytochalasin D-treated. These results suggest that influences on cell volume regulation by exposure to the magnetic field is caused as a result of changing the structure or functions of F-actin in chromaffin cells.

P4AM-24-5

C-TERMINUS OF NEBULIN BINDS TO ALPHA-ACTININ

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Nebulin is a large 773 kDa protein that binds along the full length of the actin filaments in skeletal muscle. It may regulate the length of actin filaments by binding to tropomodulin and beta-actinin/CapZ, which are capping proteins of actin filaments. The C-terminus of nebulin is located in the Z-line of the sarcomere and is co-localized with alpha-actinin. Although binding of nebulin to alpha-actinin was studied using far-western blot assay in 1990, the binding and it's site was not cleared. We analyzed the binding of the C-terminus of human nebulin (repeat 172-SH3 domain) to alpha-actinin using far-western blot, yeast two-hybrid and pull down assays. We also investigated for the binding site of the nebulin C-terminus to alpha-actinin using far-western blot and the pull down assays, and the binding site of alpha-actinin to nebulin by the yeast two-hybrid and pull down assays. Our results indicate that in skeletal muscle, nebulin fixes the actin filament into the Z-line by binding to both the actin filament and alpha-actinin. This helps in understanding the continued association between actin filaments and the Z-line when the skeletal muscles contract, and may further contribute to the understanding of nemaline myopathy, which begins with the loss of the nebulin C-terminus.

P4AM-24-6

DISUSE-INDUCED CHANGES IN FATIGABILITY IN RAT SOLEUS MUSCLE

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Disuse atrophy is a common problem of skeletal muscle associated with a lack of exercise, resulting in functional abnormalities. We recently reported that long-term hindlimb immobilization (6 weeks) lowers the expression of the giant protein titin in the soleus muscle of the rat, resulting in reduced active force production via abnormal sarcomeric organization. In the present study, we investigated how immobilization affected fatigability by using Triton X-100-treated single skinned fibers taken from the same animal model. The intracellular concentrations of inorganic phosphate (Pi) and H+ are known to increase in skeletal muscle during intense exercise, resulting in a fall in active force production. Therefore, we tested the effects of changes in pH and Pi concentration on maximal Ca^{2+}-activated force production in control vs. immobilized fibers. We found that lowering pH from 7.0 to 6.2 decreased maximal force in both muscles, with a greater magnitude in immobilized fibers. Likewise, the inhibitory effect of Pi up to 20 mM was more pronounced in immobilized fibers. These results suggest that fatigability is enhanced in disused muscle and that the mechanism includes a decrease in the fraction of force-generating cross-bridges coupled with abnormal sarcomeric organization.

P4AM-25-1

CELL BEHAVIOR IN SIZE-CONTROLLED MICRO-COMMUNITY ON NOVEL FUNCTIONAL CELL CULTURE SURFACE

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Recent progress in cell biology shows that each cell has its own individuality. However, there is less evidence about properties of single cells in size-controlled cell community or contribution of single cells to cell mass. Establishment of a functional surface for patterning cells and controlling size of cell mass should contribute to elucidate such phenomena. Here, we demonstrate a novel surface adapted for cell pattering with high efficiency. In order to realize size-controlled cell patterning, nano- and micro-scale drawings was performed by electron beam (EB) lithography on glass slide modified with EB-denaturable polymer and surfactant. Cells attached onto and were cultured stably within the patterned area adsorbed with a proper cell adhesion protein. Behavior of osteosarcoma (U2OS) cells that was emitted with GFP illumination arranged with cyclinB1, a specific expression protein on G2-M phase in cell cycle, was monitored in the microenvironment of substrate and observed the daughter cells that occurred by mitosis, genetic information was conserved till next cell divisions, has variety in cycle length. These results suggest a difference in basic cell biological property in spite of theoretically same genetic information and growth conditions.

P4AM-25-2

FUMARATED POLY ETHER-ESTER HYDROGELS THAT SUPPORT OSTEOBLAST VIABILITY, ATTACHMENT AND PROLIFERATION

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Cell-interactive polymers have widely used as synthetic extracellular matrices to regulate cell function and promote tissue regeneration. In this study, the growth of cells from different orgin have been investigated on photopolymerized Arg-Gly-Asp modified fumarated poly(ethylene glycol)-co-poly(lactic acid) hydrogels that was firstly synthesized by our study group. Osteoblast (MC3T3-E1), fibroblast (3T3) and endothelial (ECV304) cell lines were cultured on hydrogels. Cell attachment and proliferation was observed using scanning electron microscopy and light microscopy. Cytotoxicity tests were performed by MTT (methyl tetrazolium) assay. According to obtained results the fumarated poly ether-ester hydrogel matrice was only enhanced the attachment, spreading and proliferation of osteoblast (MC3T3-E1) compared with other cells and not cytotoxic. This injectable hydrogel can be used as a potential material for tissue engineering scaffold and bone tissue regeneration.

P4AM-25-3

THE EFFECT OF ELECTROMAGNETIC FIELD STIMULATION ON CHONDROGENIC DIFFERENTIATION OF HUMAN ADIPOSE-DERIVED STEM CELLS

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INTRODUCTION: Pulse electromagnetic field (PEMF) has been approved by FDA, USA. Several studies have been reported that PEMF can increase proliferation and proteoglycan synthesis on the chondrocytes. However, it remains unclear whether PEMF affects chondrogenesis of stem cells. We hypothesized that PEMF and our single pulsed electromagnetic field (SPEMF) may accelerate chondrogenesis of human adipose-derived stem cells (hADSCs) thus could be applied in articular cartilage regeneration.

METHODS: The stimulation module of PEMF was pulsed period 5 ms repeated in 15 Hz, magnetic magnitude in 20 Gauss, stimulated for 8 hrs per day. Module of SPEMF was magnitude in 1 Tesla per pulse, pulsed period 5 ms for 30 times per day. The effects of PEMF and SPEMF stimulation on chondrogenesis of hADSCs were examined by gene expressions, glycosaminoglycan synthesis, and histology.

RESULTS: Our data showed that the mRNA expressions of chondrogenesis marker genes were significantly increased after PEMF or SPEMF stimulation. The glycosaminoglycan synthesis of hADSCs was significantly increased after both PEMF and SPEMF stimulation.

P4AM-25-4

EXPLORATION OF EPINEPHRINE ACTIVATED LIVING PLATELETS BY FOURIER TRANSFORM-RAMAN SPECTROSCOPY

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Introduction: platelets activation assessment is an important step for identification of the high risk patients for cerebrovascular and cardiovascular thrombotic events. For these reason the accurate assessment of living platelets activation is useful for clinical practice. Materials and methods: we use the FT-Raman spectroscopy, a noninvasive tool, to explore the conformational switch of activated platelets with epinephrine. Liquid samples of washed platelets added to EDTA and PBS solutions (prepared from blood obtained from peripheral vein of human healthy volunteers) were analyzed before and after epinephrine activation. Results: The freshly extracted platelets revealed characteristic spectral feature in the phophodiester region. Upon epinephrine activation a new developed band has been observed (assigned to the lipids). The amide I bands exhibited a complex shape which was unaffected by the epinephrine presence. The present spectral data were correlated with the FT-IR results. Conclusions: the results of this study show that the Raman platelet assessment may be useful as a model system for activated platelets exploration and has the advantage to be a rapid, real-time technique who may explore living platelets.

P4AM-25-5

DELIVERY OF SODIUM BOROCAPTATE TO GLIOMA CELLS USING IMMUNOLIPOSOME CONJUGATED WITH ANTI-EGFR ANTIBODIES BY ZZ-HIS

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Nanoparticles are effective of delivering cargo into cells. Here, sodium borocaptate (BSH) was encapsulated in liposomes composed of nickel lipid, and anti-epidermal growth factor receptor (EGFR) antibodies were conjugated to the liposomes using the antibody affinity motif of protein A (ZZ) as an adaptor (immunoliposomes). The immunoliposomes were used to deliver BSH into EGFR-overexpressing glioma cells. Immunohistochemical analysis using an anti-BSH monoclonal antibody revealed that BSH was delivered effectively into the cells but not into EGFR-deficient glioma or primary astrocytes. In an animal model of brain tumors, both the liposomes and BSH were only observed in the tumor. Moreover, the efficiency of 10B's delivery into glioma cells was confirmed by inductively coupled plasma-atomic emission spectrometry (ICP-AES) both in vitro and in vivo. The results suggest that this system utilizing immunoliposomes provides an effective means of delivering 10B into glioma cells in boron neutron capture therapy (BNCT).

P4AM-25-6

LIPOSOMES AND LIPID MEMBRANES ON A FLAT HYDROGEL SUBSTRATE OBSERVED BY ATOMIC FORCE MICROSCOPY

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Scanning probes as atomic force microscopy (AFM) are intrinsically suitable especially to observe lipid membrane and its related biomolecules like trans-membrane proteins, since the membrane has a two-dimensional structure and thus orientation of the including proteins can be restricted by the surrounding membrane. It is important to use the substrate on which the biomolecules retain their functions. Hydrophilic polymer gel surface can be expected neither to deform nor to denature the molecules by avoiding direct contact of them to a solid surface. In this work, we report a simple preparation method of a flat agarose gel substrate and its application for AFM observation of liposomes and lipid membranes. Agarose was gelated between freshly cleaved mica and a glass plate. Flat gel surface was simply obtained by removing the mica after gelation, whereas the gel was fixed on the glass plate pre-coated with agarose including small quantity of epoxy resin. AFM observation showed 0.3 nm of its surface flatness for 0.1 mm thick gel. Liposomes could be observed unruptured on the gel substrate. As increasing adsorbed liposomes, a lipid membrane could be formed by rupturing and fusing liposomes on the gel substrate as well as the one supported on a solid surface.

P4AM-25-7

PROTEOGLYCAN STRUCTURE OF MUCUS AND EXTRACELLULAR MATRIX OF TISSUE STUDIED BY X-RAY DIFFRACTION AND FLUORESCENCE USING SYNCHROTRON RADIATION

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Various native and transformed epithelial tissues were studied by X-ray diffraction and fluorescence. X-ray patterns of biological tissue may be classified under two archetypes: no ring and ring 4.5 nm. The 4.5 nm reflection and its orders we have attributed to highly ordered proteoglycan structures and are associated with the interchain spacing of polysaccharides. The 4.5 nm spacing is a structural nanoinvariant of proteoglycans of mucus and extracellular matrix of different biological tissues. A correlation between integral intensity of X-ray pattern and elemental content in tissue was observed Ca being the major element of tissue mineral composition. Proteoglycan structure can be reversibly transformed by salt solutions or chelating agents and the 4.5 nm reflection should be considered as a marker of structural modifications of intact tissue under various endogenous and exogenous influences. It is necessary to consider mobile proteoglycan structures as the universal scaffolding providing structural homeostasis of biological tissues. Role of proteoglycan structure transformation under the influence of Ca cation in the adaptive potential of biological tissues is discussed in terms of statistical thermodynamics of mesh polymers.

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P4AM-25-8

THE HYDROGEL NATURE OF MAMMALIAN CYTOPLASM CONTRIBUTES TO OSMOSENSING AND EXTRACELLULAR PH SENSING

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Cytoplasm is thought to have hydrogel-like characteristics, including ability to absorb large amounts of water, to change volume due to alterations in external environment, and limited leakage of ions and proteins. Some gel-like behaviors have not been rigorously confirmed for mammalian cells and others should be examined under conditions where gel volume can be accurately monitored. For this reason, contributions of cytoplsam hydrogel properties to cellular processes, such as volume-sensing and regulation remain unclear. We used 3D imaging to measure volume of single substrate-attached cells after permeabilization of their plasma membrane. Permeabilized cells swelled or shrinked reversibly in response to variations of external osmolality. Volume changes were 3.7-fold greater than observed with intact cells, consistent with cytoplasm's high water-absorbing capacity. Volume was maximal at neutral pH and shrunk at acidic or alkaline pH, consistent with pH-dependent changes of protein charge density and repulsive forces within cellular matrix. Volume shrunk with increased [Mg2+] due to increased charge screening and ionic crosslinking effects. Findings demonstrate that mammalian cytoplasm resembles hydrogel and functions as a highly-sensitive osmosensor and extracellular pH sensor.

P4AM-25-9

THE DEHYDRATING EFFECTS OF SMALL ORGANIC MOLECULES ON THE STRUCTURAL INTEGRITY OF BIOLOGICAL TISSUES; SKELETAL MUSCLE AND NERVE

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Biological tissue is the association of various macromolecules such as proteins. Water molecules in the tissue mediate osmotic effects through their activity affecting the separation of the macromolecules, and therefore play a key role in maintaining the structural integrity of the tissue. That is, water in the bulk phase affects not only the folding of each macromolecules, but also the structural stability of the tissues. Strong evidence that indicate the significance of the water activity has been obtained with demembranated (skinned) cell experiments. In the case of skinned fibers of skeletal muscle, osmotic compressive force of macromolecular dextran and PVP depended on their volume% rather than molar concentration. In addition, even small organic molecules such as di-ethylene glycol efficiently compressed skinned fibers. The compression was almost exclusively observed in radial direction preserving the fine structure. We extended the study to the sciatic nerve preparation. It is indicated that the water in the anisotropic structure of nerve bundles has similar characteristics to that of skeletal muscles from the view point of restricted evaporation and transverse relaxation rate of the ^{1}H-NMR signals. Biological tissues should be viewed as a gigantic crystal in this sense.

P4AM-25-10

BOUND MAGNESIUM IS IMPORTANT TO MAINTAIN THE CARDIAC FUNCTION IN HYPOXIA-REOXYGENATION INJURY

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In Langendorff perfused rat heart, we have reported that hypoxia-reoxygenation decreases intracellular total magnesium (Mg) concentration ($[Mg]_i$), and high extracellular Mg^{2+} in hypoxic condition improves hypoxia-reoxygenation injury. Although we hypothesized that intracellular Mg plays important roles to maintain the cardiac function, it was unclear whether bound Mg or ionized Mg is crucial. We examined the intracellular ionized Mg (Mg^{2+}) dynamics during hypoxia-reoxygenation in isolated rat cardiac myocytes by using mag-fura/2 method. In control group, hypoxic perfusion (45 min) did not change the intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$), and reoxygenation (30 min) also did not change the $[Mg^{2+}]_i$. In high Mg group ($[Mg^{2+}]_o$ was elevated to 12 mM during 45 min hypoxia), $[Mg^{2+}]_i$ was elevated and reached to 1.30 ± 0.08 folds (vs basal level). Reoxygenation decreased $[Mg^{2+}]_i$, but this level was still higher than that of basal level (1.13 ± 0.07 folds). We conclude that bound Mg is important to maintain the cardiac function in hypoxia-reoxygenation injury. Our results suggest that $[Mg^{2+}]_i$ leaks from intracellular space by hypoxia-reoxygenation, but extracellular high Mg prevents Mg^{2+} leakage from intracellular space, and then inhibits Mg^{2+} dissociation from ATP and other enzymes.

P4AM-25-11

ROLE OF THE SYSTEM OF SYNTHESIS OF NITROGEN OXIDE IN PROVIDING OF LONG DURATION ADAPTATION OF ORGANISM TO MUSCULAR WORK

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It is known, that the regular physical exercises in the training mode improve the endothelium function of persons with the risk factors of cardiovascular diseases. At the same time, the information concerning the features of the system of synthesis of nitrogen oxide in persons doing exercises systematically of considerable capacity and intensity is practically absent.
Within the framework of research the boys and girls of 18-22 years old. In blood plasma of all youths and girls levels the nitrite and nitrate of anion, urea, activity of arginase, constitutive (cNOS) and inducible (iNOS) NO-sintases, nitratreductase were determined. Besides the indicated biochemical indexes of youths and girls of the volume of general physical capacity was determined.
The results of the conducted research allowed to register the objective existence of certain features of functioning of the system of synthesis of nitrogen oxide of organism of youths and girls with the long duration form of adaptation to muscular work: at the beginning and in the middle the competition period the main significance is taken to the cNOS. At the end of the competition period an oxidative way of NO formation with the participation of iNOS.

P4AM-26-1

SEMA4C EXPRESSION IN NEURAL STEM/ PROGENITOR CELLS AND IN ADULT NEUROGENESIS INDUCED BY CEREBRAL ISCHEMIA

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Sema4C is a transmembrane protein that belongs to semaphorin family. In this study, whole-mount in situ hybridization result showed that Sema4C was expressed abundantly in the areas of lateral ventricle, the striatum, the wall of midbrain, the pons/midbrain junction of E11.5 embryos brain. Neural stem/ progenitor cells (NSPs) obtained from E13.5 embryonic rat midbrain also positive for Sema4C immunoreactivity. Sema4C expression was dramatically down-regulated during induction of NSPs differentiation. We used the rat global cerebral ischemia model to make adult neurogenesis in vivo. The robust proliferative NSPs were monitored by labeling with bromodeoxyuridine (BrdU) within the subventricular zone (SVZ) and dentate gyrus (DG) that continues for at least 2 weeks. Immunohistochemistry and western-blot analysis showed that Sema4C expression was dramatically up-regulated during neurogenesis after cerebral ischemia-perfusion injury. Double-immunostaining and stereologic counting analysis indicated that a high proportion of BrdU-positive proliferative cells were Nestin positive NSPs. Sema4C was highly expressed in these proliferative populations at specific stages after ischemic injury. These support a putative role of Sema4C during neurogenesis both in vivo and in vitro.

P4AM-26-2

COMPARATIVE STUDY OF DIFFERENT ADMINISTRATIONS OF THE NEURAL STEM CELLS FOR SPINAL CORD INJURY IN MICE

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Purpose For clinical trial of neural stem cells (NSCs) transplantation for spinal cord injury (SCI), it is important to determine how to apply NSC to injured spinal cord. Therefore, we sought to determine the most optimal method of NSCs transplantation for SCI in terms of safety and efficacy.
Method We used the fetal mouse striatum-derived NSCs lentivirally transduced novel fusion protein; dVenus-Luc2. After inducing contusive thoracic SCI in adult C57BL/6 mice, we applied 5×10^5 NSCs to the injured site by three different ways; intralesional(IL group), intrathecal(IT group) and intravenous(IV group) applications. We quantitatively measured fluorescence of the grafted cells in the living mice at the several time points with a bioluminescence imaging system. At 6weeks after SCI, the grafted cells were analyzed by immunohistochemistry.
Result IL group showed the best survival rate of the grafted cells at the injured site among all groups. IT group showed that grafted cells were detected not only at the injured site but also out of the lesion site. In IV group, the fluorescence was observed at the bilateral chest and almost half of mice were dead immediately after transplantation, which suggesting pulmonary embolism.
Taken together, IL injection is the most optimal transplantation.

P4AM-26-3

ROLE OF WNT/BETA-CATENIN SIGNALING IN NEUROGENESIS IN THE ADULT MOUSE SUBVENTRICULAR ZONE

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The subventricular zone (SVZ) is the largest germinal zone in the mature rodent brain, and it continuously produces young neurons that migrate to the olfactory bulb. Neural stem cells in this region generate migratory neuroblasts via highly proliferative transit-amplifying cells. The Wnt/ beta-catenin signaling pathway partially regulates the proliferation and neuronal differentiation of neural progenitor cells in the embryonic brain. Here, we studied the role and regulatory mechanisms of this signaling pathway in the adult mouse SVZ. Retrovirus-mediated expression of a stabilized beta-catenin promoted the proliferation of transit-amplifying cells and inhibited their differentiation into neuroblasts. Conversely, the expression of Diversin, a protein involved in beta-catenin degradation, promoted their differentiation into neuroblasts. These results suggest that beta-catenin signaling plays a role in the proliferation of progenitor cells in the SVZ of the adult mouse brain.

P4AM-26-4

SLIT-ROBO SIGNALING REGULATES THE MIGRATION OF NEW NEURONS UNDER PHYSIOLOGICAL AND PATHOLOGICAL CONDITIONS

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Even in the adult brain, neural stem cells in the subventricular zone (SVZ) continue to produce new neurons, which migrate in chain toward the olfactory bulb (OB). After marked neuronal death by ischemic stroke, generated neurons in the SVZ migrate toward the damaged area, which is considered to be involved in regenerative process. However, the mechanism that controls the migration of these neurons is not well-understood.
Slit1 is a secreted protein that binds to its receptor Robo and functions as a chemorepellent for extending axons and migrating cells in developing brain. Although Slit1 is expressed in new neurons in the adult SVZ, its precise role has not been demonstrated. Here, we investigated the role of Slit1 in migration of new neurons from the SVZ.
Using Slit1 knockout mice, we found that Slit1 is required for regulation of interaction between migrating neurons and surrounding astrocytes, which is important for the fast migration of new neurons inside of the glial tube. Moreover, migration of neurons toward the damaged area after ischemic stroke is significantly impaired in these mice. These data indicate that Slit1-Robo2 signaling regulates the physiological migration of neurons toward the OB as well as the regenerative process under the pathological condition.

P4AM-26-5

HUMAN ADULT MESENCHYMAL STEM CELLS DIFFERENTIATION IN VITRO THROUGH CHONDROGENIC LINEAGE

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The aim of our study was the investigation of in vitro differentiation potential of mesenchymal stem cells (MSCs) towards chondrocytes lineage by assessment of optimal composition of chondrogenic media and morphological and functional evaluation of differentiated cells.

Methods: After obtaining the writing informed consent the bone marrow samples were harvested by femoral drilling from 5 patients undergoing surgical hip replacement. The MSCs were isolated by plastic adherence method and after two passages were placed in chondrogenic media containing either bone morphogenic protein 2 (BMP2) or tumor growth factor beta (TGF beta). The cells were analysed by optical microscopy, flowcytometry and molecular biology (RT-PCR).

Results: Cultivation of cells in micropellet technique induces a certain degree of hypoxia and activates the specific genes for chondrocytes lineage. MSCs differentiation in to chondrocytes seems to be conditioned by media supplementation with TGF beta, which can induce collagen II synthesis. The RT-PCR revealed also the expression of aggrecan and collagen X.

Conclusions: The differentiated cells can secrete the extracellular matrix components, so they can be used in tissue replacement procedures.

P4AM-26-6

TUMOR MICROENVIRONMENT INDUCES CHANGES IN HUMAN MESENCHYMAL STEM CELLS BEHAVIOR

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From the perspective of human mesenchymal stem cells (MSCs) interaction with different cell types within our body, we tried to characterize the influence of tumor microenvironment on MSCs proliferation and development. We used bone marrow-derived human MSCs, passage 4, with a well defined morphology, phenotype and differentiation potential. Tumor cells were obtained from 6 breast tumor surgical pieces of approximate 5 cm2, with the histopathological diagnosis of infiltrative ductal mammary carcinoma. After 48 hours digestion with collagenase, the cells were filtered and suspension cell cultures were grown for 2 weeks. Co-cultures of MSCs and tumor cells were obtained using different cellular densities and culture media, and further flowcytometric and immunohistochemical analysis was performed after 2 weeks. MTT assay on MSCs used conditioned tumor cells media, supplemented with 10% FCS. The results indicate that (1) morphological aspect of MSCs was seriously changed; (2) increased CD90 and CXCR4 markers expression; (3) over-expression of vimentin cytoskeleton protein in co-cultured MSCs; (4) relatively dose-dependant decrease of MSCs proliferation after 24, 48 and 72 hours. We may conclude that MSCs suffer a series of behavior changes in order to resist tumor aggression.

P4AM-26-7

HUMAN ADULT STEM CELLS AS A SOURCE FOR OSTEOPROGENITOR CELLS IN VITRO

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The cells used for bone restoration could be obtained by differentiation of human adult stem cells. Our study compared the in vitro differentiation potential of human haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs).

Methods: After obtaining the writing informed consent, bone marrow samples were harvest from 4 patients by iliac crest punction. CD34+ HSCs were selected using magnetic beads. MSCs were isolated based on plastic adherence properties. For osteoblastic differentiation various mixtures of cytokines and hormones were used. The cells were analyzed and characterized by microscopic examination, cytochemistry and RT-PCR methods.

Results: After 2 weeks in osteoinductive media both MSCs and HSCs showing polygonal shape modifications. Von Kossa staining certified the starting of mineralization process of MSCs. The RT-PCR at 21 days revealed strong expression of cbfa1 and alkaline phosphatase.

Conclusions: In vitro osteoblastic differentiation is possible for both human adult HSCs and MSCs. The key factor seems to be the presence of dexametasone and ascorbic acid in the culture media. Differentiation induces the synthesis of some bone mineralization factors enabling these cells for tissue engineering procedures.

P4AM-26-8

MESENCHYMAL STEM CELLS DIFFERENTIATION TO CARDIOMYOCYTES USING ASCORBIC ACID

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Self-renewal capacity and differentiation potential towards several cellular lineages are the theoretical and practical bases of mesenchymal stem cells (MSCs) use in cellular therapy.

Material and methods: MSCs were isolated from bone marrow aspirates obtained by iliac crest punction. The differentiation towards cardiomyocytes were tried, using ascorbic acid in comparison with 5-azacytidine. After 6 weeks, the surface markers were evaluated by flowcytometry and quantitative PCR was performed for genes that code myogenin and MYF5 myogenic factor.

Results: During the week 3, the cells organized in radial aspect colonies, adherent to the culture flask surface. The hematopoetic markers were negative (CD34, CD45), and the MSCs characteristic markers showed 66.32% expression for CD90, 36.56% for CD105, and 86.52% for CD73. The PCR results for myogenin indicate occurrence of a fluorescent signal between cycles 38 and 40, but the signal for myogenic factor MYF-5 was much weaker than in case of 5-azacytidine use.

Conclusion: Our data showed that ascorbic acid can induce both morphologic differentiation, and RNA synthesis changes, modifying the expression profile of some genes characteristic to muscle cells, induction being weaker compared to the response to 5-azacytidine.

P4AM-26-9

CARDIOMYOGENIC POTENTIAL OF SKELETAL MUSCLE-DERIVED STEM CELLS

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In regenerative therapy of the heart, transplantation of stem cells to form new functional myocardium is limited by the inability of the cells to integrate with host cardiomyocytes and conduct cardiac electrical activity. In this study, we isolated low adherent stem cells from skeletal muscle that can generate action potentials (APs) after 5 days of culture. The APs were not altered by 100nM Tetrodotoxin (TTX) but completely blocked by 3µM TTX, suggesting the presence of TTX-resistant sodium channels. The complete block of APs by 500µM Cadmium supported the presence of the cardiac specific sodium channel Nav1.5. The sensitivity to TTX of the sodium current initiating APs was further investigated. Two distinct populations of sodium channels could be separated. The TTX-sensitive fraction (IC50 ~ 6.5nM) represented 28% of the total sodium channel fraction whereas the TTX-resistant fraction (IC50 ~ 2.1µM) amounted to 70% of the total fraction. These values are consistent with the IC50 values of the skeletal muscle channel Nav1.4 and the cardiac channel Nav1.5. The expression of transcripts for Nav1.4 and Nav1.5 was confirmed by RT-PCR. We conclude that a skeletal muscle stem cell population exhibit electrophysiological properties compatible with cardiac function.

P4AM-26-10

EFFECT OF ALENDRONATE ON ENHANCING OSTEOGENESIS OF HUMAN ADIPOSE DERIVED STEM CELLS IN TISSUE ENGINEERING FOR BONE REPAIR

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Bisphosphonates are well known potent inhibitors of osteoclast activity, and used for treating osteoporosis. Recently, studies indicated that alendronate, one of the potent bisphosphonates, had a stimulatory effect on osteogenesis of bone marrow mesenchymal stem cells (BMSCs) in vitro. Our previous study also found that a short-term treatment of alendronate increased gene expressions of osteogenic markers, bone morphogenetic protein-2 (BMP2) and osteocalcin, alkaline phosphatase (ALP) activity and mineralization in human BMSCs. In this study, we further examined the effect of alendronate on osteogenesis of human adipose tissue-derived stem cells (hADSCs) in vitro and tested the effect on bone repair in a calvarial defect rat model. Our results showed that the BMP2 mRNA level was increased by alendronate treatment in a time-dependent manner. Results also showed that a 5-day treatment of alendronate significantly enhanced ALP activity 5-7 days and increased mineralization 7-14 days after osteo-induction in hADSCs. We also found that implanting combination of scaffold, hADSCs and alendronate in defect site increased bone healing observing by radiographic study. We suggest that combining stem cell and alendronate can be used for bone regeneration to repair non-union fracture.

P4AM-27-1

MICROARRAY ANALYSIS PROVIDES INSIGHT INTO EARLY STEP OF PATHOPHYSIOLOGY OF MOUSE ENDOMETRIOSIS INDUCED BY AUTOTRANSPLANTATION OF ENDOMETRIUM

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The aim of this study is characterizing the biochemical alterations that occur in early development of the mouse endometriosis model. The endometriosis model was induced by autotransplantation of endometrium. Peritoneum only (excluding the transplant) was obtained 24, 48 and 96 hours after the autotransplantation and subjected to microarray analysis. To interpret the large amounts of data generated and enable a functional analysis, genes were classified using Gene Ontology (GO) and Medical Subject Heading (MeSH) terms. Of the upregulated genes, those involved in the inflammatory response, leukocytes, cell adhesion and extracellular matrix were enriched 24 and 48 hours after autotransplantation. Those of cytokines, antibody-producing cells and inflammation were enriched after 96 hours. These results suggest that the factors occurring during early development of endometriosis are increase in adhesion molecules and inflammatory responses. Interestingly, analysis using GO and MeSH provided differing information. Particularly, MeSH showed a link between an anatomical and diseased phenotype with common genes found to be upregulated. Analysis with MeSH can provide meaningful information and contribute to further interpretation of microarray results in physiological researches.

P4AM-27-2

PROTEOMIC ANALYSIS OF THE HEAT SHOCK AND OSMOTIC STRESS RESPONSE IN LABORATORY ACCLIMATED MUSSELS (*MYTILUS TROSSULUS* AND *GALLOPROVINCIALIS*)

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M. galloprovincialis is better adapted to higher temperatures than *M. trossulus*, but the latter one is more tolerant towards low salinity. These differences may limit the distribution ranges of the congeners. We used a proteomics approach to characterize protein expression profiles in response to heat and osmotic stress. After acclimation to 11degC animals were exposed to 24 degC, 28 degC, and 32 degC or to 850 and 700 mOsm seawater. Animals recovered for 24 h before their gill tissue was prepared for protein analysis. Proteins were separated using 2D gel electrophoresis. Differentially expressed proteins were excised and prepared for mass spectrometry (MS). Matrix-assisted laser desorption ionization tandem time-of-flight MS was used to identify differentially expressed proteins. Two major groups of proteins were identified to be upregulated with temperature stress: heat shock protein 70 (Hsp70) and Hsp24. *M. trossulus* showed a limited tolerance of Hsp sysnthesis at higher temperatures. The osmotic stress response was distinct between the two congeners: greater changes in global protein expression were detected in *M. trossulus* (11% of the 477 detected protein spots) than in *M. galloprovincialis* (6%), mostly due to differences in proteins whose expression was down-regulated.

P4AM-27-3

EFFECTS OF BODY COOLING ON MYOCARDIAL PROTEIN EXPRESSION DURING EXPERIMENTAL HEATSTROKE: A FUNCTIONAL PROTEOMIC STUDY

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The aim of this study was to identify the cardiac proteins that were involved in heatstroke-induced cardiac dysfunction by using 2D gel electrophoresis coupled with mass spectrometry.

We compared the precipitate fractions of heart samples obtained from normothermic rats and untreated or body cooling treated rats and detected spots which changed more than 2-fold in the expression levels. The increased spots during heatstroke were identified as NADH dehydrogenase 1 subcomplex 10-like protein (NDUFA10) and phosphorylated alpha B crystallin. The decreased spots during heatstroke were identified as annexin III, HSP 27, adenine phosphoribosyl transferase APRTase, HSP20, cytochrome C oxidase polypeptide Vb mitochondrial precursor (MTCO1), NDUFA5, myosin heavy chain cardiac muscled α isoform (MYHC-α) and NDUFA10. Body cooling in addition to restoring the appropriate levels of HSP27, 20, APRTase, MTCO1, annexin III, NDUFA5 and MYHC-α enhances preservation of α- B crystalline and maintains appropriate cardiac function during heatstroke. This study demonstrated that the failing hearts during heatstroke were characterized by reduced mitochondrial activity and loss of the structural integrity of the cardiomyocytes and reduced anticoagulation which can be improved by body cooling.

P4AM-27-4

EFFECTS OF VARIOUS EXERCISE PARADIGMS ON THE GENE EXPRESSION PATTERN IN HUMAN LEUKOCYTES

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Exercise has broad physiological effects, which depend on the intensity, duration and chronicity of exercise. We investigated the broad spectrum of exercise effects by performing genome-wide oligo-nucleotide microarray experiments on leukocytes from human subjects exposed to a series of exercise paradigms, i.e., acute severe exercise, acute moderate exercise, exercise training, and deconditioning at the end. Our major findings were: i) different exercise paradigms, especially exercise training, predominantly down-regulated leukocyte gene expression, ii) 4-week deconditioning was relatively ineffective in reversing 8-week training effects, iii) severe exercise predominantly upregulated pro-inflammatory genes, iv) the effect patterns of acute moderate exercise and exercise training were similar, v) exercise training preferentially down-regulated genes related to metabolism and protein production. We further compared the leukocyte metabolic conditions between athletes and nonathletes and found that athlete neutrophils had higher mitochondrial membrane potential and lower spontaneous apoptosis rate. Taken together, leukocytes differentially respond to various exercise paradigms by regulating a large number of genes, especially those related to metabolism and protein production.

P4AM-27-5

TRANSCRIPTOMIC ANALYSES OF MAMMALIAN TISSUES CHARACTERIZING THEIR COMMON AND SPECIFIC PHYSIOLOGICAL ROLES

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The aim of this study is to characterize the transcriptomes of mouse tissues with the serial analysis of gene expression (SAGE) method. We identified housekeeping genes constantly expressed in all tissues. Moreover, most of these genes were not regulated by experimental conditions such as steroid hormones, adrenalectomy and gonadectomy. In addition, we report previously postulated housekeeping genes such as peptidyl-prolyl cis-trans isomerase A, glyceraldehyde-3-phosphate dehydrogenase and beta-actin, which are expressed in all the tissues, but with significant difference in their expression levels. We have also identified genes uniquely detected in each of the 15 tissues and other tissues from public databases. The results reveal several tissue-specific genes highly expressed in testis and pituitary gland. Furthermore, the main function of tissue-specific genes expressed in liver, lung and bone is the cell defence, whereas several keratins involved in cell structure are exclusively detected in skin and vagina. These results show the common and specific roles of each tissue.

P4AM-27-6

COXPRESdb: A DATABASE OF COEXPRESSED GENE NETWORKS FOR HUMAN, MOUSE AND RAT

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A database of coexpressed gene sets can provide valuable information for a wide variety of experimental designs, such as targeting of genes for functional identification, gene regulation and/or protein-protein interactions. Coexpressed gene databases derived from publicly available GeneChip data are widely used in Arabidopsis research, but platforms that examine coexpression for higher mammals are rather limited. Therefore, we have constructed a database, COXPRESdb (coexpressed gene database) (http://coxpresdb.hgc.jp), for coexpressed gene lists and networks in human and mouse. Coexpression data could be calculated for 19 777, 21 036 and 11912 genes in human, mouse and rat, respectively, by using the GeneChip data in NCBI GEO. COXPRESdb enables analysis of the four types of coexpression networks: (i) highly coexpressed genes for every gene, (ii) genes with the same GO annotation, (iii) genes expressed in the same tissue and (iv) user-defined gene sets. When the networks became too big for the static picture on the web in GO networks or in tissue networks, we used Google Maps API to visualize them interactively. COXPRESdb also provides a view to compare the coexpression patterns of the three species to estimate the coexpression conservation in mammals.

P4AM-27-7

GENE NETWORK INFERENCE IN MALIGNANT MELANOMA

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Despite recent progress in cancer research, our understanding of the molecular switches that regulate tumour growth and survival remains incomplete, limiting the development of new therapies. A novel approach to understanding the control of cellular processes that is proving successful in several cell types is to infer the underlying gene regulatory networks1,2, showing putative cause and effect relationships between large numbers of molecules within cells. These transcriptional networks are built using high-quality gene array data gathered in a large number of siRNA disruptant and time-course experiments.

We recently proposed a new method to infer gene regulatory networks from these microarray data using a differential equation modelling approach. Differential equation models relate the rate of change of expression of each gene to the expression levels of all other genes in the network, and are particularly well suited to data sets including time series data. The approach makes no prior assumption about the distribution of connections in the regulatory network, enabling us to determine the network architecture from the data. We are applying our technique to microarray data from human tumour cell lines, in order to better understand the molecular signals that underlie cancer.

P4AM-27-8

COMPARATIVE TRANSCRIPTOMIC STUDY ON PRECONDITIONING AND ISCHEMIC REPERFUSION INJURY CONDITION OF RAT HEART

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We used express sequence tag (EST) profiling to reveal genetic expression changes of whole heart genome under ischemia-reperfusion injury. The isolated hearts from 8weeks Sprague-Dawley rats were perfused on Langendorff system with three different conditions, control (CON), ischemia-reperfused (IR), and preconditioned before ischemia-reperfuse (IPC). Three cDNA libraries were constructed with CON, IPC and IR treated cardiac group. Afterward we sequenced 6336 EST, 2112 in each group and finally acquired 1402, 1356 and 1351 NCBI matched sequences in CON, IPC and IR group respectively. The acquired sequences were annotated by clusters of orthologous group especially in eukaryotic orthologous group. In macroanalysis, 'replication, recombination and repair' and 'inorganic ion transport and metabolism' related genes were commonly increased in IPC and IR. While 'amino acid transport and metabolism' and 'secondary metabolites biosynthesis, transport and metabolism' related genes were increased only in IR group. In addition, 'nucleotide transport and metabolism' related genes were commonly decreased in IPC and IR group than CON. These mass profiling of cardiac ischemia related genes provide enormous and valuable information to understand the mechanism of IR and IPC and its cure.

P4AM-28-1

MENTAL STRESS AND SLEEP: A NOVEL APPROACH TO UNFOLD THE GENETIC CONTROL OF HEMODYNAMICS IN LARGE ARABS PEDIGREES OF OMAN FAMILY STUDY

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Objectives: To determine heritability and linkage analysis for cardiac and hemodynamic components of blood pressure under resting and stress conditions. Method: 24-hr, beat-to-beat BP, cardiac and hemodynamic phenotypes were obtained in 1124 subjects, from 5 multigenerational and consanguineous Arab pedigrees. Mental and physical stress were evoked by the word conflict (WCT) and the cold pressor (CPT) tests respectively. Heritability analysis was carried out using variance decomposition method. Linkage analysis was carried out using the 400 cM Marshfield marker map. Results: Heritability estimates for daytime SBP and DBP were 0.28 and 0.38, compared sleep h2 of 0.20 and 0.17, respectively. Resting, CPT and WCT h2 for R-R intervals 0.32, 0.36 and 0.40, respectively. Stroke volume, cardiac output, cardiac contractility, left ventricular ejection time showed similar linear increases. Linkage analysis revealed 13 loci with LOD scores > 3 for sleep BP and for WCT cardiac and hemodynamic phenotypes in chromosomes 1, 3, and 8, with clusters of SBP, DBP and RRI at two Loci in chromosome 12. Conclusion: The dissection of blood pressure into its primary components with the use of mental stress provided a robust model for the detection of genetic loci for blood pressure phenotypes.

P4AM-28-2

ACQUISITION OF NOVEL CALCIUM REGULATING ORGAN DURING VERTEBRATE EVOLUTION

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The vertebrates have evolved gills and homologous organ parathyroid that regulate calcium level in the blood of their living environments. In higher teleosts, chloride cells in the skin are known to regulate calcium level, yet molecular mechanisms that control development of chloride cells during the evolution of higher teleosts remain to be investigated. We discuss a possible connection between the evolution of Gcm2 enhancers and acquisition process of a novel calcium-regulating organ. Our studies have revealed that Gcm2 was expressed not only in parathyroid and gills but also in chloride cells of zebrafish. The antisense morpholino of Gcm2 abolished development of chloride cells, suggesting that Gcm2 might be essential for their development. To examine further a role of Gcm2 in development of the chloride cells, we analyzed enhancer elements of Gcm2, and identified two enhancer regions that control Gcm2 expression in chloride cells of zebrafish. Since similar enhancers of Gcm2 in zebrafish have not been found in other vertebrates, Our results suggest that the acquisition of enhancers for the expression of Gcm2 in zebrafish may have contributed to the evolution of chloride cells in higher teleosts.

P4AM-28-3

DEVELOPMENT OF A NONAPLEX SYSTEM AND VALIDATION FOR ITS IN MEDICAL CASEWORK

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Objective: To establish a system for typing STR loci DYS434, Y-GATA-A10, DYS438, DYS439, DYS531, DYS557, DYS448, DYS456 and DYS444 by fluorescence-labeled multiplex PCR technique, by which the genetic data of Hangzhou Han population were obtained. To value its use in forensic science and other related fields. Methods: Primers of STR loci DYS434, GATA-A10, DYS438 and DYS439 were labeled with 6-FAM, DYS531, DYS557, and DYS448 labeled with HEX, DYS456 and DYS444 labeled with TAMRA respectively. The electrophoresis of PCR products was accomplished on the 310 Genetic Analyzer. Results: Each locus was successfully genotyped in all 120 samples. DYS434, Y-GATA-A10, DYS438, DYS439, DYS531, DYS557, DYS448, DYS456 and DYS444 showed 4, 5, 4, 5, 5, 8, 7, 8, 6 alleles, respectively. Gene diversity ranged from 0.4394 at DYS434 to 0.7975 at DYS557. A total of 105 different haplotypes was identified, 97 of them being unique. The haplotype diversity value calculated from all nine loci combined was 0.9968. Conclusion: The results show that the multiplex system of 9 Y-STR will be very powerful for establishing Y-STR database, the paternity testing and mixture stains identification.

P4AM-29-1

A COMPARISON OF STUDENTS' PERFORMANCE IN MULTIPLE CHOICE AND ESSAY QUESTIONS IN THE MBBS STAGE I PHYSIOLOGY EXAMINATION

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Multiple choice questions (MCQ) have long been a common type of assessment due to their reliability, validity and ease of scoring. Another common type of assessment is the essay questions. These have been shown to allow the students more flexibility in their response and reflect their individuality of approach in which interpretative skills can be evaluated. The scores for three hundred and seven (307) students were analyzed. There were one hundred and eleven (111) students in 1997, ninety four (94) in 1998 and one hundred and two (102) students in 1999. Kruskal Wallis analysis of the data revealed no statistically significant differences between the scores in the three years studied, hence the respective MCQ and essay scores were analyzed together. The overall score was 47.33±9.89% in the essay questions and 63.77±9.87% in the MCQ and this showed a statistically significant correlation ($r = 0.622$; $p < 0.01$). The result of this study suggests that the MCQ and essay questions test different aspects of the same knowledge base and that student who performed well in the essays were likely to do well in the multiple choice questions.

P4AM-29-2

STANDARD SETTING: MUCH ADO ABOUT NOTHING?

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The grading system at the Faculty of Medical Sciences, UWI, Mona was changed from absolute grading to standard setting in December 2006. Absolute grading is based on the idea that grades should reflect mastery of specific knowledge and skills. The standard setting defines the boundary between passing and failing grades. This retrospective study was aimed at comparing the performance of students using the absolute grade and standard setting methods in SF25C (Digestive System), SF26C (Endocrine System), SF27C (Cardiovascular System) and HE12B (Health and the Environment). The Wilcoxon's signed rank test for two (paired) related data showed that the median for SF25C and SF27C were in the pass (50% to 64%) range while it was in the honors (64% to 74%) range for SF26C and HE12B using the absolute grading system. In the standard setting system, the median for SF25C and SF26C were in the pass range while those for SF27C and HE12B were in the honors range. The Mann Whitney U test did not show any statistically significant difference between the scores of the students in both systems.

P4AM-29-3

PHYSIOLOGY LEARNING IN THE NEW PARADIGM OF MEDICAL EDUCATION, SCHOOL OF MEDICINE SRIWIJAYA UNIVERSITY

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The curriculum of Indonesian medical education has transformed fundamentally. The strategy has changed from teacher centered, departmental, and subject based into Harden's SPICES strategies. Undergraduate program School of Medicine Sriwijaya University, implemented the new curriculum through 22 sequential integrated blocks. The alteration has pulled out department's authorities in learning process and resulting apprehensive from the staff about their existence including the physiologist. The paper reports the role of physiology in our new curriculum.

The apprehensive of physiologists is not proved. Physiology concept is already integrated which is adapted easily in SPICES strategies. Department of physiology develop into activator and liaised among departments for integrated learning process. Physiology has special site for every block. The clinicians are more aware for the role of physiology. Even more, there is a block named homeostasis and metabolism as a character of physiology that carried out as a gate in learning clinical sciences. The new curriculum work up physiology into more exist and more dominant. The apprehensive for lose role is unreasonable. The physiologist must promote their important role for medical education in their own institution

P4AM-29-4

THE CHALLENGE OF CHANGING THE CURRICULUM IN AN ESTABLISHED MEDICAL COLLEGE

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Khartoum medical college started in 1924 as the first medical school in Sudan and the second in Africa. Since it was initiated no major changes has been introduced to the curricula although many slight modifications and additions has been included .The intake of the faculty was from the top students in a general state examination and the graduates were well recognized as efficient well trained doctors. Those attributes of its graduate constituted a real challenge for any movement to change the curriculum. Some of the staff especially those who were exposed to medical education training felt the need to that change. Mainly, to include the new approach in medical teaching and training which the evidences proved its effectiveness. A curriculum committee was formed and was supported by the recommendation of a faculty reform conference. In this presentation the major changes in the curricula and their rational are going to be highlighted. The difficulties and the way the committee managed to overcome these and to implement the curriculum will also be discussed .

P4AM-29-5

A REPORT ON STRESS AMONG FIRST YEAR STUDENTS AT MELAKA MANIPAL MEDICAL COLLEGE (MANIPAL CAMPUS), MANIPAL, KARNATAKA

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Emotional disorders are common in medical students. However, studies on emotional disorders among students in Indian medical schools are very few. The present study was undertaken to determine the prevalence of emotional disorders and the sources of stress among first year Malaysian students at Melaka Manipal Medical College (MMMC), (Manipal Campus), India. The General Health Questionnaire (GHQ) was used as the screening instrument. Based on the score, the prevalence of emotional disorders was determined. The sources of stress in students were identified by asking them to respond to a questionnaire which had items categorized under academic and non-academic problems. It was found that the prevalence of emotional disorders among the students was 37.3%. Among the sources of stress, frequent examinations and information overload were found to be the greatest sources of stress among academic problems. Among nonacademic problems, limited time for recreation and home-sickness were found to be the greatest sources of stress. The present study revealed that academic problems were greater sources of stress in first year medical students compared to non-academic problems. The study provided scope for adopting strategies intended to reduce students' stress.

P4AM-29-6

LEARNING CLIMATES CAN BE IMPROVED BY USING THE PRINCIPLES OF BRAIN/MIND BASED LEARNING: THE IMPACT OF HEMISPHERIC PREFERENCES

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Learner-related features, such as cognitive styles, hemispheric preferences, learning styles have recently received an increasing attention in the process of learning/ teaching. The hemispheric preferences of the first year students of Marmara University Medical Faculty were determined by using the Human Information Processing Survey of Torrance & Taggart (1984) and compared with the electrophysiological data recorded as Event Related Brain Potentials (ERP). Moreover, a six-week problem-based learning (PBL) program, which was re-designed by considering the principles of brain/mind based learning, was implemented in these students and the results were compared with those of the previous PBL program. Most of the students (59.9 %) preferred both hemispheres, 28.9 % preferred the right, while 11.2% declared the left hemisphere as the preferred hemisphere, and these results were partially confirmed with the ERP-based electrophysiological data. The results of the study demonstrate that learning climates can be improved by using the principles of brain/mind based learning, including hemispheric preferences, and this improvement may result in increased satisfaction of both the teachers and the students, while a higher academic success could be reached.

P4AM-29-7

FORMULATING QUESTIONS FOR PHYSIOLOGY LEARNING

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Certain aspects of Physiology present more difficulties for students learning the subject. The dynamic nature of Physiological sciences has several areas that commonly challenges the student thinking through Physiology. These include mechanisms of 'cause and effect' , quantitative and priority in homeostatic systems.

In the Malaysian Medical Schools, these hurdles are compounded by the need to have an effective command of the English language. The small group tutorials give valuable opportunites for students to tackle and think through Physiology interactively with their tutor.

Diverse styles of tutorial questions have been designed to highlight and resolve main areas that challenge the student of Physiology. The careful formulating of questions can effectively address common misconceptions and lead to better insights into Physiological processes. Working through good questions can also enhance the ability of the student to integrate their learning to understand Physiological homeostatic mechanisms in vivo.

Cheng HM (2003) Misconceptions in Physiology. Prentice Hall, Pearson Education Malaysia.

P4AM-29-8

USING OLDER ORIGINAL RESEARCH ARTICLES AS AN EDUCATIONAL TOOL IN THE TEACHING OF PHYSIOLOGY

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Journal clubs are an effective means of teaching scientific principles & methodology to science students. Traditionally journal clubs involve discussions on recent research publications but a retrospective view of the literature may be just as valuable. Older original literature may help students better conceptualise certain scientific topics. For example, the physiological adaptation to high altitude can be explored by a tutorial referencing the innovative 1946 work of Houston & Riley (1). The paper documents a simulated ascent to high altitude & demonstrates how scientists advanced the existing knowledge with creative experimental designs thus providing evidence that high altitude acclimatisation consists of a series of integrated physiological responses. A number of probing questions guide the students through an annotated figure from this paper, the information from which can be supplemented with what is currently known about high altitude adaptation. High altitude physiology remains topical & this seminal study can be used as a learning foundation: an invaluable educational tool for both undergraduate & postgraduate students.

1. Houston C.S., Riley R.L. Respiratory & Circulatory Changes during Acclimatization to High Altitude. Am J Physiol 149(3):565-588

P4AM-29-9

PHYSIOLOGY LECTURES TO VOCATIONAL STUDENTS IN THE PROFESSIONS ALLIED TO MEDICINE; SUCCESSES, FAILURES AND RECOMMENDATIONS

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Physiology is often taught as a vocational subject to students in the Professions Allied to Medicine such as Technical Staff, Nursing, and Physiotherapy. Physiology instruction of these particular cohorts presents many unique challenges. Classes are often large, and composed of students who have little previous scientific experience and may contain mature students, many years out of formal education.

The aim of this study is to investigate the problems encountered by such cohorts in Biomedical Science lectures. Survey and interview methodologies are employed to explore attitudes to learning in lectures, identify student requirements of lecturers and investigate the difficulties encountered by students while learning in large lecture classes.

The survey results indicate that students at this level favour purely didactic teaching and find interactivity in lectures intimidating. To overcome this hostility to active learning and maximise the educational value to the student of the lecture, we propose a set of guidelines. These include giving clear objectives and requirements to students, compassionately encouraging active participation through questioning and sustaining student interest through the use of modern teaching aids and innovative techniques.

P4AM-29-10

FREQUENT DISCUSSION INTERRUPTED LECTURE VS LECTURE FOLLOWED BY DISCUSSION IN PHYSIOLOGY: A TELEPHONIC SURVEY OF STUDENTS' PERCEPTION

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Context: Learning physiology, a matter of conception, can be achieved by frequent discussion. Objective: To compare the effectiveness of frequent discussion interrupted lecture (FDIL) and lecture followed by discussion (LFD) in learning physiology. Design, Setting, and Participants: A telephonic survey among randomly selected 22 medical students during November'08 in Dhaka Medical College, Dhaka. The participants were exposed to two types of lectures taken in physiology teaching. In one type the tutor taught the students for 1 hour followed by half-hour discussion among peer-students and teacher. In other type the tutor taught for 20 min followed by 10 min discussion among peer-students and teacher, this lecture-discussion cycle was repeated for 3 times in a 1.5 hour-session. The participants were asked to prefer one of these lecturing systems as an effective learning method. Ethical issues: Students were kept anonymous and free to give their opinion. Results: Eighteen (81.82%) and 4 (18.18%) students preferred FDIL and LFD respectively as an effective method for learning physiology. Conclusion: FDIL was the preferred method for learning physiology. Key words: Learning, Physiology, Facilitation.

P4AM-29-11

ON FUTUROLOGY OF PHYSIOLOGY IN CONTEXT OF ANTHROPOLOGY

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INTRODUCTION: Alfred Nobel destined 1895 prices for physics, chemistry, "domain of physiology or medicine". Human has central position (Aristoteles, Platon, Kant: physiol. anthropology). Physiol. is "mother of many...life sciences" (M Visscher/USA: plen-lect IUPS New Delhi Proc X 13 1974). CONCEPTION: Living system (from microorganisms to human) includes immense information. Future integrative anthropo-physiol to an integral anthropology must reflect essential holistic & multidimensional knowledge incl. 1. Psycho-somatic-physical (molecular to whole body) psychic mental spiritual functions (perception to consciousness); 2. ecological-influences of geo- (atmo-/hydro-/litho-), biospheres; 3. social-of society (family/school/work, etc.) reflected in 4. general physiology, i.e. philosophical (normative: epistemology/ethics/aesthetics), medical (normal/pathological). CONCLUSION: Proposed fundamental disciplines [1-4] could be base of a really human integrative physiology in context of better health, see UNO-Agenda 21. In memory of moral support of Profs R Aron/France, H Bethe*/USA, S Bergstroem*/Sweden, Y. Ikemi/Japan, Sir J Kendrew*/GB, CFvWeizsaecker/FRG (Hon.ICSD-members, *Nobel Laureate). Ref: Michailov, Neu: World Congr Phil Seoul 338-9, 363-5/2008; Faseb J 19/4, A1355/2005

P4AM-29-12

ON MEDICAL AND BIOETHICS IN PHYSIOLOGY

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INTRODUCTION: Physiology is fundamental discipline for all biological, medical, ecological sciences. According to Immanuel Kant the human has obligations to himself & sub-human beings. CONCEPTION: Future education in bio-medical sciences, beginning with physiology needs elementary essential information on epistemology. In this context ess. approaches to research include H relevancy and animal protection. Not only use of human (H) cell cultures, but also H surgical tissue (HST; enormous volume of genitourological, gastrointestinal, vascular, etc. tissue is existing from operations) could support H relevancy of observations and reduce ess. animal experiments. Fundamental differences in physiological reactivity of animal and H tissue are observed [1-3]. CONCLUSION: Large application of HST in pharmaco-physiological res. could support H relevancy of results and reduce animal experiments in accordance with medical and bioethics. In memory of moral support of Profs. J Axelrod*/USA, H. Jonas/USA, L. Pauling*/USA, A. Prokhorov*/Russia, J.H. Schroeder/Austria, M. Strell/FRG (Hon.ICSD-members, *Nobel Laureate). Ref.: [1] Michailov et al Urol int 38/4, 234-242/1983; [2] Neu et al Berichte Bayer Ges Frauenheilkunde, Muenchen, Alete 243-250/1989; [3] Welscher et al Physiol Res 48, S138/1999

P4AM-29-13

THE GLOBAL ROLE OF IUPS IN ENHANCING TEACHING PHYSIOLOGY

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Physiology - widely taught in schools and universities - underpins medicine, dentistry, nursing; health, sport, veterinary, aquacultural & agricultural sciences. Since a workshop in India in 1977, IUPS has supported the development & dissemination of evidence-based educational practices in physiology, informed by a growing international research-based literature. Educational knowledge & skills in teaching physiology are disseminated in presentations, posters & workshops at international, regional, national & local meetings; in journals, books & on the internet. Since 1986, well-attended teaching workshops of several days have been associated with all but one Congress. The first (1986) was at Jenolan Caves, Australia; subsequently in Kuopio, Finland; Inverness, Scotland; Repino, Russia; Lincoln New Zealand; Pali Mountain, USA. The next is in Kobe. Reports are published in Advances in Physiology Education. A book, on effective, economical experiments produced in 1990 will soon to be replaced with an updated, expanded web version. Hands-on workshops are regularly associated with regional meetings (e.g. FAOPS); well-attended stand-alone workshops have also been held in Africa, Asia and Oceania, Europe and the Americas.

P4AM-29-14

ON PHILOSOPHICAL FUNDAMENTALS OF PHYSIOLOGY

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INTRODUCTION: Similar to philosophy (regina scientiarum) is physiology science of science-for functions from microorganisms to human and influence of geo-/ biospheric, social factors. Immense medical and ecological problems need renewal of physiology. CONCEPTION: Creation of 1. integrative general physiology reflecting common laws and (homeostatic) properties of living system for all organisms followed by 2. general microbial, phyto-, zoo, anthropo-physiology based on 3. special disciplines such as bacterial, mycological, entomological, ornithological physiology, ethology, etc. This needs common commissions and congress sessions between scientific unions of physiology (IUPS), philosophy (FISP, IAB, ESPMH), others (FIGO, ISIM, SIU, etc.). CONCLUSION: 110 years after 1st Int. Congr. Phys. 1889 & 55 years IUPS it is last time to found an interdisciplinary Int. Journal of Physiology, also to support interdisciplinary cooperation in context of a better health, see UNO-Agenda 21. In memory of moral support of Profs Sir J Eccles*/AUS, K. Fukui*/Japan, G Kahn-Ackermann/Gen Secr aD Eur. Council/FRG, K. Lorenz*/Austria, Sir K Popper/UK (Hon. ICSD-members, *Nobel Laureate). Ref: Michailov, Neu et al: Int J Psych Berlin 43, 154&248/2008; Br J Urol SIU-Honolulu 94 UP-20.17/2004

P4PM-1-1

THE FORCE DECLINE DURING SUSTAINED MAXIMAL VOLUNTARY CONTRACTION IS ACCOMPANIED WITH THE AROUND 10-Hz OSCILLATIONS OF PHYSIOLOGICAL TREMOR

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Decline of the supra-spinal function is one of the important factors about the decline of force output during sustained maximal voluntary contraction. Physiological tremor (PT) is defined as an involuntary and continuous oscillation in every limb segment of a healthy human, and it is accompanied with a peak oscillation frequency of around 10 Hz (8-12 Hz) which reflect the supra-spinal function. The purpose of this study was to investigate that the PT during sustained maximal voluntary handgrip task. Nine healthy males (21±2 years) performed maximal voluntary handgrip task for 30-sec. PT signal of extension and flexion in the distal of middle finger was measured by an acceleration sensor. PT was analysed by the maximum entropy method, and it was compared with the ratio of force decline. At the end of the task, the handgrip force decreased to around 57% of the initial value. The ratio of force decline was not correlated with total power (1-50 Hz) of PT ($r = -.58$, n.s.). However, the ratio of force decline was significantly correlated with the sum of power of around 10 Hz ($r = -.78$, $P = .01$). These results indicate that the force decline during sustained maximal voluntary contraction is accompanied with the around 10 Hz oscillations of PT which reflect the supra-spinal function.

P4PM-1-2

ARM PEDALING MODULATES REFLEX RESPONSES FROM ANKLE DORSIFLEXOR AFFERENTS TO KNEE EXTENSOR MUSCLES

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Electrical stimulation of common peroneal nerve (CPN) was demonstrated to induce a short latency reflex response in the knee extensor muscles which was suggested to be mediated by spinal propriospinal circuitries. We investigated the extent to which the CPN-induced reflex is modulated by activation of spinal central pattern-generating elements (CPGs) controlling the upper limb. Subjects sat in an experimental chair and were asked to perform arm pedaling at 60 or 90 rpm using a stationary ergometer while performing weak isometric contraction of the vastus lateralis (VL). Control reflex from the VL was evoked without arm pedaling. Reflex responses were evoked by stimulating the CPN (1.2xMT, 2 pulses at 333Hz) at the level of the neck of the fibula. The facilitatory (mean latency 33.0±4.4 ms) and suppressive (46.7±3.4 ms) component in the VL were identifiable following CPN stimulation. The facilitatory and suppressive component was significantly increased and decreased during arm cycling compared to the control, respectively. The degree of modulation for both responses was graded by the cadence of arm pedaling. These findings suggest that that the CPN-induced reflex in the VL was under the control of the CPG system for the upper limb.

P4PM-1-3

EFFECTS OF MEDIUM LATECNCY SOLEUS AND TIBIALIS ANTERIOR STRETCH REFLEXES WITH VIBRATION DURING FEET PERTUBATIONS

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<Purpose> The purpose of this study is to examine which crural muscle spindle or ankle joint afferent signal contributes to standing postural control. <Methods> Ten male subjects(23-35 years) stood with eyes closed on a movable platform(forward; FT or backward; BT). Intensity of platform was 400ms and 4.6-6.0cm. The vibrators(92 Hz) were applied to malleolies or Achilles and tibialis anterior(TA) tendons. Soleus(Sol) and TA EMG responses during feet perturbations of about 40 times were collected under control, crural vibration(CV) and malleoli vibration(MV) conditions. EMG responses were classified as short-(SLR) and medium latency reflex(MLR) on Sol and MLR on TA. The Sol SLR, MLR and TA MLR areas and their onsets were measured. Statistical analyses were performed by AVOVA and post hoc test($p<0.05$). <Results> On the MV and CV, Sol SLR, MLR and TA MLR onsets were significantly delayed($p<0.01$). On the MV, Sol MLR and TA MLR areas significantly increased and decreased($p<0.01$), respectively. <Conclusion> It was thought that Sol activity increased to mainly act on the forward fall even if the afferent signal from ankle joint occurred functional decrement but TA activity decreased so that other muscles might compensate to prevent the backward fall.

P4PM-1-4

RESPONSES OF PRIMARY ENDINGS TO THE THREE TYPES OF INTRAFUSAL FIBERS

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Triceps surae muscle of anesthetized cats was dissected from surrounding tissue and was stretched sinusoidally for 0.5 sec by CF or upward FM vibration. Ia spikes at dorsal root and intracellular potential of a motoneuron are recorded during muscle vibration after laminectomy. Mode of frequency was altered between 10 and 120Hz in 0.5 sec. The vibration was repeated three trials every 1 sec. Ia spikes and EPSPs of motoneuron was elicited on each phase of vibration with pre-arranged amplitude. Only one Ia spike was elicited on early phase of initial trials of FM Vibration but could not be elicited any spikes at the same phase after second trial. Primary ending during 10 to 120Hz FM vibration had an adequate frequency of 80Hz and the endings showed velocity sensitivity.

It is possible that the different visco-elastic properties of the three kinds of intrafusal muscle fiber may allow us to distinguish responses of a static nuclear bag fiber from those of a dynamic nuclear bag fiber or nuclear chain fibers by using various modes of stretch. It is reported that FM vibration influences particularly the velocity sensitivity of intrafusal muscle fibers and responses of primary endings can be separated into categories corresponding to the three types of intrafusal muscle fibers.

P4PM-1-5

SPINAL NEURAL MECHANISMS IN THE GENERATION OF COORDINATED QUADRUPEDAL LOCOMOTION IN RABBITS

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In quadrupeds, coordinated movements between left and right limbs and between forelimb and hindlimbs are essential for locomotion. This study aimed to know organizing principles of the spinal neural system involved in the generation of quadrupedal locomotion in rabbits. In decerebrate rabbits, stimulation to the mesencephalic locomotor region evoked quadrupedal locomotion which was characterized by in-phase hopping of bilateral hindlimbs and left-right alteration of the forelimbs. In each locomotor cycle, the hindlimbs exhibited strong backward extensions, while the forelimbs showed simple flexion-extension movements. This may indicate that cervical and lumbar spinal central pattern generators (CPGs) respectively for forelimb and hindlimb locomotion are constituted in a different matter. Despite such differences, however, locomotor cycles of the forelimbs were always synchronized with those of the hindlimbs when the hindlimb hopping movements were evoked. This suggests that the forelimb and hindlimb CPGs are functionally coupled during locomotion and this coupling is possibly mediated via ascending propriospinal systems. Such divergent neural coupling in the spinal cord would be suitable for elaborating wider motor synergies of all limbs during locomotion in rabbits.

P4PM-1-6

WALKING-RELATED AFFERENT FEEDBACK FROM THE LEG INDUCES SHORT-TERM PLASTICITY OF UPPER LIMB SPINAL MONOSYNAPTIC REFLEX PATHWAYS IN HUMANS

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Recently, a prolonged (~20 min) suppression of the Hoffmann (H-) reflex in leg muscle was demonstrated to take place following rhythmic arm movement (Javan and Zehr 2008). However, it is less clear whether the movement-related afferent feedback or descending commands play a more important role in the prolonged suppression of the H-reflex. Therefore, we investigated amplitude modulation of the H-reflex in the flexor carpi radialis (FCR) during and after various durations of passive leg stepping (5, 10, 15, and 30 min). Passive stepping was performed employing a robotic gait trainer system (Lokomat). The H-reflex in the FCR was elicited by applying electrical stimulation to the median nerves. The amplitude of the FCR H-reflex was significantly suppressed during passive stepping, and was retained for up to 12 min after stepping for 30 min. The duration of H-reflex suppression was graded with that of passive stepping. No significant suppression of the H-reflex was noted after passive stepping for 5 min. These findings suggest that the voluntary descending command is not an essential factor for prolonged suppression of the FCR H-reflex, and that sustained walking-related afferent feedback from the leg plays an important role in the short-term plasticity of FCR H-reflex circuitry.

P4PM-1-7

INHIBITION OF MYOSTATIN EXPRESSION VIA GENE TRANSFER CAN SELECTIVELY INCREASE MUSCLE MASS IN COMPLETE SPINAL MICE

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The relationship between the adaptations in the hindlimb musculature and the ability to step after a spinal cord injury (SCI) in mammalian systems is largely unknown. The potential for the maintenance of muscle mass to improve the ability of SCI subjects to recover stepping ability and/or for the spinal cord to re-learn to control locomotion has not been examined in detail. Here, we demonstrate that blocking the expression of myostatin, a negative modulator of skeletal muscle growth, using an AAV-follistatin viral vector in the hindlimb muscles of complete spinal cord transected (ST) adult mice, can induce selective muscle hypertrophy in vector-injected hindlimb muscles, i.e., medial gastrocnemius and vastus lateralis, with lesser hypertrophy observed in some adjacent muscles, i.e., the lateral gastrocnemius, plantaris, and rectus femoris, and no effect in other muscles, i.e., tibialis anterior, soleus, and adductor longus. While there were no significant differences in hindlimb kinematics in ST mice with and without muscle hypertrophy bilaterally, of only two extensor muscles after 6 weeks of step-training, this may reflect the treatment of too few muscles or the absence of a functional effect of increases muscle mass.

P4PM-1-8

ANATOMICAL BASIS FOR CEREBELLAR FUNCTIONAL LOCALIZATION: LOBULES, STRIPES, MICROMODULES AND AXONAL CONNECTIONS

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Different areas of the cerebellum are related to various kinds of movement control. However, how this cerebellar functional localization is organized has not been fully understood beyond the three classical subdivisions of the cerebellum (vestibulocerebellum, spinocerebellum, cerebrocerebellum). The cerebellar cortex is divided transversely by its lobular folding and longitudinally into multiple stripes by expression pattern of molecules such as aldolase C (=zebrin II) in a population of Purkinje cells (PCs). These cortical divisions may be related to the functional localization by having certain organization in input and output connections to determine the functional localization. Therefore, we studied projection patterns of climbing fibers (CFs), mossy fibers and PC axons systematically in terms of the lobulation and aldolase C stripes by single axonal tracing and three-dimensional terminal mapping in the rat. These projections showed clear relationship to cortical divisions. Particularly, projections of CFs and PCs formed a micromodule by connecting small cortical divisions to corresponding small areas in the cerebellar nuclei and inferior olive, which are then connected to specific areas in the CNS. Support: KAKENHI 20300137.

P4PM-1-9

NEURONAL ACTIVITY IN PRIMARY MOTOR CORTEX OF A JAPANESE MONKEY DURING QUADRUPEDAL V.S. BIPEDAL LOCOMOTION ON THE TREADMILL

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To elucidate cortical mechanisms related to the control of bipedal (Bp) locomotion in humans, we trained a Japanese monkey to walk on a moving treadmill belt with transforming its locomotor patterns from quadrupedal (Qp) to Bp and vice versa. We then recorded single-unit activity from primary motor cortex (M1) of the monkey and compared it between two modes of locomotion. Tungsten microelectrodes were inserted into M1 trunk/hindlimb regions using a custom-made micro-manipulator. EMG activity was simultaneously recorded from a total of 15 muscles in the trunk and four limbs. To date, 72 neurons were recorded and analyzed. During Qp locomotion, majority of neurons modulated their discharge phasically, or phasically and tonically time-locked to the step cycle. Frequency of the peak activity ranged from 17.4 to 98.4 (42.2 ± 24.4) spikes/s at a speed of 1.0 m/s. When the monkey converted its locomotor pattern from Qp to Bp, almost all the neurons increased their discharge frequencies (60.2 ± 24.5 spikes/s) with the firing period shortened. These results suggest that the monkey M1 provides common neural substrates for the control of both Qp and Bp locomotion, but the latter mode requires stronger activity of M1, a part of which is sent to the brain stem and spinal cord.

P4PM-1-10

HYPOTHALAMIC REGULATION OF MUSCULAR TONUS THROUGH THE OREXINERGIC AND NON-OREXINERGIC SYSTEMS

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The hypothalamic area is involved in, as well as regulation of autonomic nervous systems or sleep-wake regulation, in the regulation of muscular tonus or of locomotion. We have recently shown that the orexinergic neurons in the lateral hypothalamus (LHA) have a role in maintaining muscular tonus through activating the GABA ergic neurons in the substantia nigra pars reticulata, which inhibit the muscular atonia system in the pedunculopontine tegmental nucleus. Using urethane anesthetized rats, we examined effects of electrical stimulation to the hypothalamus on the soleus muscular tonus. Blood pressure and EEG were also recorded. The tonic increase of muscular tonus from 70 seconds to more was evoked mainly from the LHA which was rich in the orexinergic neurons. The phasic activation accompanied by the rhythmic leg movement was evoked from the dorsal part of the hypothalamus including zona incerta and anterior hypothalamus which were devoid of the orexinergic neurons. The most effective are for inducing muscular activity was dissociated from the area for inducing blood pressure changers or EEG activation. These results suggest that the different areas in the hypothalamus have different roles in the regulation of muscular tonus which were closely involved in locomotion.

P4PM-1-11

ESTIMATION OF RAT LOCOMOTION PROCESS THROUGH MULTIRECORDING OF EPIDURAL POTENTIALS

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Epidural recordings are a possible method to develop a low-invasive brain-machine interfaces (BMIs) that reintegrate motor functions of physically changed persons. To examine the possibility in rats, we simultaneously measured epidural potentials at six electrodes (15-20 kΩ) on the brain surface and paws motion during exploratory locomotion in an open-field cage (45 cm square) provided constant white noise. Focusing on the functional relations among the recorded signals, the signal differences were analyzed off-line using short-time Fourier transform, and the power spectra were classified into the motion (e.g., static, right/left forepaw, or rearing). The highest accuracy of the estimation was approximately 65-75% in two-motion classifications at the electrodes' locations by using an artificial neural network, being 75-80% by using a support vector machine. In addition, specificity of electrode position was not observed and daily estimations were varied. On the basis of these results, we further discuss the information characteristics of the recorded epidural potentials behind the difference between the discrimination circuits and the feasibility of low-invasive BMI using epidural electrodes.

P4PM-1-12

POSTURAL ADJUSTMENTS TO COMBINED VISUAL SCENE VELOCITY AND INCLINED BASE OF SUPPORT

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In this study we have explored the relative contributions of surface tilt and visual velocity on the process of realignment to the vertical. Pitch motion of the visual field was presented at varying velocities to eight subjects (29.9 ± 2.8 yrs) standing quietly on a 3 deg toes-up tilt of the base of support. Trials in which the platform was inclined consisted of a pre-incline period of 5 sec when the support surface was held horizontal, an incline period of 30 sec when the surface was maintained in the toe-up position following the 30 °/s ramp, and a post-incline period of 30 sec when the surface slowly returned to the horizontal at a constant velocity of 0.1 °/s. Onset of virtual scene and platform movement were synchronized in all trials. We hypothesized that motion of the visual world would affect the ability to return to the initial vertical position while the surface slowly returned to the horizontal.

P4PM-1-13

CHARACTERIZATION OF ZEBRAFISH MOTILITY MUTANT DEFECTIVE IN VOLTAGE-GATED SODIUM CHANNEL

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Zebrafish (Danio rerio) is a good vertebrate model to study motor development. A new mutant, which was isolated by ENU mutagenesis does not respond to mechanosensory stimulation at 2 day post fertilization (dpf). In teleost and amphibian, Rohon-Beard (RB) neurons, a subset of HuC-positive neurons in dorsal spinal cord, function as primary mechanosensory neurons at early developmental stage, but eventually disappear up to 4 dpf by programmed cell death and replaced by dorsal root ganglion neurons. This cell death of the RB neurons is induced in an activity-dependent manner. Our in vivo whole-cell recordings revealed that the RB neurons in the mutant did not exhibit full spikes following current injections due to reduced voltage-gated sodium channel function. Agreeing with the reduced activity, many mutant RB cells did not undergo apoptosis and survived at 4 dpf. Thus, the present study suggests that the mutant has defects in the voltage-gated sodium channel in the RB neurons, thereby mitigating activity-dependent cell death and touch-induced behavior.

P4PM-2-1

CIRCADIAN LOCOMOTOR ACTIVITY AND FEEDING ACTIVITY RHYTHM IN JAPANESE SEA CATFISH, PLOTOSUS JAPONICUS

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To find out whether or not the locomotor and feeding activity of the Japanese sea catfish, Plotosus japonicus, shows a rhythmicity, the locomotor and the feeding activity of this catfish was recorded in a laboratory. All of the catfish recorded showed a nocturnal locomotor activity rhythm under a light-dark (LD) cycle (LD 12:12). Locomotor activity persisted within a constant darkness (DD) and the average free-running period of locomotor activity under DD lasted for 24.2 ± 0.4 hours (mean ± SD, n=8). In ad lib demand feeding condition under the LD cycle, all the catfish show nocturnal feeding activity. The feeding activity increased in the presence of food and the increased activity level lasted for several days after food removal. During restricted food availability in the light period, the catfish showed food-anticipatory feeding activity, both before and after the food restricted time under LD and constant light (LL). Thus, the present results demonstrate that the Japanese sea catfish, P. japonicus, has an endogenous circadian oscillator entrained by an LD cycle controlling locomotor activity and exhibits circadian feeding activity rhythms entrained by food.

P4PM-2-2

COMPARISON OF THE TWO KINDS OF MUSCLE HARDNESS METER

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We have already studied the effects of physical exercise or stretching on the objective muscle stiffness by a newly devised muscle hardness meter. In this study we compared the muscle hardness meter with a commercial muscle hardness meter. Measuring part of our device was made up with three bars and the commercial device was one bar and a round plate. Tip toe standing for 2min was loaded twice with one min rest on the lateral leg as physical exercise. The physical exercise increased the hardness of soleus muscle measured by both devices. There were no statistical difference between data obtained by both devices. This result demonstrates objectivity of the data obtained by these devices. Our device shows the tendency of smaller statistical variation than the commercial device.

P4PM-2-3

THE CONTRACTILE, BIOCHEMICAL AND HISTOLOGICAL PROPERTIES OF A NOVEL IN VITRO THREE-DIMENSIONAL SKELETAL MUSCLE MODEL

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The tissue-engineered skeletal muscle has the potential to realize generating the three-dimensional in vitro model for studying skeletal muscle exercise physiology. In this study, we investigated a variety of contractile force, protein expression of a novel tissue-engineered skeletal muscle model with cultivation period. Elastase treated porcine aorta were used as an artificial tendon for measurement of isometric contractile force. C2C12 cells were embedded in type-I collagen gel solution at density of 1.0×10^7 cells/ml. The suspension was added to two artificial tendons, and cultured for 2 days in growth medium. The medium was shifted to differentiation medium for 19 days. At 5 days after differentiation induction, the protein accumulations of α-actin and slow MHC in tissue-engineered skeletal muscle were evident and the isometric contractile force was evaluable by electrical pulse. At 12 days, the expression of the contractile proteins and the isometric contractile force increased dramatically. At 19 days, the isometric contractile force was higher than that at 12 days and the sarcomere structures were evident by electron microscopy. These results suggested that this novel three-dimensional culture can be used as a tool for studying in vitro skeletal muscle model.

P4PM-2-4

NEURONAL ACTIVITY ORIGINATING IN THE BRAINSTEM CAN TRIGGER LOCOMOTOR-LIKE ACTIVITY VIA DESCENDING PATHWAYS IN THE LOWER SPINAL CORD

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We employed a decerebrated and arterially perfused in situ mouse preparation (P7-21) to examine the interaction between brainstem respiratory activity and locomotor-like activity generated by the lumbar spinal cord. The perfusate was an oxygenated ($95\%O_2/5\%CO_2$) Ringer's solution (pH 7.40-7.45) containing an oncotic agent. The pattern and frequency of the motor nerve discharges depended on the perfusion flow rates. At high flow rates (>10x total blood volume), the phrenic and motor discharges became organized into episodes punctuated by quiescence. The episodes comprised rhythmic motor bursts that were correlated with the respiratory discharge recorded from the phrenic nerve and which could include periods of alternating left/right activity characteristic of locomotion. When the preparation was transected at the spino-medullary junction, respiratory-related motor discharges were not observed. However, under these conditions rhythmic motor discharges in hindlimb motor nerves could be induced by drugs or by electrical stimulation of the tail. We conclude that under certain conditions respiratory related activity can propagate into the lumbar cord and activate the locomotor circuitry.

P4PM-2-5

MECHANISMS UNDERLYING THE REGULATION OF DIFFERENT MODES OF LOCOMOTOR BEHAVIOR IN ADULT CHRONIC SPINAL RATS

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The neural mechanisms involved with the transformation from one mode of locomotor behavior to another mode in complete spinal cord transected (T9) adult rats during slow rotation of the treadmill have been evaluated by electrophysiological, kinematics, and immunocytochemical analyses. Seven complete spinal rats were trained for 6 weeks to step forward bipedally on a treadmill while stabilized in a body weight-support system and in the presence of epidural spinal cord stimulation (ES) at L2-S1 at 40 Hz and quipazine (a 5-HT agonist, 0.3 mg/kg i.p.) treatment. We assessed changes in the stepping pattern and in the pattern of coordination of motor pools during rotation of the treadmill with discrete 15-sec stops at 0, 45, 90, 145, and 180o to record forward stepping, sideward stepping, and backward stepping. After treatment all rats demonstrated good coordinated hindlimb stepping during ES and quipazine at 0o (forward stepping) as well as at 45o. At 90 and 180o we observed irregular non-coordinated rhythmic sideward and backward hindlimb movements, respectively. The patterns of c-fos labeling identifying the spinal neurons that generate the different modes of locomotor behavior also will be presented.

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P4PM-2-6

NEURAL CLOUDS FOR CLASSIFICATION OF TREMOROGRAMS FOR IDENTIFICATION OF HUMAN MOTOR SYSTEM DISORDERS

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One of the methods, implemented in the human motor system diagnostics, is registration and analysis of the static tremor (Romanov S.P., Manoilov V.V., 2003). Here person under study presses strain sensor for some fixed time period. Electrical signal, produced by the strain sensor - tremorogram -, is being converted into digital form and saved for further analysis.

In our work we use novel intelligent methods to separate recordings, made from healthy subjects, from recording, made in cases of motor system disorder (e.g. Parkinson decease). These methods are based on the trained artificial neural networks - self organized Kohonen maps and so-called neural clouds (technology elaborated by Siemens Corporate Technology AG). Tremorogram in case of Kohonen maps were preprocessed using the EMD approach (empirical mode decomposition).

The trained neural cloud was tested on tremorogram of 10 healthy subjects and 10 subjects with motor system pathology. As a result 8 of 10 diseased patients were classified by neural cloud as diseased and 8 of 10 healthy subjects were classified by neural cloud as healthy.

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P4PM-2-7

SINUSOIDAL ELECTRICAL WAVES ALONG THE SPINAL CORD DURING SPONTANEOUS FICTIVE LOCOMOTION IN PRECOLLICULAR-PREMAMMILARY DECEREBRATE CATS

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Previously we reported that the spinal interneurons generate a sinusoidal electrical wave during scratching in precollicular decerebrate cats. (Manajarrez et al., 2005, SFN. Abstr. 753.14). The purpose of the present study was to investigate whether the same phenomenon occurs during fictive locomotion. Experiments were carried out in 9 precollicular-premammilary decerebrate cats, paralyzed and artificially ventilated. Cord dorsum potentials were recorded by means of a multielectrode array of 32 channels, simultaneously with the electroneurographic activity of flexor and extensor nerves. We observed that during spontaneous locomotion the electrical field potential of spinal neurons takes the shape of a sinuous electrical wave that was highly correlated to the alternated electroneurographic activity of the left and right hindlimbs. Furthermore, we found that the lumbosacral (L1-S4) distribution for the amplitude of the sinusoidal cord dorsum potentials follows a bell shape, very similar to the amplitude distribution for the sinusoidal electrical waves associated with scratching. These results suggest that the sinusoidal electrical field potentials recorded on the spinal cord represent a common phenomenon underlying the electrical activity of the central pattern generator network.

P4PM-2-8

GASTROINTESTINAL RHYTHMIC MOVEMENT REGULATES HUMAN MOOD

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Patients with depression or other mental illness are always accompanied with gastrointestinal (GI) rhythmic disorder. 2% Serotonin (5-hydroxytryptamine: 5-HT) is distributed in brain, while over 95% is distributed in gut.

Through a 10-year trace investigation on healthy people in Japan, Chinese Traditional Taijiquan is helpful to reduce stress, anxiety and improve mood , meanwhile maintain energy balance. According to Diagnostic and Statistical Manual of Mental Disorders (DSM-TV-TR-Forth Edition), we got to know Chinese Traditional Taijiquan could regulate GI tract rhythmic movement by questionnaire. Probably large amounts of 5-HT in gut play a vital role in controlling mood, maybe the mechanism of regulation is similar to the function of 5-HT in brain. Our results suggest that Chinese Traditional Taijiquan prevents depression, Irritable Bowel Syndrome (IBS) or other mental disorders for healthy people by modulating the mood. A deeper insight into the serotonin along the brain-gut axis is a new target to antidepressants.

P4PM-2-9

WHAT IS THE MOST EFFECTIVE METHOD TO GET THE RELAXANT CONDITION ?

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This study aimed to investigate the most effective method to get relaxation. Subjects were 11 normal people (23.7±3.6 years old). Four methods, abdominal respiration (AR), gradual advancement muscle relaxation (GA), listening to the music (LM), and smelling a fragrance (SF), were selected to get relaxation. Finger-floors distance (FFD), diastolic and systolic blood pressure (DBP, SBP), heart rate (HR), breathing number (BN), and the range of straight leg rising (SLR) in all subjects were measured before and after 4 methods in a soundproofing room at 25˚C. This experiment was carried out getting informed consent from all subjects. FFD and SLR increased and SBP, HR and BN decreased after AR, significantly. FFD increased and SBP decreased after GA, significantly. FFD and SLR increased and SBP decreased after LM, significantly. FFD increased and DBP, SBP and BN decreased after SF, significantly. Differences in changes of HR, BN and SLR were shown significantly, and those in AR and SF were more effective than those in GA. This study suggested that all methods could have the effects on the relaxation of the body. It was considered that AR and SF led parasympathetic nerve to be dominant with composed respiration. The relaxant condition can be affected by control of respiratory system.

P4PM-2-10

EXCLUSIVE APPROACHES TO THE ESTIMATION OF ORGANISM FUNCTIONAL PREPAREDNESS

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Objectively existing nowdays insufficient effectiveness of functional testing for sportsmen is explained by absence of the complex system, and the process of testing itself comes to particular parameters registration with the whole battery of tests.

In connection with this problem we have developed the computer program SHVSM. The originality of the proposed programme is in the fact that practically all organism functional preparedness parameters can be calculated just on the basis of the 10-minute submaximal test. It is necessary to note that PWC170 and OMC values calculation is made according to well-known formulas, whereas calculation of such values as alactate and lactate anaerobic power (ALACp, LACp), alactate and lactate anaerobic capacity (ALACc, LACc), anaerobic metabolism threshold (AMT), cardiac systoles frequency at the AMT level (CSFAMT), general metabolic capacity (GMC) and functional preparedness level (FPL) is made accordance with the authors' exclusive formulas. It is necessary to note, that results of special clinical and biochemical examinations were taking into account the process of formulas development. The results allowed to establish a high degree of the correlation dependence between the indexes defined by experimental and calculating methods.

P4PM-2-11

ARECOLINE EXCITES THE DISTAL COLONIC CONTRACTION IN RATS VIA M_3 RECEPTORS - Ca^{2+} STORE RELEASE- EXTRACELLULOR Ca^{2+} INFLUX PATHWAY

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We investigated the effects of arecoline, the most efficient component of Chinese herbal medicine areca, on the motility of distal colonic smooth muscle strips in rats. In longitudinal muscle of distal colon (LMDC) and circular muscle of distal colon (CMDC), arecoline increased the contraction in a dose-dependent manner. Tetrodotoxin (TTX, 10 μM) did not inhibit the effects of arecoline. The contractile response to arecoline was completely antagonized by atropine (10μM). Selective muscarinic M_3 receptor antagonist, 4-diphenylacetoxy-N-methylpiperidine-methiodide (4 - DAMP, 0.4 μM), severely depressed the response for arecoline. Selective muscarinic M_2 receptor antagonists, gallamine (1 μM) or methoctramine (3 nM) did not influence the effects of arecoline. Selective inositol-1,4,5-trisphosphate (IP_3) receptor antagonist, 2-aminoethoxydiphenyl borate (2 - APB, 30 μM), L type Ca^{2+} channel blocker, nifedipine (0.1 μM) and Ca^{2+} free Krebs solution partly inhibited the effects of arecoline. Pretreatment with Ca^{2+} free Krebs solution and 2-APB simultaneously blocked the effects of arecoline completely. These results suggest that arecoline stimulates distal colonic contraction in rats via muscarinic M_3 receptors - Ca^{2+} store release - extracellular Ca^{2+} influx pathway.

P4PM-2-12

EFFECTS OF WATER ON THE FOOD BOLUS FORMATION DURING MASTICATION

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The sufficient saliva during mastication is essential for the food bolus formation. To evaluate the role of fluid including saliva on the masticatory behavior, we examined the effects of fluid on the relationship between the number of chewing strokes (NCS) and the physical properties of the bolus at the time of swallowing. Thirteen healthy subjects were instructed to chew the two test foods, biscuit (B) and rice cake (RC) without and with the tap water (2 ml). The electromyographic (EMG) activities were recorded from the masseter muscle during mastication using surface electrodes. The NCS immediately before swallowing was counted on the EMG recordings, and the triturated food bolus was spat into the cup. Three texture parameters (hardness, adhesiveness, cohesiveness) of each collected bolus were measured by the texture profile analysis. The added water significantly reduced the NCS until swallowing for both B and CR. However, there were no significant effects of added water on three texture parameters of the bolus. These results suggest that the water added to food can facilitate the bolus formation for swallowing.

P4PM-2-13

THE ANABOLIC ACTIVITY OF TURKESTERONE FROM THE SILENE LINCOLA

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In this study the anabolic activity of turkesterone from the plant Silene linicola were investigated. This compound shows the expressed influence on anabolic processes in the mouse organism. During 10 days injection turkesterone in the dose of 5 mg/kg (orally) immature rats (mass 80-90 g) are detected to increase their mass of the body (in mg/g/day) up to 121 %. The known anabolic steroid - nerobol increased the weight of the body in these experiments up to 113%. So expressed augmentation of the mass of the body of animals under influence of turkesterone was determined by augmentation of the common protein content in organs and tissues. In the liver, heart, kidneys and skeletal muscle the augmentation of the total protein content (in relation to the control) accordingly made 36.8 and 34.8 %. The mass of organs grow from 27.8 up to 34.3 % ($p<0.05$). The nerobol shows similar action, but in some more weak degree. However differing from nerobol, the turkesterone did not show specific androgenic actions, which are characteristic for all known steroanabolics that limits their application in clinical practice, especially in women and children. Turkesterone can be considered as perspective anabolic means which is not having accidental hormonal-specific effects on a whole organism.

P4PM-3-1

ENDOTHELIUM-DEPENDENT AND -INDEPENDENT VASORELAXATIONS TO 3,5,7,3',4'-PENTAMETHOXYFLAVONE IN THE RAT AORTA

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The extract of Kaepmferia parviflora rhizomes (KPE) has been generally used as a tonic for rectifying male impotence, and to treat hypertension, allergy and diarrhea. Our recent studies have shown that KPE and its methoxyflavone derivatives exert vasorelaxant effects in the rat aorta (Tep-areenan et al., 2008a; 2008b). In this study, we aims to investigate mechanisms of vasorelaxation induced by 3,5,7,3',4'-penta methoxyflavone (PMF), isolated from KPE, in the rat aorta. We found that PMF (1 - 100 microM) induced acute vasorelaxations, which were reduced by endothelial denudation and NG-nitro-L-arginine methyl ester (300 microM). However, vascular responses to PMF were not affected by indomethacin (10 microM). A high concentration of KCl (60 mM) inhibited vasorelaxations to PMF at high concentrations in both endothelium-intact and -denuded rings. In denuded rings, pretreatment with 4-aminopyridine (1 mM), barium chloride (30 microM), glibenclamide (10 microM), or tetraethylammonium (5 mM) reduced the responses to PMF. In this study, we have demonstrated that PMF causes vasorelaxation partly via endothelium-derived NO. Moreover, endothelium-independent vasorelaxations to PMF are largely mediated by increasing K+ efflux, at least in part, through KV, KIR and KATP channels.

P4PM-3-2

SUPEROXIDE DISMUTASE INHIBITION PARTIALLY ATTENUATES ANTI-DIURETIC AND ANTI-NATRIURETIC RESPONSES TO ANGIOTENSIN II IN ANESTHETIZED RATS

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To evaluate the interaction between superoxide dismutase (SOD) and NADPH oxidase activity during angiotensin II (AngII) administration, we assessed renal responses to intra-arterial infusion of AngII (0.5 ng/kg/min for 45 min) before and during infusion of SOD inhibitor, diethyldithiocarbamate (DETC, 0.5 mg/kg/min) in rats (n=8) pretreated with enalaprilat (33 μg/kg/min). Total and regional renal blood flows (RBF) were measured by Transonic and laser-Doppler flowmetry. Prior to DETC, AngII caused reductions of 19±3% in RBF (baseline, b, 6.0±0.3 mL/min/g), 32±4% in GFR (b, 0.90±0.05 mL/min/g), 33±3% in urine flow (V, b, 10±0.7 μL/min/g) and 51±3% in sodium excretion (UNaV, b, 1.3±0.1 μmol/min/g). DETC itself caused reductions of 14±3% in RBF, 24±5% in GFR, 12±2% in V and 29±5% in UNaV. During DETC infusion, AngII caused similar decreases in RBF or GFR but significantly less decreases in V (-12±2%) and UNaV (-25±3%) as compared to AngII alone. Application of DETC (10 μM) also reduces NADPH oxidase activity in renal cortical (573±43 to 67±4 RLU/mg protein) and medullary (1607±28 to 148±5 RLU/mg protein) tissue slices (n=6). These data suggest that a downregulation of NADPH oxidase limit AngII induced anti-diuretic and anti-natriuretic responses in the condition SOD inhibition.

P4PM-3-3

EFFECT OF ORCHIDECTOMY ON VASCULAR RELAXATION RESPONSES TO cAMP AND K CHANNEL ACTIVATION IN MALE SPRAGUE DAWLEY RATS FED A HIGH SALT DIET

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To assess the effect of testosterone deficiency on adenylyl cyclase and potassium channel activities on isolated aortic rings in male Sprague Dawley rats by using forskolin an adenylyl cyclase activator, and diazoxide a potassium channel activator. 24 weanling male rats aged 8 weeks with an average weight of 100g were divided into 4 groups of 6 rats each. Groups A, (Intact + normal diet), B (Intact + high salt diet), C (Orchidectomised + normal diet) and D (Orchidectomised + high salt diet). 3mm Aortic rings were suspended in organ baths containing Hepes buffer bubbled with 100% oxygen. Relaxation responses to forskolin and diazoxide were studied in noradrenaline pre-contracted rings. Arterial blood pressure was determined before and weekly during the experiment using non-invasive tail cuff method. There was a significant increase ($P < 0.01$) in the MABP of group B rats when compared with both groups A and D rats. There was no significant difference in the relaxation response of all the groups to forskolin but salt diet reduced the relaxation response to diazoxide and this effect was ameliorated in group D rats. Modulation of potassium channel appears to be part of the mechanism by which high salt diet increases vascular tone, and this effect was counteracted by orchidectomy.

P4PM-3-4

ATTENUATION OF THE DEVELOPMENT OF EXPERIMENTAL SALT-SENSITIVE HYPERTENSION BY THE AQUEOUS CALYX EXTRACT OF *HIBISCUS SABDARIFFA*

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Hibiscus sabdariffa (HS) extract has antihypertensive properties (1). A strategy in the prevention of hypertension is use of K+-rich diets. We studied the effect of HS on the development of hypertension. An HS concentration of 6mg/ml was prepared and its K+ content measured. Rats (n=8 each) were treated for 12 weeks as follows: control (normal diet + water), salt-loaded (8% salt diet + water), HS (normal diet + 6mg/ml HS) and salt+HS (8% salt diet + 6mg/ml HS). Their blood pressure was measured under anaesthesia. Bilateral carotid occlusion (BCO) and responses to noradrenalin or acetylcholine (0.05mg/kg each) were performed. The K+ concentration of 6mg/ml HS was 840mmol/l. The mean arterial pressure (MAP±SEM; mmHg) of salt loaded rats (184.6±29.8) was significantly ($P<0.05$) higher than control (113.2±3.0), HS (90.0±7.4) and salt+HS (119.4±8.9). The MAP of salt+HS and control rats did not differ significantly nor did the response to noradrenalin or acetylcholine. The BCO-induced blood pressure rise in rats given HS (-14.2±18.4) and salt+HS (-9.8±23.5) was reduced compared to control (53.0±12.1; $P<0.05$). These suggest that HS may prevent the development of salt-induced hypertension through the inhibition of sympathetic activation.

1. Mojiminiyi F et al, Fitoterapia 78, 292-297, 2007

P4PM-3-5

THE EFFECT OF RENAL DENERVATION ON VASCULAR RESPONSIVENESS TO SYMPATHOMIMETICS IN NORMAL AND HYPERTENSIVE RATS

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To investigated the influence of adrenergic and angiotensin II receptor blockade on responses of renal vascular bed to dopamine and isoprenaline or neural stimuli in denervated kidney of spontaneously hypertensive (SHR) and Wistar-Kyoto (WKY) rats treated with losartan, carvedilol or losartan+carvedilol for 7 days. Rats underwent acute unilateral renal denervation at the day of experiment. SHR respond with higher ($P<0.05$) vascular responsiveness to dopamine and neural stimuli but not to isoprenaline, as compared with WKY rats. Denervation aggravates responses to dopamine and isoprenaline during hypertension. Losartan alone attenuates ($P<0.05$) the renal vascular responses to dopamine, neural stimuli and isoprenaline in denervated WKY and SHR as compared to untreated counterparts. Carvedilol or losartan+carvedilol blunt responses to dopamine and isoprenaline but not to neural stimuli in denervated WKY and SHR. The effects of dopamine and isoprenaline are independent on intact renal innervation but on intact renin-angiotensin system (RAS) component and an interactive relationship between RAS and sympathetic nervous system can be suggested in the absence of normal sympathetic tone to the kidney of normal and hypertensive rats.

P4PM-3-6

RESVERATROL PROTECTS AGAINST HYPERTENSION-INDUCED OXIDATIVE INJURY OF THE AORTA AND IMPROVES CARDIOVASCULAR FUNCTION IN RATS

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The effect of resveratrol (RVT) on impaired cardiovascular functions was studied in an angiotensin II-dependent renovascular hypertension model in Wistar albino rats with surgically placed renal artery clips (two-kidney, one-clip; 2K1C), while sham rats did not have clip placement. Rats received either RVT (10 mg/kg/day) starting by the 3rd week of the operation. Blood pressure (BP) and echocardiographic recordings were made before, at the 3rd and 9th weeks of surgery. At the end of the 9th week, aortic tissues were removed to study the contraction and relaxation responses and to determine malondialdehyde (MDA) and glutathione (GSH) levels. 2K1C led to increases in BP, left ventricular (LV) posterior wall thickness, LV end-diastolic and end-systolic dimensions, while ejection fraction was significantly decreased. Aortic MDA levels were increased with concomitant decreases in GSH levels, while phenylephrine-induced vascular contraction was enhanced and relaxation response to acetylcholine was reduced. On the other hand, oxidative injury and the altered contractile activity of the aorta were prevented by RVT, which also reduced BP and improved LV function. In conclusion, RVT improved cardiovascular function and protected against hypertension-induced oxidative injury of the aorta.

P4PM-3-7

SHIFT IN THE FUNCTIONAL IMPORTANCE OF α_1 ADRENOCEPTOR SUBTYPES IN PRESSURE OVERLOAD-INDUCED LVH IN SPRAGUE DAWLEY RATS

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This study aimed to investigate the altered functional contribution of renal α_1 adrenoceptor subtypes in the control of renal haemodynamics in pressure overload induced LVH rats.

Male SD rats were randomized into three Control and three aortic banded groups (AB), (n=6 each). Supra renal aortic stenosis was produced in the AB group under Ketamine and Xylazine (6mg/Kg & 10mg/Kg respectively). Using a laser doppler probe, the cortical blood perfusion (CBP) changes in the kidney caused by close intra renal administration of NA, PE and ME were determined before and after selectively antagonizing renal α_{1A}, α_{1B} and α_{1D} adrenoceptors by 5MeU, CEC and BMY7378 respectively. Data, means ±S.E.M were subjected to ANOVA with significance at $P<0.05$.

Baseline MAP was reduced by BMY7378 to about 10% in the control group vs 20% in AB group $P<0.05$ with no change in baseline CBP. The study showed decreased responsiveness of renal α_1 adrenoceptor subtypes to adrenergic agonists in AB group. A shift in functional importance from α_{1A} in the Control group to α_{1D} adrenoceptors in the AB group with a minimal role of α_{1B} adrenoceptors (all $P<0.05$) in the control of renal haemodynamics is the major conclusion of this study.

P4PM-3-8

ROLE OF HYPERLEPTINEMIA AND HYPERINSULINEMIA IN INDUCING RESISTANCE TO ACUTE NO-MIMETIC AND EDHF-MIMETIC EFFECTS OF LEPTIN

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Background: Leptin activates sympathetic system and vasodepressor mechanisms and has no acute effect on blood pressure. We have demonstrated that in experimental obesity leptin-induced NO production is impaired and is replaced by EDHF-dependent vasorelaxation (Life Sci 2006; 79: 63). We examined mechanisms involved in the resistance to vascular effects of leptin.

Methods: Effect of leptin was studied in 5 groups of rats: (i) control, (ii) and (iii) obesity induced by high-calorie diet for either 1 (O1) or 3 (O3) months, characterized by hyperleptinemia/normoinsulinemia, and hyperleptinemia/hyperinsulinemia, respectively, (iv) experimental hyperleptinemia (EHL) induced by chronic administration of exogenous leptin (hyperleptinemia/normoinsulinemia), (5) fructose-fed (hyperinsulinemia/mild hyperleptinemia).

Results: Leptin-induced NO production was impaired in the O1, O3 and EHL but not in fructose-fed rats. In contrast, EDHF-mimetic effect of leptin was impaired in only the O3 and fructose-fed groups.

Conclusions: Hyperleptinemia induces resistance to vascular NO-mimetic effect of leptin but not of insulin, whereas chronic hyperinsulinemia and insulin resistance are responsible for the attenuation of EDHF-mimetic effect of leptin.

P4PM-3-9

EFFECT OF ACUTE INFLAMMATION ON RESPONSIVENESS OF BETA-2 ADRENOCEPTORS OF RAT KNEE JOINT BLOOD VESSELS WAS DECREASED IN DIABETIC ANIMALS

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Introduction: The aim of this study was to investigate the vasodilatory response of knee joint blood vessels to salbutamol (Beta-2 adrenoceptor agonist) in presence of inflammation in diabetic rats.

Methods: Acute inflammation was induced by intra-articular injection of kaolin 4% and induction of diabetes was performed by streptozotocine (55 mg/kg). Wistar rats weighting 200-300 g were used. The animals divided into 4 groups as: control, diabetic, inflammatory and diabetic- inflammatory. Blood flow of knee joint was measured using laser Doppler flowmetry technique (LDF). Vasodilatation of articular micro vascular was measured in response to topical application of different concentration (10 -11 -10 -1) of salbutamol.

Results: 1- Increased knee joint diameter and perimeter due to acute inflammation in diabetic rats were significantly lesser than that of inflammatory rats.

2- Responsiveness of Beta-2 adrenoceptors was increased in kaolin- induced acute inflammation.

3- The response of beta-2 adrenoceptors in acute inflammation was inhibited in presence of diabetes. Conclusion: Based on the mentioned results, we conclude that diabetes inhibits the effects of acute inflammation on responsiveness of Beta-2 adrenoceptors.

P4PM-3-10

NS309 RESTORES EDHF-TYPE RELAXATION IN MESENTERIC SMALL ARTERIES FROM THE ZDF RAT MODEL OF TYPE II DIABETES

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This study investigated the EDHF-type relaxation in mesenteric small arteries from Zucker Diabetic Fatty (ZDF) rats using NS309, a potent activator of small- and intermediate-conductance calcium activated potassium channels (SK3 and SK4).
Methods: The EDHF type relaxation to acetylcholine (ACh) was investigated in the presence of 3 μM indomethacin and 100 μM l-NAME. The role of SK3 and SK4 was investigated by incubation with 0.5 and 1 μM NS309 and selective inhibition using 50 nM apamin and/or 1 μM TRAM-34. Expression was assessed by Western blotting and changes in membrane potential were recorded along with changes in intracellular endothelial calcium.
Key results: The EDHF-type relaxation was abolished and the ACh relaxation and the related hyperpolarization were attenuated in arteries from ZDF rats. After incubation with NS309, the EDHF-type relaxation was restored in arteries from ZDF rats without changing endothelial intracellular calcium. The restored EDHF-type relaxation was more sensitive to TRAM-34 than to apamin, and protein expression was unaltered for SK3.
Conclusion: We conclude that the attenuated EDHF-type relaxation in mesenteric small arteries from ZDF rats can be restored by NS309 without changing intracellular endothelial cell calcium.

P4PM-3-11

PI3-K DELTA ISOFORM UP-REGULATES L-TYPE Ca2.1 CURRENTS AND INCREASES CONTRACTILITY IN STREPTOZOTOCIN-INDUCED DIABITIC MICE AORTA

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The aim of this work was to investigate the molecular mechanism underling vascular dysfunction in streptozotocin (STZ)-induced diabetic mice. 3 weeks old C57BL/6 mice received an i.p. injection of STZ or vehicle. Muscle tension was measured in aortic rings, ten weeks later. Ca^{2+} currents through L-type Ca2.1 channels (I_{Ca}) were measured in freshly dissociated aortic smooth muscle cells. Western blot was used to measure protein expression and oligodeoxynucleotides antisense was used to knockdown PI3K delta. Plasma glucose was ~4 times higher in STZ-treated animals. Histological analysis did not show structural differences. Contractile responses to phenylephrine and KCl were strongly enhanced in diabetic mice and were endothelium-independent. Patch clamp experiments showed increased magnitude of I_{Ca} in smooth muscle cells from diabetic mice. Increased contractile response and I_{Ca} were restored by the PI3K inhibitors LY204002, wortmanin, IC87114 a selective inhibitor of PI3K delta, and by antisense knockdown of PI3K delta. PI3K delta expression was increased in diabetic animals. It is concluded that diabetic mouse aorta showed an increased contractility that is due to PI3K delta mediated-increase in Ca^{2+} currents through L-type Ca2.1 channels. Support: FAPEMIG, CAPES and CNPq.

P4PM-3-12

DIABETES MELLITUS IMPAIRS VASODILATATION INDUCED BY PINACIDIL IN AORTIC RINGS WITHOUT ENDOTHELIUM IN RATS

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It is known that ATP-sensitive potassium channels (K_{ATP} channels) of vascular smooth muscle participate in the control of vascular tone. It is also known that K_{ATP} channels in cardiac myocytes from diabetic animals have a reduced sensitivity to ATP. In order to study the arterial dysfunction in diabetes mellitus, the tension of thoracic artery rings was recorded by means of an isometric force transducer in control rats and diabetes-induced rats by streptozotozin administration (60 mg/Kg, one i.p. application). As control for vascular dysfunction, arterial rings were exposed to acetylcholine (Ach) and phenylephrine (PE). Standard drug concentration-response analysis was performed. In diabetic rats Emax for Ach was reduced (88.1 to 61.1 %), EC_{50} for PE was reduced (10.1 to 3.5 μM) and the EC_{50} for pinacidil, a K_{ATP} channel opener, was increased (0.43 to 2.56 μM). Therefore, it is possible that K_{ATP} channels in diabetic animals could present a lower sensitivity to ATP as in the cardiac myocytes. This would explain the reduced sensitivity to pinacidil, because the K_{ATP} channels would be in the open state for long time periods.

P4PM-3-13

EFFECTS OF GLUTAMATE ON SYSTEMIC AND SPLANCHNIC CIRCULATIONS

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To determine the effects of glutamate on the systemic circulation and the superior mesenteric artery (SMA) blood flow response to a protein meal, we compared the responses of mean blood velocity (MBV) in the SMA, heart rate (HR), and mean arterial pressure (MAP) between glutamate-enriched and control liquid meals. After 5 min of baseline, nine subjects ingested a meal for 3 min and rested for 60 min as the postprandial state. The MBV in the SMA increased significantly relative to baseline throughout the postprandial state in both the glutamate and control trials. The peak in the MBV response in the SMA occurred significantly later in the glutamate trial than in the control trial (21 ± 4 vs. 14 ± 2 min, mean ± SEM) and tended to be smaller in the glutamate trial (75 ± 7 vs. 88 ± 8 cm/s, $p = 0.08$). Both HR and MAP increased significantly during the ingestion in both trials. In the control trial, the significant increase in HR lasted until 25 min after the ingestion, and the MAP was significantly increased at 20-25 and 35-30 min after the ingestion, whereas increases in HR and MAP returned to the baseline within 5 min after the ingestion in the glutamate trial. These results suggest that glutamate moderates the systemic and splanchnic circulatory responses to a protein meal.

P4PM-3-14

EFFECT OF GRAPE SEED EXTRACT ON ISOLATED AORTA RESPONSE TO PHENYLEPHRINE IN LEAD EXPOSED RATS

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Chronic exposure to low level of lead results in increased contractile response of aorta to vasoactive agents in rats. Several studies indicated the involvement of reactive oxygen species in lead-exposed animals. On the other hand, grape seed is a good source of antioxidants and have free radical scavenging properties. The main objective of this study was to determine the effect of grape seed extract (GSE) on isolated aorta response to phenylephrine. Forty adult male Wistar rats were divided into four groups. Control group, received tap water (A). The other three groups received tap water contained 100 ppm lead acetate alone (B), GSE alone (100 mg/kg, orally, C) or 100 ppm lead acetate in drinking water + GSE (100 mg/kg, orally, D) for 45 days. The isolated aorta (with or without endothelium) response to phenylephrine was recorded and compared in different groups. The results indicated that the aortic response of lead-treated group (B) was increased significantly compared to other groups, and GSE administration prevent this increment in response (group D), indicating that the GSE could prevent the lead induced changes in vascular response.

P4PM-3-15

ROLE OF eNOS FOR LIPOPOLYSACCHARIDE-MEDIATED VASORELAXATION AND BLOOD PRESSURE DECREASE IN VIVO AND IN VITRO

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During sepsis cardiovascular failure and increased COX-2 levels are observed. We therefore hypothesized that lipopolysaccharide (LPS)-induced suppression of vasoreactivity is caused by increased vascular COX-2 and activation of eNOS by PGE2. Chronic indwelling catheters were placed in femoral artery and vein in wt and eNOS-/-mice. An LPS bolus (2 mg/kg) significantly reduced MAP by 29 ±2% (wt) and 31±4% (eNOS-/-) and HR by 50±3% (wt) and 52±2% (eNOS-/-) with no significant difference between wt and eNOS-/-.
Mouse aorta was incubated with LPS (50μg/mL) or vehicle for 18h, 37oC and the effect of $2*10^{-7}$mol/L phenylephrine was recorded with a myograph. LPS exposure reduced contractility by 84% compared to vehicle in wt and by 76% in eNOS -/-. Removal of the endothelium in rings from wt mice before incubation with LPS and L-NAME (10^{-4} mol/L) or LPS alone reduced contractility by 26% and 76% compared to vehicle. We conclude that eNOS is not critical for the acute blood pressure decrease after LPS treatment. Furthermore, the LPS depressed vascular function is mediated by NO independent of the endothelium and eNOS in murine models of LPS endotoxemia.

P4PM-3-16

SLOW SKIN WARMING YIELDS INCREASES IN SKIN AND MUSCLE BLOOD FLOW VIA AXON REFLEX

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Axon reflex (AXR) can mediate an increase in skin blood flow (SkBF) via the signals from primary afferent neurons. We examined whether or not AXR could further yield the simultaneous increase in blood flow in the muscle layer in humans. A steaming pad (Megurizumu, Kao Corp.) was attached onto the shoulder skin to produce slow rising phase of shoulder skin temperature (Tshoulder) in female (f, n=8, 68±2 yr) and male (m, n=8, 73±2 yr) subjects. After resting for 10 min, shoulder was warmed with the steaming pad for 30min, followed by a removal of the pad for 20min. Monitoring was performed for Tshoulder, SkBF along with the regional oxygenation for trapezius muscle blood flow (MBF) by near-infrared spectroscopy. Tshoulder gradually increased in a logarithmic fashion from 33.4C to 40.7C (f), or 40.4C (m) over 30min. During warming, SkBF began to rise at 2.5min when Tshoulder was 38.3C (f) or at 4.5min when Tshoulder was 38.2C (m). MBF began to rise at 3.4min (f, m). Correlation analysis revealed that there were significant correlations between latencies for SkBF and MBF for female or male subjects (r=0.72 and r=0.84, respectively). These results strongly suggest that skin warming can elicit increases of both SkBF and MBF via AXR at lower thresholds of Tshoulder.

P4PM-3-17

REGULATION OF POST-EXERCISE HAEMODYNAMICS FOLLOWING HYPEROXIA IN MAN: ROLE OF ATRIAL NATRIURETIC PEPTIDE

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Hyperoxic exercise blunts post-exercise vasodilatation independent of reduced NO (New et al, 2008). The present study investigated the influence of the non-adrenergic vasoactive metabolites ANP, AVP and angiotensin II (ANG II) on post-exercise haemodynamics in ageing men. 9 males, MAP = 106 ± 5 mmHg (50 ± 10 yr), not on medication, were studied following 30-minutes of cycle exercise at 70% maximal oxygen consumption in hyperoxia (50% O_2) and normoxia (21% O_2). Subjects were followed post-exercise for 2-hours. Left ventricular haemodynamics were assessed via echocardiography and systemic vascular resistance (SVR)/vascular conductance (SVC) determined by the quotient of MABP/Q and Q/MABP, respectively. Peripheral venous blood was sampled from an antecubital vein and metabolite concentrations corrected for plasma volume shifts. ANP, AVP and ANG II were determined in plasma via radioimmunoassay.

Hyperoxic exercise blunted post-exercise haemodynamics by attenuating the reductions (from normoxic baseline) in SVR and MAP (P<0.05). Only ANP bioavailability was attenuated following hyperoxia (P<0.05).

Hyperoxic exercise has a deleterious effect on post-exercise haemodynamics which corresponds with a diminished concentration of circulating ANP.

P4PM-3-18

PEAK OXYGEN UPTAKE DOES NOT CORRELATE WITH BLOOD VOLUME IN PERSONS WITH CERVICAL SPINAL CORD INJURY

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To investigate the limiting factors of the decreased peak oxygen uptake (VO_2peak) in subjects with cervical spinal cord injury (CSCI), we determined the relationship between blood volume (BV) and the endurance performance during arm exercise in able-bodied subjects (AB) and CSCI. After determination of BV by Evans Blue dilution technique, Eight CSCI (age; 26.4 ± 2.0 years old, C6, the lowest normal motor segment) and nine age-matched AB (age; 25.4 ± 1.3) conducted incremental wheelchair propulsion exercise until exhaustion. Peak oxygen uptake (VO_2peak) was 589.6 ± 58.4 ml/min in CSCI and significantly lower than that in AB ($p<0.05$). BV did not correlate with either VO_2peak and peak oxygen pulse (VO_2peak/peak heart rate) during arm exercise in CSCI, however, peak oxygen pulse in AB was well correlated with BV. These results suggested that oxygen supply limited endurance performance during arm exercise in AB but not in CSCI. This might be due to dysfunction in sympathetic nervous system below the level of the lesion and/or lack of muscle pump in leg muscles in CSCI, so that blood flow to the exercising muscles does not depend on BV.

P4PM-3-19

CHANGES IN THE RENAL RESISTANCE ARTERY RESPONSE TO AGONISTS IN TRAINED RATS

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It's well known that renal blood flow is affected by exercise. There is no study about the effect of regular physical activity on alterations in renal resistance arteries response to vasoactive hormones. The aim of the present study was to investigate the responses of renal resistance arteries to vasodilator and vasopressor agents in trained rats. The exercise group performed swimming exercise for (5d/w,6w) whereas controls did not. Resistance arteries were isolated from the kidneys at the end of the study and transferred into the organ baths of a wire myograph to determine the responses of the vessels to vasopressor (noradrenalin, vasopressin, endothelin-1, KCl, dopamine, TxA2) and vasodilator agents (ach, bradykinine, adenosine, isoproterenol, SNP). Relaxation responses didn't show a significant difference between groups whereas contractile responses to noradrenalin and dopamine were increased significantly in the exercise group. According to the results of our study, renal resistance arteries of trained rats, in contrast to previous studies on conduit-type renal artery, showed a different behavior and exhibited an increased contractile response to sympathetic stimuli. This finding explains the marked decrease in renal blood flow during exercise in trained individuals.

P4PM-3-20

ROLE OF CENTRAL COMMAND IN CEREBRAL BLOOD FLOW REGULATION DURING STATIC EXERCISE

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The purpose of this study was to examine the role of central command in the exercise-induced increase in cerebral blood flow. Eleven subjects performed static elbow flexion for 2-min at 30% MVC with the manipulation of central command by vibration applied to the biceps brachii tendon (EX+VIB) and without vibratiaon (EX). The influence of the central command can be decreased without altering the muscle mechanoreflex activation by tendon vibration to assist an exercising muscle in developing a given force. We recorded cardiovascular responses, the mean blood flow velocity of the middle cerebral artery (VMCA), and blood flow of the vertebral artery (QVA). Cerebrovascular resistances (CVR) were calculated by dividing the mean arterial pressure (MAP) by VMCA and QVA. The muscle fatigue sensation and the increases in heart rate and MAP were significantly lower in EX+VIB than that in EX indicating a substantial decrease of central command during EX+VIB. Similar responses was also found in the vertebral artery that CVRVA in EX+VIB was lower than that in EX, whereas the responses of CVRMCA were identical in both EX+VIB and EX. These results suggested that central command affects the flow-regulation in the vertebral artery during exercise but not in the middle cerebral artery.

P4PM-3-21

BLOOD FLOW RESPONSE TO PLANTAR FLEXION IN ANTIGRAVITY LEG AFTER 3WK OF UNILATERAL LOWER LEG SUSPENSION IN HUMANS

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Blood flow in exercising muscle increases to meet the oxygen demand, which is a key factor to perform exercise. Influence of physical inactivity on the blood flow in exercising muscle remains uncertain. We therefore examined whether 3wk of unilateral lower leg suspension alter the blood flow response to exercise in the antigravity leg in human. Before and after leg suspension, we measured blood flow of the popliteal artery with Doppler ultrasound during planter flexion exercise in the suspension leg. Plantar flexion exercise was dynamically performed (2-s on and 2-s off) for 7 min. The exercise intensities of plantar flexion were 25kg, and 50% of maximal voluntary muscle contraction (MVC) measured before and after leg suspension. 3wk of leg suspension reduced the MVC force of plantar flexion and attenuated the popliteal blood flow response to plantar flexion exercise at 50% MVC. In contrast, leg suspension did not alter the blood flow response to plantar flexion exercise at 25 kg. These results suggest that leg suspension attenuates the blood flow response to exercise in antigravity leg performed at the same relative MVC force, but does not affect the blood flow response to exercise at the same weight workload.

P4PM-3-22

CHRONIC FEMORAL ARTERY OCCLUSION AUGMENTS EXERCISE PRESSOR REFLEX IN DECEREBRATED RATS

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Although the exercise pressor reflex evoked by static muscle contraction has been reported to be potentiated by acute ischemia of skeletal muscles, the effect of chronic muscle ischemia on the exercise pressor reflex remains unclear. We, therefore, determined if ligation of the femoral artery 72 hours prior to static contraction augmented the exercise pressor reflex in male rats. 72 hours after ligation of one femoral artery, rats were decerebrated at midcollicular level. The pressor responses to static muscle contraction were compared between the side ligated previously for 72 hours and the contralateral side. The pressor response to contraction of the side whose femoral artery was ligated 72 hours previously averaged 28 ± 8 mmHg whereas the pressor response to contraction of the contralateral freely perfused side averaged 10 ± 8 mmHg (n=4, $P<0.05$). Likewise the pressor response to contraction on the side whose femoral artery was ligated for 72 hours previously averaged 28 ± 8 mmHg, whereas the pressor response to contraction on the contralateral side whose femoral artery was ligated for only 3 minutes averaged 8 ± 3 mmHg (n=4, $P<0.05$). We conclude that chronic femoral arterial occlusion augments exercise pressor reflex.

P4PM-3-23

RELATIONSHIP BETWEEN BLOOD PRESSURE AND ATMOSPHERIC PRESSURE IN THE JAPANESE ELDERLY

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The purpose of this study was to clarify the relationship between blood pressure and the atmospheric pressure in the Japanese elderly. Sixty-two elderly volunteers participated in this study (Age:63.5 yrs). A sample number was 19,196 data, which have been collected for 12 years. Blood pressure was always measured using a manometer at the fixed time in a day. The subjects were divided into three groups by the response of systolic pressure; 1) a group that increased depending upon changes in atmospheric pressure, called valley type, 2) a group that decreased depending upon changes in atmospheric pressure, called hill type, 3) a group that indicated no change according to changes in atmospheric pressure, called horizontal type. Systolic pressure increased by 5-10 mmHg in valley type and decreased by 5 mmHg in hill type, when the rate of changes in the atmospheric pressure in the previous day was around 0.8 hPa per hour. Percentage of subject number of each group was 51.7 % in valley type, 31.7 % in hill type and 16.6 % in horizontal type, respectively. These data suggest that atmospheric pressure would influence to autonomic nerve activity, and consequently, the lowered of autonomic nerve and arterial stiffness would induce the variability of blood pressure of the elderly.

P4PM-3-24

ACUTE MODERATE HYPOXIA ALTERS CARDIOVASCULAR RESPONSES TO STRESS

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It is generally accepted that hypoxia induces sympathetic dominancy during exercise. To our knowledge, no studies have assessed whether hypoxia affects cardiovascular response to mental stress. For this study, we hypothesized that acute moderate hypoxia can modulate cardiovascular responses at mental task as well as during exercise stress.

Seven middle agers were exposed to normoxic and moderate hypoxic (FiO_2=12.7%) conditions on a separated day. On each exposure, they were kept in a resting for 10 min, and performed a mental test for 15 min. After a recovery from mental task, a graded maximal cycle ergometer bout comprising 3 min by 30 W followed until exhaust. HR, BPs and the indices of cardiovascular autonomic activity were evaluated and compared.

1) Mental stress induced significant increases in HR, systolic BP and mean BP in both conditions. 2) All indices of cardiac autonomic nerve activity were unchanged. In contrast, the vasomotor sympathetic nerve activity decreased significantly during mental stress in only hypoxic condition.

In our subjects, acute normobaric moderate hypoxia had negative effect on vasomotor sympathetic nerve activity during mental task, whereas HR and BPs during mental stress had been kept a elevating in hypoxic condition.

P4PM-3-25

EFFECTS OF GENDER ON CORONARY VASOCONSTRICTOR RESPONSES DURING EXERCISE

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Premenopausal women are protected from exercise-induced angina. The mechanisms by which gender influences coronary tone during exercise are unclear. One theory is that sympathetic coronary constriction is less in women than in men. To examine this issue we measured coronary flow velocity (CBV) in young men (n=12; 28±1yrs) and women (n=14; 30±1yrs). CBV (Duplex Ultrasound), heart rate (EKG) and blood pressure (BP; Finapres) were measured during static handgrip (HG; 20 sec) exercise at 70% maximum voluntary contraction and during lower body negative pressure (LBNP). A coronary vascular resistance index (CVR) was calculated as diastolic BP/CBV. Increases in CVR and decreases in CBV were greater in men vs. women during HG (CVR, 1.25±.49 vs. 0.26±.38 units; $P<.04$ and CBV, -0.9±0.9 vs.1.7±0.8 cm/s; $P<.01$, men vs. women, respectively). In women changes in CBV were linked to the myocardial oxygen consumption during HG; no correlation was seen in men. CBV reductions were also greater in men vs. women during graded LBNP ($P<.04$). We conclude that during HG exercise and LBNP, coronary constrictor tone is attenuated in premenopausal women vs. men. Whether the reduced sympathetic responses in women contribute to reduced angina in women is unclear.

P4PM-3-26

CHANGES OF GENITAL ORGANS BLOOD FLOW AND EFFECTS OF ELECTRO-ACUPUNCTURE DURING THE ESTRUS CYCLE IN ANAESTHETIZED FEMALE RATS

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[Purpose] To evaluate the effects of electro-acupuncture (EA) on gynecological disorder, genital organs blood flow were measured by microsphere technique in female rats during the estrus cycle. First, the change of blood flow into uterus, ovary and oviduct were compared among at estrus cycle. In the next, the effects of EA on these genital organs blood flow were investigated. [Methods] 51 female SD rats were used. To evaluate the changes of blood flow, radioactive microsphere (^{51}Cr) was injected to right carotid artery, and reference blood was withdrawn from the left femoral artery under the pentbarbital anesthesia. Additionally, EA to abdomen was performed for 30 minutes. [Result] All of the genital organs, especially uterus and oviduct, confirmed that blood flow decreased in estrus. Decrease of ovarian blood flow induced by EA was observed in estrus and diestrus without significance. [Conclusion] Specific changes of the genital organs blood flow were obtained during estrus cycle. Additionally, decrease of ovarian blood flow was observed in estrus and diestrus after EA, which is much similar to previous reports. In these results, we considered that EA had been shown more effectiveness in estrus cycle then other periods at ovarian.

P4PM-3-27

ABOUT THE RELATION BETWEEN THE AUTONOMIC NERVOUS FUNCTION AND THE CONTRAST BATH

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The contrast bath is used to improve the edema, the reflection sexual intercourse feeling nerve dystrophy, and circulatory dynamics. However, the physiology target machine beginning in the contrast bath is not clarified. To clarify the autonomic nervous activity executing alternation, this experiment was done.

The method of catching the change of the autonomic nervous activity was do a Fourier transform of the R-R interval change and analyzed the frequency. Because the high frequency element (HF element) reflected only a parasympathetic activity, the high frequency element was taken out and the autonomic nervous activity was evaluated.

A significant rise was not admitted since the third times though the rise was caused in the HF element in the HF element value when the second taking a bath when the first taking a bath.

The contrast bath is thought that an autonomic parasympathetic activity becomes dominant if the doing cold stimulation and the Onnets stimulation alternately join the body.

P4PM-3-28

AN ASSESSMENT METHOD FOR REGULATORY ACTIVITY OF BLOOD FLOW IN HUMAN FINGER

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To investigate regulatory changes in peripheral circulation of human, both oxygen level & flow of finger arterial blood were measured respectively with hemoglobin oxy-meter & flow meter (ADInstruments, MLT320 & MLT1020PPG). When the finger arteries were pushed for a minute by 40 mmHg, the distal blood flow usually stopped immediately. After 15-30 sec of the stop of flow, the oxygen level decreased. Hypoxia lower than 70%O2 continued during the occlusion. In some instances, however, the hypoxia was sometimes broken; oxygen level returned intermittently to normal during the continuous occlusion. This enhanced circulation occurred more often when examined by repetitive occlusion. The increased rate of occurrence of enhanced blood flow was from 15-20% to 50-59% in 56 persons. The data suggest that by the transient occlusion, the blood flow in human finger increases upto 4 times than normal. This measurement of enhanced change of finger blood flow may be useful to assess the regulatory function of peripheral circulation.

P4PM-3-29

EFFECT OF COMBINED ADMINISTRATION OF ESTROGEN AND PROGESTERONE ON BRAIN EDEMA AFTER TRAUMATIC BRAIN INJURY RATS

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This study was performed on 8 groups of female rats as follows: 1: control,2: sham, 3: ovarectomized trauma, 4: vehicle, 5: physiologic dose of estrogen+physiologic dose of progesterone , 6: physiologic dose of estrogen+pharmacologic dose of progesterone , 7: pharmacologic dose of estrogen+physiologic dose of progesterone, 8: pharmacologic dose of estrogen+pharmacologic dose of progesterone). Hormones were injected i.p a half hour after diffuse traumatic brain injury through marmarou model.

The results showed a significance decrease in water content in 8 group comparing to vehicle and 6 groups respectively and a significance decrease in water content in 5 group comparing to 6 group. Evans blue level found a significance decrease in 6 and 7 groups comparing to vehicle. Neurological score showed a significance increase in 5 group comparing to vehicle and 3 groups at 1 hour after TBI respectively, a significance increase showed in all groups comparing to 3 group at 4 and 24 hours after TBI. Scores showed significance increase in 7 and 8 groups comparing to vehicle at 24 hours after TBI.It can be concluded that combined administration of estrogen and progesterone have benefical effect..

P4PM-4-1

K_v CHANNEL EXPRESSION BY TGF-BETA1-INDUCED DIFFERENTIATION OF MESENCHYMAL STEM CELLS TO VASCULAR SMOOTH MUSCLE CELLS

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TGFbeta-1 induces differentiation of human adipose tissue-derived mesenchymal stem cells (hADSCs) to vascular smooth muscle-like cells. In order to explore whether TGFbeta-1 induces differentiation of hADSCs to functional vascular smooth muscle cells (SMCs), we determined ion channel (Kv) properties and subtypes of the SMCs differentiated from hADSCs (hADSC-SMC) by using RT-PCR, western blot, and whole cell patch clamp. The hADSC-SMC exhibited increased expression of voltage-dependent K^+ (Kv) channel subtypes such as Kv1.1, 1.2, 1.6, 3.2, 3.4, 6.3, and 9.2 in TGFbeta-1 induced differentiation of hADSCs. Western blot analysis also revealed that specific subtypes of Kv (Kv1.1, 1.6, 3.2) were increased the expression level. Kv currents of native vascular smooth muscle rise rapidly activated and then slowly and partially inactivated during repolarization above +10 mV. Consistent with these facts, TGFbeta-1-induced differentiation of hADSCs had strong inactivation process above 0 mV, but not observed in undifferentiated hADSCs. These results suggest that TGFbeta-1-induced differentiation of hADSCs have very similar Kv channel properties of native vascular smooth muscle cells.

P4PM-4-2

ACETYLCHOLINE-INDUCED MEMBRANE CURRENTS IN VASCULAR ENDOTHELIAL CELLS IN SITU

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Whole-cell voltage clamp experiments in vascular endothelial cells *in situ* were difficult and inaccurate because of dense electrical coupling among cells. In the present experiments, we tried to clarify the properties of ACh-induced currents using the conventional whole-cell clamp technique in freshly isolated vascular endothelial layers. Voltage control of the patched cell within a multicellular preparation could be achieved using mefloquine, which effectively blocked gap junctions at concentrations of 40-50 μM. This compound, unlike many other gap junction blockers, did not seem to have major side effects including the membrane depolarization. When ACh (1 μM) was applied while the membrane potential was clamped at -15.5 mV, a sustained outward current was induced. The I-V relationship of ACh-induced current was examined by repetitive applications of a ramp from +50 to -120 mV. The reversal potential of ACh-induced current was more negative than -120 mV at first, and then gradually increased to a value close to the estimated E_K (-87.4 mV) after 1 min. When this current was blocked by charybdotoxin, a current was revealed, which had a positive reversal potential. ACh-induced currents could be precisely analyzed using mefloquine as a gap junction blocker.

P4PM-4-3

EFFECTS OF DIABETES AND INSULIN RESISTANCE IN PREGNANT RATS ON EX VIVO VASCULAR REACTION TO MAGNESIUM

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The study investigated effects of diabetes and insulin resistance (IR) during pregnancy on the ex vivo vascular reaction to magnesium. Female Sprague-Dawley rats were made diabetic by IV injection of alloxan, or insulin resistant by fructose feeding. The rats were allowed to mate and sacrificed on Day 19 of pregnancy. Aortic rings were mounted in organ baths for measurement of isometric tension. The rings were contracted with 10-7 M phenylephrine and cumulative concentration-response curves for magnesium (1-12 mM) were determined in the presence and absence of 10-4 M L-NAME or 10-5 M indomethacin.

The relaxation response to magnesium was significantly decreased in pregnant rats compared with non-pregnant rats. Pregnant rats with diabetes or IR had greater impairment in the relaxation responses to magnesium compared with normal pregnant rats. The effects of diabetes and insulin resistance on magnesium-induced relaxation in pregnant rats were not altered in the presence of L-NAME and indomethacin. The results suggest that diabetes and IR aggravate the alteration in magnesium-induced vascular relaxation observed in pregnancy, and this may be due in part to impairment to mechanisms other than the nitric oxide-cyclic guanosine monophosphate and cyclcooxygenase pathways.

P4PM-4-4

ENDURANCE TRAINING ENHANCES CIRCULATING PLASMA VEGF AND b-FGF IN OPEN WATER SWIMMERS

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Aim: To investigated the changes in circulating plasma VEGF and b-FGF in response to acute endurance exercise.

Methods: 8 open water swimmers (16± 1 years) exercised for 5 kilometers. Antecubital vein plasma was collected at rest and at 0, 2, and 4 h post exercise. Plasma VEGF was measured by ELISA analysis. b-FGF levels has been measured in serum by ELISA analysis.

Results: Acute endurance exercise significantly increased VEGF and b-FGF at 2 and 4 h postexercise in open water swimmers.

Conclusions: Endurance exercise can greatly increase plasma vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF) in open water swimmers.

That physical activity enriched the bFGF response is consistent with the hypothesis that hemodynamic factors are important contributors to collateral vessel enlargement.

The use of either plasma or serum for the measurement of VEGF and bFGF should yield similar conclusions on circulating VEGF. In addition, increases of both VEGF and bFGF enhance open water swimmers performance.

P4PM-4-5

RND3 PROMOTES ENDOTHELIAL BARRIER RECOVERY BY INHIBITING RHO

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The endothelial barrier plays a key role in normal tissue function. Signals that control cytoskeletal dynamics, such as those from Rho family small GTPases, modulate endothelial permeability. We hypothesized that the novel Rho family member Rnd3 promotes endothelial barrier integrity by inhibiting Rho-mediated actin polymerization. To test this hypothesis, we evaluated the permeability of cultured human umbilical vein endothelial cells (HUVEC) before and during 1 U/ml thrombin treatment, with either overexpressed MAT-FLAG-Rnd3 or selective siRNA depletion of endogenous Rnd3. We also evaluated Rho activation by ELISA and the F/G-actin ratio with FITC-phalloidin/Texas Red-DNAseI labeling. Rnd3 depletion significantly extended the time-course of thrombin-induced hyperpermeability, whereas Rnd3 overexpression significantly inhibited it. Rnd3 knockdown also extended the time-course of thrombin-induced Rho activation, while Rnd3 overexpression shortened it. Likewise, thrombin-induced actin polymerization was extended by Rnd3 knockdown, and inhibited by Rnd3 overexpression. These findings suggest a time-dependent role of Rnd3 in promoting endothelial barrier integrity, likely as a signal for barrier recovery. Supported by NIH RR-018766 and a grant from the American Heart Association.

P4PM-4-6

THE PATTERN OF THE RESPIRATORY MODULATION OF TISSUE-BLOOD-VOLUME MEDIATED BY SYMPATHETIC ACTIVITY

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Respiratory fluctuations in the cardiovascular system can originate from mechanical effect of thoracic pressure changes or from changes in vagal or sympathetic activity. We studied the temporal relationship between respiratory phase and tissue blood volume by using two noninvasive sensors: photoplethysmographic (PPG) device, which measured the infrared light transmission through the finger and a depth-of-breath sensor. Changes in the PPG baseline reflect changes in tissue blood volume.

15 healthy subjects were examined during long breathing of 12s period. In 11 examinations tissue blood volume increased during inspiration and decreased during expiration. The effect can be related to the increase in sympathetic activity during expiration, previously described in other studies. It was found that the finger blood volume started to decrease within 2s after the start of expiration, indicating fast sympathetic response. In two examinations tissue blood volume decreased during inspiration, probably due to the mechanical effect of lower thoracic pressure during inspiration. The measurement of the changes in tissue blood volume during inspiration and expiration can provide better understanding of the autonomic regulation of the respiratory and the cardiovascular systems.

P4PM-4-7

ADENYLYL CYCLASE TYPE 2 AND 6 DIFFERENTIALLY PROMOTE VASCULAR TONE AND REMODELING IN THE DUCTUS ARTERIOSUS

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Prostaglandin E (PGE)-adenylyl cyclase (AC)-cyclic AMP (cAMP) signaling plays two opposing roles in ductus arteriosus (DA); opening by vasodilatation and closing by hyaluronan-mediated neointimal thickening. However, distinct roles of AC isoforms in DA unsolved. Results: The expression of type 2 and 6 AC (AC2 and AC6) mRNA was higher in DA than in aorta (2.8-fold, $P<0.01$, n=60) at the perinatal period. The AC5/6-selective activator, 6-[3-(dimethylamino)propionyl]-14 15-dihydroforskolin, and AC2-selective activator, 6-[N-(2-isothiocyanatoethyl)aminocarbonyl]forskolin produced similar amounts of cAMP to PGE1. The AC5/6, but not AC2, activator promoted hyaluronan production in DA smooth muscle cells (4.8-fold, $P<0.01$, n=6). AC6-targeted siRNA negated PGE1-and AC5/6 activator-induced hyaluronan production. In organ culture, adenovirus-mediated gene transfer of AC6, but not AC2, promoted intimal thickening of DA explants (2.4-fold vs LacZ, $P<0.01$, n=8). The ductus-dilating effect of PGE1 was disappeared at 2 h in rat neonates. However, the neonatal DAs were widely open without apnea at 8 h by 8 μg/g of AC2 activator *in vivo* ($P< 0.01$, n=4). Conclusions: AC2 dilates DA without hyaluronan production while AC6 plays a role in hyaluronan-mediated vascular remodeling in DA.

P4PM-4-8

THE EFFECT OF EXPERIMENTALLY-INDUCED METABOLIC ACIDOSIS ON ENDOTHELIAL PERMEABILITY IN NORMAL AND HIGH CHOLESTEROL FED RABBITS

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The aim of this study was to evaluate the effect of experimentally-induced metabolic acidosis on coronary vascular and aortic endothelial permeability in normal and high-cholesterol fed rats. 24 male rabbit were divided into normocholesterolemic (NC) and hypercholesterolemic (HC) groups and each group was divided into normal and acidemic groups. After 6 weeks, blood samples were taken for serum pH, bicarbonate and base excess and endothelial permeability was measured using Evans blue dye method. Results showed that pH, bicarbonate and base excess were significantly lower in acidosis groups. HC diet significantly increased serum cholesterol and LDL levels, however, acidosis didn't alter serum cholesterol level (HC: 507.70±28.31, HC+Acidosis: 569.67 ±26.01mg/dl; $p>0.05$). HC animals had significant higher aortic endothelial permeability compare to NC groups (17.77± 0.91 vs. 13.60±0.68 μg EB/gram tissue) and acidosis significantly increased it in these group ($p<0.05$). Coronary vascular permeability was not different between groups and acidosis didn't change it. Experimentally metabolic acidosis didn't change serum lipids in NC and HC animals, however, it increased aortic endothelial permeability which can affect progression of atherogenesis.

P4PM-4-9

RESPONSES OF SPINAL CORD BLOOD FLOW TO VARIOUS TYPES OF SOMATOSENSORY STIMULATION IN ANESTHETIZED RATS

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Responses of spinal cord blood flow (SCBF) to various types of somatosensory stimulation were investigated in anesthetized rats. Regional SCBF was measured with a laser Doppler flowmeter probe placed on the dorsal surface of the L4 - 6 region of the spinal cord. SCBF was increased by brushing of the hind leg and hindpaw, but not by brushing of the forepaw, fore leg, chest or abdomen. None of these brushing stimuli produced significant changes in systemic arterial blood pressure. These results indicate that brushing can produce a segmentally-organized increase in regional SCBF. Pinching of the forepaw (non-segmental stimulation) or hindpaw (segmental stimulation) increased SCBF, coincident with increases in arterial blood pressure. After treatment with phenoxybenzamine, an alpha-adrenoceptor blocker, the responses of arterial blood pressure to pinching of the forepaw or hindpaw became negligible; however, the responses of SCBF to pinching of the hindpaw (but not forepaw) persisted. Thermal stimulation (3 - 53 degrees centigrade) of the hindpaw had no influence on SCBF. These results indicate that increases in SCBF are dependent on the modality of somatosensory input.

P4PM-5-1

ESTROGEN ATTENUATES RESPONSES OF FOOD INTAKE AND PERIFORNICAL OREXINERGIC NEURON' S ACTIVITY TO GLUCOPRIVATION

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We examined the effect of estrogen (E_2) replacement in ovariectomized rats on responses of food intake and lateral hypothalamic neuron's activity to glucoprivation induced by i.v. 2-deoxy-D-glucose (2DG) administration. Rats were ovariectomized and implanted E_2 or vehicle (cholesterol) subcutaneously. Two weeks after the replacement, rats were i.v. infused with 2DG (400 mg/kg) and food intake was measured for 3 hours. The same experiment was performed for immunohistochemical examination of c-Fos, melanin concentrating hormone (MCH), and orexin A expressions in the lateral hypothalamic area (LH) and c-Fos in the arcuate nucleus (Arc). Glucoprivation induced c-Fos expression in the orexin A neurons locating at the perifornical region of LH, but did not activate MCH neurons. Glucoprivation significantly stimulated food intake, and food intake in E_2 group was significantly lower than vehicle group. The number and fraction of c-Fos expressed orexinergic neurons was significantly lower in E_2 group than vehicle group. The number of c-Fos ir cells was less in E_2 group than vehicle rats. These data indicate that E_2-induced reduction of food intake stimulated by 2DG is possibly due to the reduced perifornical orexinergic neuron' s activity and to the reduced Arc neuron's activity.

Poster Session

P4PM-5-2

SHORT FASTING DECREASES DUODENAL MUCOSAL AJP RECEPTOR mRNA AND INHIBITS APELIN INDUCED STIMULATION OF DUODENAL BICARBONATE SECRETION

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Apelin is the endogenous ligand of the APJ receptor and the peptide as well as APJ mRNA is expressed in several tissues. We have studied effects of apelin on bicarbonate secretion by the duodenal mucosa in fed and fasted rats. Lewis x Dark Agouti rats had free access to water and, unless fasted overnight, free access to food. Proximal duodenum was cannulated in situ after anesthesia, bicarbonate secretion titrated (pH stat) and apelin-13 administered by close intra-arterial infusion. Total RNA was extracted from mucosal specimens, reverse transcripted to cDNA and expression of APJ receptor measured by quantitative real-time PCR. Results: Apelin increased the duodenal secretion in fed animals. The lowest dose infused (6 pmol/kg, h) caused a 35% rise in secretion and 60 pmol/kg,h induced a further slight increase. Pretreatment with atropine did not affect the secretory response. No stimulation was observed in fasted animals, even with the highest dose of apelin tested (600 pmol/kg,h). Overnight fasting caused a 8-fold decrease in the expression of APJ receptor mRNA. Very low doses of apelin thus stimulate the duodenal secretion in the fed state. Stimulation does not involve muscarinergic pathways and, as previously found with orexin-A and GIP, markedly depends on feeding status.

P4PM-5-3

REGIONAL DISTRIBUTION AND THE DYNAMICS OF n-DECANOYL GHRELIN, ANOTHER ACYL-FORM OF GHRELIN

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n-Decanoyl ghrelin (D-ghrelin), a member of ghrelin-derived peptides, is found in plasma and the stomach; however, there have so far been no studies describing its dynamics. A D-ghrelin-specific radioimmunoassay was established to examine the tissue distribution and the kinetics of D-ghrelin in mice. The effect of D-ghrelin on 2 h food-intake was also examined and compared to n-octanoyl ghrelin (O-ghrelin). D-ghrelin was detected throughout the gastrointestinal tissue and plasma with highest level in the stomach. Immunofluorescent studies and electormicroscopic findings revealed the co-localization of D- and O-ghrelin in the same stomach cells. Upon fasting, the levels of D-ghrelin in the stomach and plasma significantly increased, while that of O-ghrelin in the stomach declined. D-ghrelin increased the 2 h food-consumption in mice as O-ghrelin does. These findings indicate that D-ghrelin is mainly produced in the stomach to work in concert with O-ghrelin. The different kinetics of D- and O-ghrelin in the stomach implies the possibility of D-ghrelin-specific bioregulation.

P4PM-5-4

FUNCTIONAL CHARACTERIZATION OF CULTURED A-LIKE CELLS ISOLATED FROM RAT GASTRIC MUCOSA

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The regulation of food intake in the stomach is the initial important process in the recognition of nutrient status and regulation of nutritional homeostasis in the brain. Previous studies revealed that stomach produces important peptide hormones such as orexigenic ghrelin and anorexigenic leptin and nesfatin-1 and then regulates food intake in an endocrine manner. In addition, interesting finding was recently shown that A-like cells in the gastric mucosa are the main source of nesfatin-1 as well as ghrelin. This suggests the important roles of A-like cells in the regulation of food intake and satiety. However, the mechanisms in the direct regulation of ghrelin and nesfatin-1 secretion are still unknown. Here, we established A-like cell culture isolated from rat gastric mucosa using a counterflow elutriation. In addition, we successfully detected enough amounts of ghrelin and nesfatin-1 in cell culture supernatants stimulated by some reagents. These results indicate that our A-like cell culture is quite valuable model to clarify the direct regulation of ghrelin and nesfatin-1 secretions from the cells. This method would help to explore the new therapeutic targets for the prevention of metabolic syndrome by regulating food intake and satiety via gastric hormones.

P4PM-5-5

FUNCTIONAL ROLES OF GLUTAMATE RECEPTORS/ TRANSPORTERS ON CULTURED D CELL SOMATOSTATIN RELEASE ISOLATED FROM RAT GASTRIC MUCOSA

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Glutamate is known as the taste receptor agonist for umami. We have previously reported that luminal application of glutamate into the stomach stimulates the activity of afferent vagus nerve. This suggests the important role of glutamate as the nutrient signaling molecule from the stomach to the brain. To clarify the glutamate chemosensing pathway, we fractionized different kinds of cells from rat gastric mucosa using a counterflow elutriation and a density gradient centrifugation and then analyzed the expressions of possible glutamate sensors in these cell fractions. We also tested the effects of glutamate on the functions of fractionized cell culture. The expressions of glutamate receptors/transporters were diverse among these cells, however, D cell fraction showed relatively specific expressions for some Gi-coupling mGluR subtypes and excitatory amino acid transporters. In addition, D cell fraction was specific for taste cell markers such as PLCb 2 and TRPM5. Interestingly, glutamate decreased both the basal and the stimulated somatostatin release. From these results, we propose that the D cells are the possible candidates for luminal glutamate sensors in the stomach and regulate gastric functions via endocrine (somatostatin release) and neural (vagus nerve) pathways.

P4PM-5-6

ROLE OF APPETITE HORMONES GHRELIN, OREXIN AND OBESTATIN IN THE MECHANISM OF GASTRIC ULCER HEALING

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Ghrelin isolated from gastric mucosa, obestatin encoded by the ghrelin gene and orexin-A (OX-A) belong to the family of appetite hormones but their effect on the healing of preexisting gastric ulcers remains unknown. Gastric ulcers were induced by serosal application of acetic acid (ulcer area=28 mm^2) in Wistar rats treated for 9 and 15 days with: 1) vehicle (saline), ghrelin, orexin-A and obestatin; and 2) blockade of ghrelin GHS-1a and orexin OX-R1 receptors with D-Lys^3-GHRP-6 or SB 334867 (5 mg/kg i.p.). Both, ghrelin and orexin-A dose-dependently reduced the area of gastric ulcers and these effects were accompanied by the rise in gastric blood flow (GBF) at ulcer margin and RIA plasma ghrelin and OX-A levels and the fall in mRNA IL-1β and TNF-α levels. Obestatin by itself failed to influence the area of gastric ulcers. The ulcer healing effects of ghrelin and OX-A were significantly inhibited by D-Lys^3-GHRP-6 or SB 334867, COX-1 (indomethacin) and COX-2 (rofecoxib) inhibition and by vagotomy and capsaicin denervation. COX-2 mRNA and protein were upregulated at the ulcer margin in ghrelin- and OX-A-treated rats. We conclude that ghrelin and OX-A accelerate ulcer healing via mechanism involving activation of specific receptors, PG-COX-2 system, vagal and sensory nerves.

P4PM-5-7

IMPORTANCE OF PERIPHERALLY AND CENTRALLY APPLIED GHRELIN IN THE MODULATION OF RESISTANCE OF THE INTESTINE TO THE ISCHEMIA AND REPERFUSION

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The aim of present study was to evaluate the effects of exogenous ghrelin in the modulation of the intestinal mucosa resistance against damage induced by ischemia and reperfusion (I/R). Wistar rats were used. Short lasting (30/60 min) and long lasting (60/120min) ischemia /reperfusuion was applied with central ghrelin (CG) and peripheral ghrelin (PG) administration prior and after ishemia. Mesenteric and intestinal microcirculatory blood flow, intestinal oxygen uptake were measured. Planimetrical and histological assessment of intestinal damage was performed.

PG evoked marked increase of intestinal and oxygen uptake and significant reduction of mucosal ulcer area by 38 %.

In case of PG when ghrelin was administered prior or at the end of reperfusion period of short I/R protective effect with marked reduction of ulcers area was observed. This effect also observed with CG administered prior to short I/R but not after I/R or in case of long I/R. Sensory denervation blocked protective effects of PG. Whereas CG protective activity was abolished after vagal denervation. We conclude that CG and PG evoked intestinal protection which is blood flow and oxygen uptake dependent and mediated by intestinal sensory and vagal innervation.

P4PM-5-8

OPTIMIZING METHODS FOR ISOLATING AND MAINTAINING NODOSE GANGLION NEURONS FOR Ca^{2+} IMAGING

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Vagal afferent neurons play a key role in relaying peripheral information to the brain. Vagal afferent fibers originate from the nodose ganglion (NG) neurons. To elucidate the neuronal mechanisms for vagal afferent sensing of visceral factors, functional analysis of NG neurons is essential. The aim of this study was to optimize the methods for isolation and culture of NG neurons and measurement of cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) with fura-2 fluorescence imaging in NG neurons.

NG neurons were isolated from Wistar rats and treated with dispase II, collagenase and DNase II for 20 min at 37 degree C. Isolated NG neuronswere plated onto poly-L-lysine-coated coverslips and cultured in MEM or nutrient-rich DMEM containing 10% FBS. Effect of brain-derived neurotrophic factor (BDNF) added in culture was also examined.

Cholecystokinin-8 (CCK8) (10 nM) and capsaicin (CAP) (100 nM) increased $[Ca^{2+}]_i$ in NG neurons after culture for 1 day. These responses to CCK8 or CAP occurred in, respectively, 37-47% and 75-79% of NG neurons, irrespective of the type of culture medium and presence of BDNF. We found that as much as 30-80% of NG neurons responded to CCK8 and CAP, thereby establishing the optimal method for analyzing the regulation of isolated NG neurons.

P4PM-5-9

AGING EFFECT ON EXERCISE-INDUCED ALTERATIONS OF THE SECRETION OF METABOLIC HORMONES IN MALE RATS

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Ghrelin is a hormone produced mainly by the fundus of the stomach. Ghrelin levels increase before meals and decrease after meals. This study was to investigate the change of active ghrelin before and after swimming exercise. The changes of secretion of leptin, insulin, corticosterone before and after exercise between young and middle-age male rats were also examined. The rats at different ages divided into exercise and control groups, and catheterized via right jugular vein (RJV) before an overnight fast. The rats of exercise groups swimmed in 20ºC water for 20 min. Blood samples were collected from RJV at different time intervals. The results showed that the concentration of plasma leptin increased after exercise, which rised relatively less after exercise and returned back to basal levels earlier in middle-age rats than in young rats. The downward trend of plasma active ghrelin was relatively compared with the young rats after exercise while the middle-age rats returned back to basal level faster. These data suggest that swimming exercise led to decrease plasma level of active ghrelin and insulin but enhance leptin level, whereas middle-age male rats had less effect.

P4PM-5-10

FASTING PLASMA LEVELS OF NESFATIN-1 IN PATIENTS WITH TYPE 1 AND TYPE 2 DIABETES MELLITUS

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Objective: A recent study indicates that nesfatin-1 (nesf-1) decreases food intake and body weight in rodents after icv injection. We investigated the relationship between fasting plasma nesf-1, glucose and insulin concentrations in the patients with type 1diabetes mellitus (T1 DM) and type 2 diabetes mellitus (T2 DM).

Methods: Fasting levels for plasma nesf-1, insulin and glucose were measured and analyzed in healthy subjects and in patients with T1 and T2 DM.

Results: The mean fasting plasma nesf-1 levels were slightly but not significantly higher for patients with T1 DM compared to the healthy subjects .However, fasting plasma nesf-1 levels were significantly lower in the patients with T2 DM compared to the healthy subjects and the patients with T1 DM.

Conclusion: We showed that fasting nesf-1were significantly lower in the patients with T2 DM compared to the healthy subjects. The significance of this result is unclear but the reduction in fasting nesf-1 level may be one of the appetite-related hormones involved in diabetic hyperphagia. A significant reduction in the fasting plasma nesf-1 level in the patients with T2 DM could be due to the fact that these patients were overweight, had a far greater risk of developing insulin resistance than the patients with T1 DM.

P4PM-5-11

ADIPONECTIN HYPERPOLARIZES PROOPIOMELANOCORTIN NEURONS IN SLICES OF THE HYPOTHALAMIC ARCUATE NUCLEUS

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Aim: An adipokine, adiponectin, facilitates insulin sensitivity and regulates energy expenditure in the peripheral organs. Recent studies have suggested that serum adiponectin secreted from adipocytes enters and exerts effects in the brain. Adiponectin receptors are expressed in several regions of the brain including the hypothalamic paraventricular and arcuate nucleus (ARC), the centers regulating feeding and energy expenditure. Although intracerebroventricular injection of adiponectin was reported to alter energy expenditure and food intake, discrepancy exists between reports: adiponectin either facilitated or inhibited both energy expenditure and food intake. The aim of this study was to determine the effect of adiponectin on the activity of proopiomelanocortin (POMC) neurons in the ARC.

Methods: Slices containing ARC were produced from the hypothalamus of POMC-GFP mice. The effect of adiponectin on the membrane potential and firing in POMC neurons in slices was recorded under current clamp mode.

Results: Bath application of adiponectin (100 ng/ml) hyperpolarized membrane (4.7±1.6 mV, n=5 cells) and decreased firing in ARC POMC neurons.

Conclusion: Adiponectin inhibits POMC neurons in ARC slices, which may be related to suppression of satiety by adiponectin.

P4PM-5-12

PARAVENTRICULAR HYPOTHALAMIC AMPK REGULATES MACRONUTRIENT SELECTION OF CARBOHYDRATE AND FAT DIETS

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AMP-activated protein kinase (AMPK) in the hypothalamus regulates food intake by responding to hormonal and nutrient signals. The paraventricular hypothalamus (PVH) is implicated in the regulation of food preference as well as total calorie intake, while the regulatory mechanism remains unclear. Here, we report that neuron specific expression of constitutively active (CA)-AMPK in the PVH using lenti virus increases the intake of high carbohydrate diet (HCD) rather than high fat diet (HFD), by enhancing fatty acid oxidation (FAO) in the PVH. CA-AMPK mice then became massive obese under HCD, while they were lean under HFD. Refeeding after overnight fasting and treatment with melanocortin receptor (MCR) agonist and antagonist, which change AMPK activity in the PVH, also altered food preference depending FAO in the PVH. KK-Ay mice, which inhibit MCR activity by ectopically expressing agouti protein and thereby cause late-onset obesity, revealed high carbohydrate intake in young age but increased fat intake according to the body weight gain, which paralleled with the paradoxically decreased AMPK and FAO activity in the PVH under the obesity. Thus, our results suggest that AMPK in the PVH regulates macronutrient selection of carbohydrate and fat diets by changing FAO in the PVH.

P4PM-5-13

A LOW-CALORIE DIET INFLUENCE ON THE BODY WEIGHT REDUCTION AND ADIPO-METABOLISM

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Obesity is the greatest risk factor for the metabolic syndrome, a cluster of insulin resistance, hypertension, dyslipidemia, Type 2 diabetes, and atherosclerotic vascular diseases. Thus, weight reduction is valuable and essential to prevent the lifestyle-related disease and to maintain health.

To clarify the influence of the low-calorie diet (LCD) on health, changes in hematological and biochemical plasma markers including leptin and adiponectin were monitored using healthy overweight subjects (BMI, kg/m^2, ≥25).

Subjects (15 men, age 30.4 ±5.6 years, BMI 27.7 ±1.5 kg/m^2 and 12 women, age 28.6 ± 6.0 years, BMI 27.7 ±2.4 kg/m^2) consumed marketed SLIMTOPS for breakfast and supper, while lunch was free intake (800 kcal), so that total daily intake fell within 1500 kcal. Body weight, BMI, blood pressure, and measures of general plasma hematological and biochemical markers were taken before the study and at study weeks 4 and 9. Body weight, BMI, and biochemical markers, such as triglyceride, LDL-cholesterol, total cholesterol, GOT, GPT, cholinesterase and γ-GTP were improved at weeks 4 and 9. Visceral fat decreased significantly and more than subcutaneous fat. In conclusion, the diet resulted in improved glucose and lipid metabolism.

P4PM-6-1

ORGAN PROTECTIVE AND ANTI-LIPIDEMIC EFFECT OF OCIMUM SANCTUM L. LEAVES IN RATS FED WITH HIGH CHOLESTEROL DIET

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The present study was conducted to elucidate anti-hyperlipidemic and organ protective effect of OS leaves in rats fed with high cholesterol diet. OS leaves were extracted by water. Three groups of male Wistar rats were used including normal control group, high cholesterol (HC) group and HC group treated with aqueous extract of OS. HC rats were fed with 2.5% cholesterol supplemented in diet for 7 week. OS was fed during the last three weeks. The results showed that HC diet raised serum total cholesterol, triglyceride, LDL-C and atherogenic index (AI) whereas it decreased HDL-C. Liver and cardiac lipid peroxide (LPO) were significantly enhanced in HC rats. OS significantly depressed high serum levels of total cholesterol, triglyceride, LDL-C and AI but it raised HDL-C. High serum levels of ALT, AST, LDH and CK-MB were suppressed by OS. Similarly, high levels of LPO content in liver and heart were attenuated in HC rats treated with OS. It can be concluded that treatment of aqueous extract of OS during the last 3 weeks decreased high serum lipid profile and AI, and expressed hepatoprotective and cardioprotective effects in rats fed with HC diet for 7 weeks.

P4PM-6-2

EFFECT OF CALCIUM-ENRICHED HIG-FAT DIET ON CALCIUM, MAGNESIUM AND ZINC RETENTION, IN MICE

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Aims: The aim of this work was to assess the effects of a high-fat diet enriched in Ca, which accompanies lower body fat deposition, on mineral depots, as well as to assess the potential role of adaptive thermogenesis in mice. Approach: Male mice were fed ad libitum a high-fat (43%) diet with a Ca content of 4 g/kg from calcium carbonate (control group) or 12 g/kg (42% from milk powder and the rest from calcium carbonate) (Ca group) for 56 days. Body weight, food intake and urine, were periodically collected. Tissue samples were collected ata sacrifice and composition was determined. Expression of uncoupling proteins was determined by Western blotting. Mineral content was measured by flame atomic absorption spectrometry. Results: Lower body weight gain and fat accretion was found in Ca group. This could not be attributable to lower gross energy intake or to activation of adaptive thermogenesis. Although significant urine mineral loses were found in Ca group, preservation of mineral depots in bone was seen. Conclusions: Our data support the fact that adding more calcium to the diet, using a combination of calcium carbonate plus milk powder containing among other things higher Zn and Mg, contributes to counteracting obesity and improving lipid metabolism.

P4PM-6-3

THE INFLUENCE THAT A MILK INTAKE CUSTOM GIVES TO THE BONE MINERAL DENSITY OF THE ATHLETE

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This study examined influence of the milk intake customs and the education promoting milk intake on bone mineral density of athletes.

Subjects are 172 male students of physical education. For them, the body composition including the bone mineral density were measured by dual-energy X-ray absorptiometry (DXA). The milk intake customs and the education promoting milk intake was assessed by a questionnaire.

Based on whole body bone density, subjects were divided into three groups named high density, medium density and low density group. The milk intake customs of High density group showed higher than low density group.

The group that experienced the cessation of milk drinking after elementally school or junior high school showed significantly low bone mineral density than the group with consistent milk intake from elementally school to present. While more than half of subjects experienced the promotion about milk drinking in the elementally school to junior high school period, it is decreased to forty percent during high school period.

Generally, it is said that high bone mineral density are observed in athletes.

This study suggest that the milk intake custom not a little participated of the bone mineral density of athletes.

P4PM-6-4

ANORECTIC ACTIVITY OF UROCORTIN 2 IS ATTENUATED BY ACCESS TO HIGH FAT DIET ACCESS AND OBESITY RISK GENOTYPE IN FEMALE RATS

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Corticotropin-releasing factor type 2 (CRF_2) receptor agonists reduce food intake. We tested the hypothesis that third ventricle infusion of urocortin 2 (Ucn 2, 0.1-3 μg), a CRF_2 agonist, retains anorectic activity in a genetic model of obesity risk, despite high-fat (HF) diet history. Female DIO and DR (N = 122) rats were provided ad lib access to chow or to HF diet or binge-like access to HF (1h/day, 3 days/week), with chow otherwise provided. Food intake and body composition were measured for 3 weeks. *Ad lib* HF-fed DIOs showed greater baseline 1h and daily caloric intake, gaining 2.5x more weight and fat mass by the 3rd week, than HF-fed DRs. During binge-like access to HF, DIO and DR binge rats equally overate relative to *ad lib*-fed animals. However, binge-fed DIOs ate more chow in the other 23h than DRs, yielding greater total daily intake. Despite eating more during the "binge" hour, total daily intake, weight and fat content of "binge" groups did not differ from chow-fed rats by the 3rd week. ICV Ucn 2 reduced chow intake with lower potency in DIOs, than in DRs. However, Ucn 2 did not reduce HF diet intake in either genotype under ad lib or binge access conditions. Thus, the anorectic activity of Ucn 2 was attenuated by HF diet and by obesity risk genotype.

P4PM-6-5

PROLACTIN-RELEASING PEPTIDE IS IMPORTANT FOR RELAYING LEPTIN SIGNALING TO REDUCE FOOD INTAKE AND BODY WEIGHT

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Food intake is controlled by signals from the brainstem that mediate signals from the gut. Prolactin-releasing peptide (PrRP) neurons are localized in the brainstem. We have reported that food intake activates PrRP neurons in the nucleus of tractus solitarii, and that PrRP-deficient mice develop late onset obestity associated with metabolic disorders due to hyperphagia reflecting an increase in meal size. These mice also show an attenuated response to the peripheral satiety signal, cholecystokinin (CCK). However, mice deficient in CCK_A receptors do not show obesity, suggesting that the abnormality in CCK_A receptor signaling might not necessarily cause obesity observed in PrRP-deficient mice. Leptin is important for long-term metabolic homeostasis. We thus investigated whether leptin activates PrRP neurons, and examined effects of leptin in PrRP-deficient mice. Leptin induced phosphorylation of STAT3 in PrRP neurons in the nucleus of tractus solitarii and dorsomedial hypothalamus. Reduction in food intake and body weight observed following i.p. or i.c.v. leptin at the lower dosage was impaired in PrRP-deficient mice. These data suggest that PrRP is important for relaying leptin signaling to reduce food intake and body weight.

P4PM-6-6

OBESE IN INFANT MICE LACKING CHOLECYSTOKININ-A RECEPTORS

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Although cholecystokinin (CCK)-A receptors are known to control satiety, their functions for milk sucking behaviors in pups remain unclear. Therefore, gene knockout mice lacking CCK-A receptors ($CCKAR^{-/-}$) were used to analyze (i) post-natal body weight and (ii) CCK-A receptor expressions in the posterior hypothalamus. Although newborn body weights were not significantly different between genotypes, body weights increased faster in $CCKAR^{-/-}$ during lactating period. The X-gal staining signals, which visualize lacZ reporters expressed by the CCK-A receptor gene knockout cassette, were abundant in the dorsal medial hypothalamus and arcuate nucleus in adult brains whereas the signals were scattered in infant brain parenchima. Alternatively, third ventricular epidermal cells represent staining signals in infants. The epidermal CCK-A receptors are functional since the CCK-A agonist (CCK-8s) mobilized cytosolic Ca^{2+} most significantly at this locus in infant brain slices. These results suggest CCK peptides and CCK-A receptors are functioning to control feeding behaviors not only in adults but also in infants. Dramatic changes in CCK-A receptor expressions in the posterior hypothalamus may intermediate switching of feeding behaviors from sucking to eating during post-natal development.

P4PM-6-7

EFFECTS OF COLD ACCLIMATION AND DEACCLIMATION ON ENERGY BALANCE OF GENETICALLY DIFFERENT OBESE RATS; OBESE ZUCKER AND OLETF RATS

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Cold acclimation (C) and deacclimation (D) resulted in good effects on lipid metabolism and energy balance of obese rats. Different effects were observed in genetically different obese rats: OLETF rats (cholecystokinin-A receptor defective) and obese Zucker rats (leptin receptor defective). Weight-reducing effect (13.8%) was pronounced with less increment of food intake (13.6%) in obese Zucker rats. While, in OLETF rats, a remarkable increase in food intake (35.8%) and less weight-reducing effect (3.1%) were observed. We compared properties of the brown adipose tissues (BAT) and serum hormones, lipids and adipocytokines. Glucose and insulin were higher in obese rats. Insulin was usually decreased during C and restored to the control level after D. In OLETF rats, it was strikingly reduced during C and D. Changes in BAT and blood level of lipids and hormones differed in both obese rats. Thyroxine was higher and corticosterone was lower in OLETF rats than in LETO rats, contrary to Zucker rats. UCP1, GLUT4, insulin receptor, leptin receptor and PGC1 of BAT were differentially expressed in both rats during C and D. As a result, obese Zucker rats mainly use stored fat and reduced blood triglyceride and OLETF rats preferred to use food for heat production during cold acclimation.

P4PM-6-8

ALTERED METABOLIC RESPONSES TO HIGH-FAT DIETS IN NOP RECEPTOR DEFICIENT MICE

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Central administration of nociceptin/Orphanin FQ (NOP) causes hyperphagia and chronic administration accelerated body weigh gain in rodents. The aim of this study is to test how chronic NOP receptor deficiency affects body weight gain under both regular diet (RD) and high-fat diet (HFD). NOP receptor knockout (KO) mice exhibited significantly lower body weight gain than those in wild-type (WT) mice with RD for 19 weeks. When HFD was given to both genotypes, the difference in body weight gain between WT and KO mice was exaggerated. The NMR study and measurement of retroperitoneal white adipose-tissue weight revealed that NOP receptor deficiency seemed to prevent fat accumulation especially in the mice fed HFD. In addition, the KO mice exhibited lower food intake and increased O_2 consumption measured by the indirect calorimetry study. Results in glucose tolerance test revealed that NOP receptor deficiency prevented larger increases in plasma glucose, thus the progress of HFD-induced insulin resistance might be delayed. These results suggest the possibility that the NOP is an important regulator of the energy metabolism and that inhibition of the NOP would be a potential therapeutic target for obesity and subsequent metabolic disorders.

P4PM-6-9

MICE OVEREXPRESSING HUMAN PREPRO-OREXIN SHOW INCREASED METABOLIC HEAT PRODUCTION AND ELEVATED PPARS AND UCP2 MRNA IN WAT

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Orexins are peptides involved in energy homeostasis. They have been shown to act peripherally and affect thermogenesis. Orexins increase PPARs mRNA in WAT, which are known to affect UCPs expression. We used a transgenic approach to investigate if overexpression of the human prepro-orexin (hPPO) changes the phenotype of mice. Transgenic (tg) mice were basic characterized, and metabolic performance, home cage activity as well as drinking and feeding behavior were measured using an automated monitoring system. mRNA expression of UCP 1-3 and PPARs delta and gamma from WAT/BAT and skeletal muscle from fed mice were analyzed. Tg mice showed significantly increased hPPO/orexin-A levels in several tissues including the WAT. The presence of orexin-A in WAT was verified in wt mice, suggesting a role of orexins in adipose tissue metabolism. Tg mice exhibited an increased heat production with no change in 24hr eating and drinking behaviour or activity. However, there was a significant increase in cumulative daytime food intake in tg mice. PPARs mRNA levels were upregulated 3-fold, and UCP2 2-fold in WAT in tg mice, but not in other tissues. In conclusions, orexins increase heat production, mRNA expression of PPARs and UCP2 in WAT thereby affecting overall body energy homeostasis.

P4PM-7-1

LONG CHAIN n-3 FATTY ACID SUPPLEMENTATION INCREASES THE RATE OF MUSCLE PROTEIN SYNTHESIS IN YOUNG ADULTS

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In growing steers long chain n-3 polyunsaturated fatty acids (LC n3 PUFA) improve insulin sensitivity and increase whole-body amino acid disposal during hyperinsulinemia-hyperaminoacidemia. The effects of LC n-3 PUFA on human protein metabolism are unknown. To fill this gap, we measured rates of whole-body protein breakdown (WBPB) and muscle protein synthesis (MPS) during basal postabsorptive conditions and intravenous infusion of insulin and amino acids in eight adults (40±1.7 y; BMI: 26±1.0 kg/m^{-2}; means ± SEM) before and after 8 weeks of supplementation with LC n3 PUFA (4 g/d). LC n-3 PUFA supplementation increased MPS by ~40% ($P< 0.05$), both during basal conditions (from 0.032±0.003 to 0.050±0.007 %/h) and during hyperinsulinemia-hyperaminoacidemia (from 0.054±0.006 to 0.072± 0.007 %/h); the anabolic response (i.e., the stimulation of MPS by hyperinsulinemia-hyperaminoacidemia), was however not different before and after LC n-3 PUFA supplementation (0.022±0.006 vs. 0.021±0.008 %/h; $P= 0.99$). Measurement of phosphorylation of P70s6K, mTOR and eif4BP1 showed no differences in basal or fed state responses after LC n-3 PUFA. WBPB was not affected by LC n3 PUFA ($P> 0.35$). We conclude that in humans LC n-3 PUFA have anabolic properties in muscle.

P4PM-7-2

COMPARISON OF RESTING METABOLIC RATES MEASURED WITH TWO DIFFERENT METHODS

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The aim of this study was to compare the resting metabolic rates (RMRs) measured by bioelectrical impedance analysis and portable indirect calorimeter methods.

A total of 99 subjects (59 females and 40 males) which were 20-58 years old were included in the study. Subjects who participated in this study were asked not to take any food in 12 hours and not to perform exercise in 24 hours in advance. After resting 15 minutes, the measurements were applied to the subjects in laboratory which was silent, lightless and at room temperature. The electrodes of bioelectrical impedance analysis device (Bodystat 1500) were placed on the right hand and foot to take the measurements for RMR. Secondly RMR was measured from breathing gases with portable indirect calorimeter (Bodygem). Data were analyzed using Wilcoxon test and Spearman correlation. The mean values for age, body weight, height, body mass index in subjects were determined. Mean RMRs were found as 1637.5±225.7 Kcal/day by bioelectrical impedance analysis, 1577.2±286.8 Kcal/day by portable indirect calorimeter. The difference between the two measurements was significant ($p=0.008$). We suggest that RMR measurements with bioelectrical impedance analysis can not be used instead of the ones measured with portable indirect calorimeter.

P4PM-7-4

TISSUE DISTRIBUTION IN RATS OF TRANS-RESVERATROL, A NATURAL ANTIOXIDANT FROM GRAPES

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trans-Resveratrol, a polyphenol from grapes, is being recognized as a bioactive compound with potential beneficial effects on health. However, little is known of its availability to tissues, so extraction methods of trans-resveratrol from organs with HPLC analysis have been developed. The methods were validated by the analyses of tissue samples spiked with pure resveratrol. Precision (CV) ranged from 3.7 to 13.2%, in testicle and lungs. Recoveries were 98.5 ± 3.2% (liver), 100.1 ± 1.8% (kidney), 96.5 ± 7.6% (lungs), 99.0 ± 0.7% (brain) and 103 ± 2.7% (testicle). The limits of detection ranged from 5.5 to 11.2 nM in testicle and kidney. The methods were applied to the analysis of rat tissues after the intravenous administration of 15 mg/kg (n=6). At 90 min, trans-resveratrol and its glucuronide and sulfate conjugates were widely distributed, with the highest concentrations (nmol/g tissue) in kidney (resveratrol: 1.45 ± 0.35; glucuronide: 2.91 ± 0.19; sulfate: not detected), and the lowest in brain (resveratrol: 0.17 ± 0.04; glucuronide: not detected; sulfate: 0.04 ± 0.01). In conclusion, accurate and reproducible methods have been used to identify target tissues of resveratrol, which may help to understand its mechanisms of action in vivo. Supported by AGL2005-05728 (MCT)

P4PM-7-5

THE EFFECTS OF VITAMIN BEVERGE ON THE FUNCTION OF THE ENDODERMIS IN ABLE- BODIED PEOPLE AFTER SHORT TERM CONSUMPTION

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Aim: As VCRESC (NUTRI CO.Ltd.) is now widely used in medical facilities for micronutrient assistance and preventative medicine, this study examines the effects on well bodied peoples' vitamin value, homo-cysteine (Hcy) value and vasodilator factor after short term consumption of VCRESC.

Methods: Twelve well bodied subjects were selected for the study (n=12), and blood vitamin and mineral levels, Hcy and Asymmetric Dimethylarginine (ADMA) values were measured. From lunchtime the following day, subjects drank 125ml of VCRESC after lunch each day for a period of three days. Patients were tested once again on the forth day for comparison and analysis.

Results: After three days of VCRESC consumption, subjects' vitamin B1, B2, B6, B12 and folic acid found within blood samples significantly rose ($p<0.01$)with ADMA values significantly decreased. Anti- oxidant vitamin C also significantly rose ($p<0.01$).

Conclusion: The short term consumption of a vitamin micronutrient replenishment beverage VCRESC effects blood density, effects coenzymes, and particularly the function of the endodermis. Kanazawa Medical University Hospital began giving all in-patients (18 years and over) VCRESC from September 2003.

P4PM-7-6

THE EFFECTS ON VITAMIN AND HOMOCYSTEIN (Hcy) LEVELSIN HEALTHYPEOPLE

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Aim: The nourishment evaluation in the periodic health check-up is evaluated overall by a part of the body measurement, blood test. In the periodic health check-up of a general enterprise, the influence on the change of the blood inside vitamin value and value of the Hcy when the movement was taken was examined.

Methods: Diseased factor Hcy of blood inside vitamin B1 value before and after moving, vitaminB6, vitaminB12, folic acid, vitaminC, and cells that line the blood vessels was assumed to be a measure -ment item to 14 person general deskwork. The condition of the movement was to have used it for fitness club three times or more during the week.

Results: A blood vitamin B6 and bloody inside neither inside vitamin B12 nor blood folate had a significant difference to move. After it had moved before it moved of the low value tendency, vitamin B1 and blood inside vitaminC inside blood intentionally reached a low value. ($p<0.01$) Inside Hcy blood intentionally indicated a low value. ($p<0.01$)

Conclusion: When the nourishment of the vitamin is evaluated, it is necessary to evaluate it in blood overall by the measurement and the meal investigation of the vitamin value. It thinks about metabolizing by the movement the Hcy value in blood and it is possible to have been improved.

P4PM-7-7

THE EFFECTS OF A VITAMIN BEVERAGE ON CORONARY-ARTERY ATHEROSCLEROSIS PATIENTS' VITAMIN AND HOMOCYSTEINE AFTER LONG TERM CONSUMPTION

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Aim: There are three causes associated with clot formation in Coronary-Artery Atherosclerosis; artery damage, fibrous plaque and hard plaque. This study examines blood vitamin levels (B6, B12), folic acid levels, and Hcy values after consuming VCRESC on a long term basis.

Methods: Fifteen arteriosclerosis subjects were selected for the study (54.4±3.0 years old). Blood samples were taken from each subject, and vitamin B6, B12, folic acid and Hcy values were measured. Subjects then consumed 125 ml/day of VCRESC, over a six month period. Blood samples were taken again after six months and were measured, compared and examined with the original blood samples.

Results: In primary blood samples, levels of vitamin B6, after the trial period showed significant increase into middle and higher regions. ($p<0.01$) B12 levels were showed significant increase in the second test. ($p<0.05$) Folic acid readings were low, showing considerable difference between subjects. ($p<0.01$) Because, levels Hcy values were low. ($p<0.01$)

Conclusion :When an atherosclerosis patient's diet is not balanced and lacks nutrients, the inclusion of VCRESC 125ml into a daily diet can be considered as a significant means to improve the intake of necessary micronutrients.

P4PM-7-8

WRESTLER STUDENTS TO INVESTIGATE TRAINING AND NUTRITION CONDITION

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Nutrition is very important for athletes.This type of cross-sectional type of study was students in Samsun in Kavak (n:19) (Group I), Ordu and Amasya (n:17) (Group II) have been involved in the study n:36 in these wrestler schools in 2007 2008 . An appropriate nutrition programme was given to the Group I wrestlers, but was not given to Group II.The data has been evaluated with the version of SPSS 12.0. The averages is given aritmetic average and the standard deviation is given . Chi-square test and Student T test was used method.The statistical relation level is $p<0,05$.The results showed that statistically difference daily training , threeday food intake between energy (kkal), proteine (g),fat (g), carbohydrate (g), lif (g), cholestrol (mg), A vitamine (mcg), B1 vitamine (mg), B2 vitamine (mg) ve B6 vitamine (mg), folic acid (mcg), B12 vitamine (mcg), sodium (mg), potassium(mg), calcium (mg), magnesium (mg), phosphor (mg), iron (mg), zinc (mg), pantothenic acid(mg). There were statistically difference boys variation after before study($p<0,05$).It was found that the group I boys have a greater average than group II.The aim of this study was to determine training, nutrition position of the wrestler students.

P4PM-8-1

GENETICAL VISUALIZATION OF AMPAR DYNAMICS IN MICE

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Synaptic trafficking of GluR1 subunit-containing AMPA receptor (AMPAR) has been intensively studied because changes in the strength of glutamate-dependent excitatory synapses are believed to underlie cognitive functions such as learning and memory. The critical question is whether such changes really occur in the intact adult brain during memory formation and storage. One of the difficulties to study the molecular and cellular basis of cognitive behaviors is that such changes are assumed to be limited in a small and sparse subset of neurons and synapses in the brain. c-fos and other immediate-early genes (IEGs) are rapidly and transiently induced in response to neuronal activity and have been widely used as activity markers for mapping neurons involved in a variety of animal behaviors including learning and memory. To study the AMPAR dynamics in behaviorally activated neurons, we have developed transgenic mice expressing GFP-GluR1 under the control of c-fos promoter and tetracycline-regulated inducible system. The transgenic mice were used to address several important questions whether AMPAR dynamics is regulated by cognitive animal behaviors in the hippocampus and whether specific limited subsets of synapses are preferentially labeled.

P4PM-8-2

A STUDY OF SYNAPTIC PLASTICITY BY GLUTAMATE RECEPTOR CHANNEL KINETICS MODELS -- FUTURE APPLICATION OF 2-PHOTON LASER PHOTOLYSIS

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We investigated AMPAR channels kinetics in order to characterize the excitatory synaptic transmission and its plasticity by 2-photon laser photolysis and theoretical methods. We constructed the computer-generated EPSCs based on AMPAR kinetics models proposed by the experimental results obtained from the membrane patches (by out-side out patch configuration) of both hippocampal pyramidal and cerebellar Purkinje cells. These simulated EPSCs were analyzed in order to estimate the possible rate constants that could cause to the enhancement and reduction of EPSC amplitudes during LTP and LTD, respectively. Moreover, by using whole-cell current recording technique, we have recorded spontaneous miniature EPSCs and current responses activated by 2 photon laser photolysis. It was shown that the miniature EPSCs and laser-evoked current responses have the same physiological properties and are mediated by the proposed AMPAR channels. We proposed the possible changes in rate constants that could induce LTP/LTD and the biological factors which correspond to these rate constants. It was also concluded that the combination of the experiments of 2-photon laser photolysis and theoretical analysis of channel kinetics models could be useful for the investigation of synaptic plasticity.

P4PM-8-3

ENLARGEMENT AND SHRINKAGE OF DENDRITIC SPINES WITH TWO-PHOTON UNCAGING OF IDENTIFIED SPINES

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Dendritic spines are the major post synaptic sites for excitatory synaptic inputs to the pyramidal neurons. Long-lasting synaptic plasticity, such as long-term potentiation (LTP) and long-term depression (LTD), is associated with increase and decrease in spine-head volumes, respectively. Spine shrinkage has less characterized relative to spine enlargement, because conventional electrical stimulation for LTD induction does not permit identification of stimulated spines. We have overcome this difficulty by stimulating single spines in CA1 pyramidal neurons of rat hippocampal slices by repetitive two-photon uncaging of MNI-glutamate each associated with postsynaptic spike. The spike-timing protocol allowed us to induce spine shrinkage by the LTD induction timing in a particular type of spines. We found that spine shrinkage was associated with reduction in glutamate sensitivity, and often spread into neighboring spines, unlike spine enlargement induced by the LTP-induction protocol. Thus, we found that spine enlargement and shrinkage showed distinct spatial organizations.

P4PM-8-4

IN VIVO TWO-PHOTON UNCAGING OF GLUTAMATE TO INVESTIGATE THE PROPERTIES OF DENDRITIC SPINES IN ADULT NEOCORTEX

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A tight correlation has been demonstrated between the functional expression of synaptic AMPA receptors and the volume of dendritic spines in pyramidal neurons of hippocampal slice using two-photon (2P) photolysis of caged-glutamate (caged-Glu) compound. It has also been established that repetitive 2P Glu administration at single spines induces the enlargement of spine heads which is associated with long-term potentiation (LTP). Thus, although 2P uncaging technique is powerful in slice preparations, it has never been successfully applied to in vivo brain.

Here, we introduced it to the adult mouse neocortex in vivo. Mice were deeply anesthetized, layer 2/3 pyramidal neurons were whole-cell clamped, caged-Glu was applied from the surface of the brain, and 2P uncaging-induced currents were elicited at identified spines. We have confirmed the tight structure-function relationship of spines, indicating that individual spines were precisely stimulated in vivo with a spatial resolution as in slice preparations. Repetitive Glu uncaging in the surface layer (layer 1) could also induce enlargement of spines. We are now quantifying the differences between the structural plasticities of spines in adult neocortex in vivo and those in adult hippocampal and neocortical slices.

P4PM-9-1

A COMPUTIONAL STUDY ON THE INTERDEPENDENCE OF NEUROUNAL IMPULSE PATTERN AND SYNCHRONIZATION

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Neuronal synchronization plays a crucial role in many physiological functions such as information binding and wake-sleep transitions as well as in pathophysiological processes like Parkinson's disease and epileptic seizures. The occurrence of synchronized activity is often associated with significant alterations of the neuronal impulse pattern, mostly with a transition from tonic firing to burst discharges. We have used Hodgkin-Huxley type simulations to study how alterations of individual neurons' dynamics influence the synchronization in electrotonic coupled networks. The individual neurons have been tuned from tonic firing to bursting with chaotic dynamics in between. Our results demonstrate that these transitions have significant impact on the neurons' synchronization. Vice versa, the synchronization state can essentially modify the impulse pattern. The most remarkably effects appear when the individual neurons operate in a periodically tonic firing regime close to the transition to chaos.

P4PM-9-2

SYNAPTIC NOISE MODULATES THE PROBABILITY OF CLIMBING FIBER INDUCED STATE TRANSITIONS IN CEREBELLAR PURKINJE CELLS

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Cerebellar Purkinje cells (PC) exhibit bistability within a limited range of low firing frequencies where a unidirectional climbing fiber (CF) input is able to toggle the cell between a firing (up) and rest (down) state. We have identified several factors that contribute to bistability in vitro. However, input conditions that determine whether a PC expresses bistability in vivo have not been determined. A key difference in vivo is the presence of background input to the dendrites from parallel fiber (PF) and stellate cell (SC) inputs. We tested the hypothesis that dendritic depolarizing and hyperpolarizing currents affect the ability of the cell to enter the bistable regime and thus the ability for CFs to induce toggling of PC output.

Presentation of mixed excitatory and inhibitory dendritic current noise to a two-compartment model of the PC showed that mixed noise can cause up-down transitions when the model is in the bistable regime and can increase the probability of CF-invoked down transitions. We identified dendritic currents associated with different types of CF-induced transitions. The size and time course of these currents suggest that properly timed PF and/or SC inputs could affect the ability for CFs to invoke Purkinje cell transitions between up and down states.

P4PM-9-3

SPATIAL NOISE AND CORTICAL FUNCTIONS: EFFECTS OF THE SPATIAL VARIABILITY OF THE INPUTS AND THE MEMBRANE PROPERTIES OVER THE DENDRITIC TREE

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The neuronal input-output function, namely, the relationship between the average input and the average firing rate has significant impacts on the behavior of neurons and neural populations. It has been proposed that the temporal fluctuation of the input, termed the temporal noise, in the in vivo cortex changes the shape of the input-output function so as to stabilize a low-activity spontaneous state of the recurrent circuit for working memory or to implement the multiplicative gain modulation of neural responses by top-down attentional signals. Contrary to the temporal noise, little has been known about how the spatial noise, that is, the spatial variability of the applied inputs and/or the membrane properties over the neuronal dendritic tree could affect the input-output function, because of the inferior spatial resolution, compared with the temporal resolution, of the experiments as well as of the models. I have addressed this issue by computational modeling. I show that given the nonlinear input integration in individual dendritic branches, the spatial noise could also change the shape of the input-output function in a similar way to the temporal noise and thus provide alternative or additional mechanisms of the stable spontaneous firing and the multiplicative gain modulation.

P4PM-10-1

TARGET-REACHING AND SWITCHING MOVEMENTS UNDER SUPPRESSION OF THE VISUAL CORTEX IN HUMANS

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During target-reaching movements, humans can make mid-flight adjustments (switching) when the target jumps to new positions. In the present study, we examined involvement of the primary visual cortex (V1) in the switching movement. Five normal human subjects participated in experiments. They sat 40 cm in front of a CRT monitor, to reach for a target in the center of the screen after sound GO signal. After 25 ms from the movement onset, the center target sometimes disappeared, and a new left or right target could appear either transiently (duration<10 ms) or constantly, to induce switching movement. Transcranial magnetic stimulation (TMS) was sometimes delivered 70 or 100 ms after appearance of the new target. When TMS was delivered, subjects sometimes missed perceiving the new target. However even in such cases, they occasionally made switching toward the new target. The switching movement was deteriorated in both frequency and amplitude, when using TMS with 70 ms delay. We conclude that switching movements could be induced without conscious visual experiences of the object, though V1 could partly be involved in the movement.

P4PM-10-2

DIFFERENT FUNCTIONAL DIVERSITY OF EXCITATORY AND INHIBITORY NEURONS ACROSS CORTICAL LAYERS IN VOLUNTARY MOVEMENT

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The motor cortex neurons are activated or inactivated at a variety of timing around the execution of voluntary movement. However, it has been poorly understood how excitatory pyramidal cells and inhibitory interneurons in distinct layers of the motor cortex participate differentially in sequential phases of voluntary movement (i.e., preparation, initiation, execution, termination/switch etc.). Here we demonstrate, using a novel behavioral and electrophysiological approach (juxtacellular and multiunit recordings from behaving rats), that pyramidal cells play diverse functional roles in voluntary movement across cortical layers, whereas fast-spiking (FS) interneurons, a major interneuron subtype, are limitedly involved in motor execution in conjunction with a group of pyramidal cells producing command-like activity. Pyramidal cells with a similar preferred direction of movement (pull or push) had a tendency to cluster together spatially. FS interneurons were activated less selectively by the movement direction than pyramidal cells. These results suggested 1) multi-layer motor processing for each of sequential motor phases, rather than layer-by-layer conversion of motor information, and 2) motor command shaping, rather than command gating, by FS interneurons.

P4PM-10-3

PHYSIOLOGICAL FEATURES OF PYRAMIDAL CELLS IN RAT INSULAR CORTEX REVEALED BY IN VIVO WHOLE-CELL PATCH CLAMP RECORDING

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Our optical imaging study revealed the characteristic properties of excitatory propagation in the insular cortex (IC), which receives axonal projections from the sensory thalamic nuclei and limbic structures. However, optical images were integrated to improve signal-to-noise ratio, and cellular basis of excitatory propagation such as firing properties and synaptic transmission are still unclear. To elucidate the electrophysiological features of the IC, we performed *in vivo* whole-cell patch clamp recording from layers II/III pyramidal cells in the dysgranular zone of the IC.

All recorded cells showed slow oscillation of the membrane potential around -70 to -80 mV (down state) and -50 to -60 mV (up state). In the up state, pyramidal cells frequently fired spontaneously, often with periodicity. The evoked excitatory postsynaptic potentials (eEPSPs) were induced by electrical stimulation applied through an electrode placed at the caudal site of recording cell at the distance 1 mm. During the up state, distinct inhibitory postsynaptic potentials (IPSPs) were observed, whereas only EPSPs were recorded during the down state, suggesting that eEPSPs were mediated by glutamatergic and GABAergic receptors. These cellular mechanisms shed light on the physiological roles of the IC.

P4PM-10-4

FUNCTIONAL CONNECTIVITY IN RAT INSULAR CORTEX REVEALED BY IN VIVO OPTICAL IMAGING

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The insular cortex (IC) receives various sensory inputs including visceral, gustatory and somatosensory information from sensory thalamic nuclei. Axonal projections from the limbic structures, which may play a critical role for induction of epileptic activities, also converge onto the IC. However, little is known about the functional connectivity of excitatory propagation in the IC. To elucidate functional local network in the IC, we performed optical imaging using voltage-sensitive dye in urethane-anesthetized rats. Repetitive electrical stimulation of the granular zone of the IC (GI), motor or somatosensory cortices evoked round-shaped excitatory propagations, which often extended to adjacent areas. On the other hand, repetitive stimulation of the agranular (AI) and dysgranular zones (DI) evoked characteristic flattened patterns of excitation along the rostro-caudal axis, and often evoked excitation in the dorsolateral orbital cortex. The excitatory propagation was primarily mediated by local cortico-cortical circuits where AMPA and $GABA_A$ receptors play critical roles in regulating spreading patterns of excitation. The features of excitatory propagation provided new clues about the physiological and pathological roles of the IC.

P4PM-10-5

CORTICAL PATHWAYS FROM THE PRIMARY SENSORY AREAS TO MULTIMODAL ASSOCIATION CORTICES REVEALED BY SEQUENTIAL TRANSCRANIAL STIMULATION IN MICE

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Cortical pathways from the primary to higher sensory areas are important for multimodal sensory processing. Natural stimuli elicit activities in the primary sensory cortices, and these activities are easily visualized using transcranial flavoprotein fluorescence imaging in mice. However, the cortical pathways to higher areas are hardly activated in anesthetized mice. To investigate the cortical pathways from the primary to higher areas, we developed a new technique of transcranial electrical stimulation. First, the skull was shaved with a blade of a dental bar. Next, a dulled tip of a needle was gently pushed on the thinned skull, so that the skull was deformed and the subdural space between the skull and the cortex was minimized around the tip. Stimulus currents applied to the needle could directly flow into the cortex through the thinned skull. With this technique, we sequentially visualized the cortical pathways starting from the primary visual, auditory and somatosensory areas to higher areas. From these results, it was indicated that area 2, which is surrounded by the three areas, received inputs from all of them, and sent the outputs to the anterior parts of the parietal association cortex. Area 2 may have an important role in multimodal sensory integration in mice.

P4PM-10-6

X-RAY NANO-TOMOGRAPHY FOR NEURAL CIRCUIT RECONSTRUCTION

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Neural circuits in the central nervous system build our various higher brain functions. Anatomical structures of neuronal networks in the brain will provide us with fundamental views to elucidate their information processing mechanisms. Some studies are progressing forward figuring out a brain wiring diagram with synapse-resolution of the electron microscopy called "Connectome Project". Here we aim at developing a three dimensional (3D) atlas of neural circuits using nano-resolution X-ray tomography by synchrotron radiation. A full-field X-ray microscope has been built at the BL20XU of SPring-8, working with a fresnel zone plate as objective lens and a Talbot interferometer for phase contrast imaging. We stained neurons of whole mouse brain with Golgi-Cox and cobalt method. Heavy metals used in our procedure enhance X-ray absorption and phase contrast. X-ray computed tomography method reconstructed 3D isotropic voxel images of fibriform axons and dendrites of various neurons. X-ray microscopy combined with Talbot interferometry revealed sharp stereographic structures of pyramidal neurons in the cerebral cortex. This observation probably serves as a foundation for deciphering a set of mammalian neuronal circuits in the near future.

P4PM-10-7

TRANSCRANIAL OPTOGENETIC STIMULATION FOR FUNCTIONAL MAPPING OF THE MOUSE MOTOR CORTEX

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We developed a method that uses Channelrhodopsin-2 (ChR2) for non-invasive mapping of brain circuitry in living animals. Photostimulation of the motor cortex of mice expressing ChR2 in pyramidal cells induced limb movements with millisecond precision and could be induced at frequencies up to 20 Hz. Such transcranial optogenetic stimulation allowed simultaneous definition of the motor maps controlling multiple limbs and reproducible measurement of these maps over periods of weeks.

P4PM-10-8

RECORDING AND SPECTRUM ANALYSIS OF THE PLANARIAN EEG

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Many animals produce continuous brainwaves as the EEG, but it is not known at what point in evolution the EEG developed. Planarians possess the most primitive form of brain, but still exhibit learning and memory behaviors. Here, we observed and characterized the EEG continuous waveform of the planarian at sub-microvolt amplitudes by inserting a monopole electrode into the head of a planarian on a cold stage. The frequency spectrum of the EEG was observed in the range of 0.1 to 5 Hz, showing a broad rise below 0.5 Hz and a monotonic decrease above 1 Hz, apparently following the 1/f law. The intensity of the total EEG diminished during anesthesia by cooling to 2 to 3°C, and recovered when the sample was warmed to about 10°C. The EEG signal was sustained for 30 to 40 min, and gradually weakened as the animal died. Stimulation of the planarian with water vibration at 0.5 to 2 Hz induced chaotic resonance with a broad peak spectrum of around the stimulation frequency.

The continuous EEG waveform suggests the existence of feedback loop circuits in the neural network of the planarian. However, because of the broad band character of chaotic resonance observed, these loops appear to be loose couplings between ganglia.

P4PM-10-9

EFFECT OF ACTIVATION TYPE ON CORTICO-SPINAL INHIBITORY CIRCUITS - A TMS STUDY

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Cortical inhibitory systems play a crucial role in modulating motor cortical output. Trancranial magnetic stimulation was used here to evaluate the activation of cortical inhibitory network.

The effect of different hand muscle activation modes - cocontraction (Co) and isometric abduction (Ab) on the magnitude of short intracortical inhibition (SICI) and the duration of the silent period (SP) was examined. To investigate any hemispheric differences dominant and nondominant hands of right-handed (RH) and left-handed (LH) subjects were tested separately during activation of first dorsal interosseus m. at about 20 % of the maximal voluntary contraction.

In RH group the SP was significantly shortened during Co compared to Ab only in the dominant hand. In LH group significant SP shortening during Co was observed in dominant and nondominant hands. SICI was reduced in the dominant hand compared to the nondominant in both RH and LH during Ab and Co. In contrast, no effect of the activation mode was found on SICI.

SICI and SP are influenced differentially by the activation type and hand preference. These results should be taken into account in neurorehabilitation practice since inhibition may mediate cortical plasticity.

P4PM-11-1

ROLE OF NUCLEUS PARABRACHIALIS FOR RESPIRATORY NEURAL NETWORK IN NEONATAL RAT

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The nucleus parabrachialis complex (NPB) of the pons is known as a respiratory modulating center and the NPB plays a crucial role in the inspiratory off-switch. First of all, we examined how the NPB participates in the inspiratory off-switch using brainstem-spinal cord preparations obtained from 0-4 days old rats. First, the effects of NPB electrical stimulation on C4 ventral nerve inspiratory activity using hemi-sectioned the pons preparation were examined. The electrical stimulation induced a transient depression or termination in C4 inspiratory activity. This inhibition of C4 inspiratory activity was greatly reduced by perfusion of NMDA antagonists and the inhibition was blocked by perfusion of a $GABA_A$-antagonist. When NMDA-antagonist was microinjected into the NPB, the inhibition of C4 activity by the NPB stimulation was reduced. Inspiratory-expiratory (I-E) neurons were found in the NPB. When phentolamine as an adrenergic antagonist was superfused, the firing pattern of I-E neuron changed to pre-inspiratory neuron. These results suggested that NPB is involved in the inspiratory off-switch mediated by NMDA receptors in neonatal rat. I-E neurons in the NPB might be projected from pre-inspiratory neurons and receive adrenergic modulation.

P4PM-11-2

LONG DURATION FAST EEG RIPPLES INDUCED BY TETANIZATION OF THE RIGHT CAUDATE PUTEMEN OR BY INTRAVENOUS INJECTION OF NIKETHAMIDE IN RATS

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Fast neocortical EEG ripples reflects local neural network behaviour that mirrors physiological homeostasis in cerebral cortices. It might be considered as a special pattern of neocortical network information that multiple neural networks outside neocortices might be involved in. Some experimental evidence has been obtained in our lab. The experiments were performed on SD rats. Long duration fast EEG ripples (about 10s) were induced by acute tetanization (60Hz,2s) of the right caudate putamen (ATRC) or accumulative intravenous injection of nikethamide (AIIN). These fast EEG ripples had very widen bandpass from 80Hz to 500Hz or upto 800Hz. A possible Cpu-neocortical circuits were bilateraly involved in tetani-induced fast EEG ripple genesis. The resemblant fast EEG ripples were induced by AIIN. Differently, a very strong toxic effect of AIIN was observed on peripheral organs and central nervous system. The respiratory irregularity, sighing or postsigh apnea, and bradycardia were observed in AIIN model. 4~5Hz hippocampal EEG oscillations coupled with sighing, postsigh apnea with or without fast EEG ripple following. It implied much more complex networks were integrated in AIIN-induced fast EEG ripple genesis.

P4PM-11-3

RESPIRATORY RHYTHM GENERATION AND ITS RAPHE MODULATION IN IN VITRO MEDULLARY HALF-SLICE PREPARATIONS OF NEONATAL MICE

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The site of respiratory rhythm generation has been hypothesized to reside in the pre-Botzinger complex (PBC) localized in the medulla. To analyze the changes of respiratory bursts of the bilateral PBC after severing the mutual connections, we employed reduced medullary slice preparations (a midline transection or a para-midline transection which spared the caudal raphe nuclei). Respiratory bursts which originated in the PBCs were recorded from the hypoglossal nerve before, and after, a midline or para-midline transection. After transection, desynchronized respiratory bursts in both half-slices continued but with slightly decreased amplitudes and frequencies. This confirmed that the PBC can generate respiratory bursts independently, and without mutual connections. In the half-slice preparations the respiratory bursts were facilitated with an addition of serotonin (5-HT). The result suggests that 5-HT neurons localized in the caudal raphe nucleus, predominantly in raphe pallidus, exert facilitatory influences on PBC neuronal activity. The present results clearly demonstrate that PBC neurons in a single half-slice are capable of independently generating rhythmical respiratory bursts and that 5-HT neurons in the caudal raphe nuclei modulate this rhythm.

P4PM-11-4

PATTERNS OF COUPLING BETWEEN NEURONAL DISCHARGE IN THE MEDULLA AND CARDIORESPIRATORY OSCILLATIONS IN FREELY MOVING RATS

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Direct neuronal recordings from structures involved in cardiovascular regulation in freely moving animals are almost nonexistent. Rhythmic single neuron firing synchronized with blood pressure and/or sympathetic nerve oscillations have been demonstrated in a number of central nuclei but with a few exceptions all these studies used anesthetized animals. In the present study neuronal recordings have been performed in 6 unanesthetized rats together with ECG and polysomnography. Simultaneous recordings of multiple single neurons were obtained using tetrode electrodes aimed at the rostral ventrolateral medulla (RVLM) and in surrounding structures located dorsal to this nucleus. We found that neurons located in close vicinity to each other exhibited different levels of coupling with cardiorespiratory oscillations. Several discharge patterns were found in simultaneous recordings of different neurons, including cardiac and respiratory rhythmicities or patterns in which cardiac-related modulation was periodically enhanced at specific phases of the respiratory cycle. The coupling between R-waves and neuronal discharge outside the RVLM was intermittent and showed slipping of the relative phase indicating relative coordination and was frequently modulated by the level of vigilance.

P4PM-11-5

THALAMOCORTICAL PROJECTIONS OF THE VAGUS-RESPONSIVE REGION OF THE BASAL PART OF THE VENTRAL MEDIAL NUCLEUS IN MONKEYS

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The basal part of the ventral medial nucleus (VMb) in monkeys receives gustatory input and projects to both the anterior insular and ventral precentral cortex. To elucidate whether this pathway also conveys vagus viscerosensory information as has been suggested, we recorded vagal evoked potentials in VMb and injected anterograde tracers. In pentobarbital-anesthetized cynomolgus monkeys, the cervical vagus nerve was electrically stimulated and evoked potential responses were mapped in the thalamus. Vagal evoked potentials at both A delta- and C-fiber latencies were focused in VMb, anteromedially to the taste-responsive site; tracer injections were made into the focus. Dense terminal projections were found consistently in and around the fundus of the superior limiting sulcus at the anterior end of insula and in and around the fundus of the inferior precentral sulcus (areas 3a/b). These results are consistent with the vagal activation of both the insula and ventral sensorimotor cortex in rats, cats and humans. The dual cortical projection to the insula and area 3 is common to gustatory VMb and pain / thermosensory thalamus (the posterior part of the ventral medial nucleus), suggesting close relationships among these three apparently distinct sensory thalamocortical systems.

P4PM-11-6

GASTRIC ARRHYTHMIA AND NAUSEA OF MOTION SICKNESS INDUCED BY VIEWING AN OPTOKINETIC ROTATING DRUM IN HEALTHY SUBJECTS

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[Object] The purpose of this study was to observe whether nausea and gastric dysrhythmia of electrogastrograms (EGG) induced by optokinetic motion sickness in Japanese healthy humans.

[Methods] Twelve healthy Japanese volunteers participated in this study. Subjects sat in an optokinetic drum was rotated around the subject for 45 min: 15-min before drum rotation period, 15-min during drum rotation and 15-min after drum rotation periods. EGG was continuously recorded before, during and after the drum rotation. The severity of nausea was rated by visual analogue scale at before, immediately after and 15-min after the drum rotation.

[Results] Ten subjects complained nausea immediately after finishing the drum rotation. The VAS score of nausea was significantly increased in immediately after the drum rotation and 15-min after the drum rotation compared to before drum rotation period. On the during drum rotation period, normogastria of EGG decreased gradually. Instead, tachygastria has increased certainly.

[Discussion and conclusion] It is concluded that the vection-induced motion sickness with optokinetic drum is a non-invasive neurological stimulus that may use for the investigation of the mechanisms of nausea accompany with gastric dysrhythmia in healthy subjects.

P4PM-11-7

CENTRAL VASOPRESSIN INDUCED BY CENTRAL SALT-LOADING PARTICIPATES IN BODY FLUID HOMEOSTASIS THROUGH MODULATORY EFFECTS ON THE PVN IN RATS

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We examined whether central endogenous AVP is involved in the control of body fluid homeostasis. We studied neuronal activity in the paraventricular nucleus of the hypothalamus (PVN), periventricular parts of the PVN, and limbic brain areas, as well as AVP mRNA expression in the PVN, and the peripheral secretion of AVP after central salt-loading in rats that had been pretreated i.c.v. with the AVP V_1 receptor antagonist OPC-21268. Neuronal activity in the PVN evaluated in terms of Fos-like immunoreactivity (FLI), especially in the parvocellular subdivisions, was suppressed. FLI was enhanced in the lateral septum, the bed nucleus of the stria terminalis, and anterior hypothalamic area. AVP mRNA expression was enhanced in the magnocellular part of the PVN despite the lack of a significant difference in the peripheral AVP level. We recorded renal sympathetic nerve activity (RSNA) as sympathetic nerve outflow. The suppression of RSNA during central salt-loading was significantly attenuated by i.c.v. pretreatment with OPC-21268 and this response might be ascribed to a decrease in neuronal activity in the parvocellular subdivisions of the PVN. The subdivisions of the PVN might show different responses to central salt-loading due to the modulatory role of central endogenous AVP.

P4PM-12-1

EFFECTS OF AN ANGIOTENSIN II RECEPTOR BLOCKADE ON BRAIN RENIN-ANGIOTENSIN SYSTEM

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We have previously demonstrated that brain tissue angiotensin II (AngII) contents were decreased by systemic acute infusion of an AngII receptor blocker (ARB). However, the mechanisms by which an ARB decreases brain AngII levels remain unclear. To determine the RAS components and AngII accumulation in the brain, vehicle, AngII or Val^5-AngII was subcutaneously infused for 14 days in SD rats with or without an ARB (candesartan; 10 mg/kg/day, p.o.). AngII or Val5-AngII concentrations in brain tissues and plasma were measured by radioimmunoassay or HPLC, respectively. As compared with vehicle-infused rats, AngII or Val^5-AngII-infused rats showed increased systolic blood pressure; effects were markedly suppressed by an ARB. However brain AngII levels were not changed by AngII or Val^5-AngII infusion. Treatment with an ARB significantly decreased brain AngII in both vehicle- and AngII-infused rats, while Val^5-AngII in brain was undetectable. In these animals, ARB-induced reductions in brain AngII levels were associated with decreases in mRNA levels of angiotensinogen and ACE in cortex and basal ganglia. Theses data suggest that peripheral treatment with ARBs suppresses brain AngII generation at least partly via inhibiting the gene expression of ACE and angiotensinogen.

P4PM-12-2

EFFECTS OF ANGIOTENSIN BLOCKADE ON BLOOD-BRAIN BARRIER PERMEABILITY AND COGNITIVE DISORDERS IN DAHL SALT-SENSITIVE HYPERTENSIVE RATS

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We examined the effects of an angiotensin II receptor blocker (ARB), olmesartan, on blood-brain barrier (BBB) and cognitive disorders in Dahl salt-sensitive (DSS) hypertensive rats. Six-weeks-old DSS rats were treated with vehicle + 0.3% NaCl diet (n=6), vehicle + 8% NaCl diet (n=8) or olmesartan (1 mg/kg/day, p.o.) + 8% NaCl diet (n=8) for 4 weeks. Horse radish peroxidase was infused for determining the BBB permeability. Cognitive functions were evaluated by passive avoidance test. High salt diet developed the hypertension in DSS rats. As compared with low salt-treated normotensive DSS rats, a significant leakage of the brain microvasculature, especially in hippocampus and corpus callosum, and reduced cognitive functions were observed in high salt-treated DSS hypertensive rats. Treatment with olmesartan did not alter systolic blood pressure. However, olmesartan significantly ameliorated the BBB leakage and improved the cognitive functions in DSS hypertensive rats. These data indicate that there is a significant correlation between BBB leakage and cognitive disorders. Furthermore, protective effects of ARBs on BBB permeability may results in neuroprotective effects during the development of salt-dependent hypertension, which are independent of blood pressure changes.

P4PM-12-3

THE AMINO ACID L-PROLINE PREFERENTIALLY ACTIVATES SUPRAOPTIC VASOPRESSSIN NEURONS OF THE CONSCIOUS RAT BRAIN TO RAISE THE BLOOD PRESSURE

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Intracisternal injection of the non-essential imino acid L-proline increases an arterial blood pressure in conscious freely moving rats. The present study examined whether vasopressin neurons in the hypothalamus are involved in the L-proline produced hypertension using the double immunohistochemical staining. Seven conscious rats increased arterial pressure and decreased heart rate for 20-30 min in response to intracisternal stimulation of L-proline, as in the previous study. The brain was obtained 100 min after L-proline injection. Double staining of c-Fos as a marker of activated neurons and vasopressin was robustly observed in majority of supraoptic but not paraventricular vasopressin neurons, although the region close to the paraventricular vasopressin neurons had varying degrees of many c-Fos spots dissociated from vasopressin neurons. Control experiments using artificial cerebrospinal fluid showed no change in blood pressure and heart rate, and few c-Fos spots in vasopressin neurons of both supraoptic and paraventricular nuclei. The results indicate preferential activation of supraoptic vasopressin neurons with L-proline, provably resulting in hypertension.

P4PM-12-4

INCREASED EXPIRATORY ABDOMINAL MOTOR NERVE ACTIVITY CORRELATES WITH INCREASED SYMPATHETIC NERVE ACTIVITY IN SPONTANEOUSLY HYPERTENSIVE RATS

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Rats submitted to chronic intermittent hypoxia (CIH) develop hypertension and an altered pattern of respiratory-sympathetic nerve activity (SNA) coupling that correlates with enhanced late expiratory (LE) discharge recorded in the abdominal nerve (ABN)[1]. We aimed to assess if such alterations in respiratory-sympathetic coupling were also found in the spontaneously hypertensive rat (SHR). Simultaneous recordings of phrenic, sympathetic, ABN and cervical vagus nerves were made using the working heart brainstem preparation of 5-week-old SHR and Wistar Kyoto rats (WKY). SHRs showed significantly higher levels of ABN activity overall compared to WKY (n=4 each; $p<0.01$) with an additional burst in LE and a smaller burst of post-inspiratory activity. The LE ABN discharge in SHR was coupled with an additional SNA burst in this phase of the respiratory cycle that was not evident in WKY rats. During increased respiratory drive with hypercapnia (10% CO2), expiratory ABN activity was revealed in the WKY rat (and coupled to SNA) and elevated in the SHR. We have found there is recruitment of LE ABN activity (active expiration) that provides additional respiratory modulation to SNA in the SHR which may contribute to the higher vascular resistance in this animal model. 1 Zoccal et al., 2008.

P4PM-12-5

EFFECTS OF MENTAL STRESS APPLIED DURING ORTHOSTATIC STRESS

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Aim: Passive Head up tilt (HUT) induces orthostatic stress and elicits complex cardiovascular and hormonal changes. We studied the effects on cardiovascular and salivary responses when orthostatic stress is combined with mental stress. Material and methods: Twenty healthy young males were subjected to two randomized protocols: a) only orthostatic stress and b) mental stress combined with orthostatic stress. Each protocol was separated two weeks. Beat to beat continuous hemodynamic variables were measured and analyzed by statistical models; serial saliva was also collected for hormonal assay. Results: From baseline to orthostatic application, increases in heart rate (60 ± 8 to 80 ± 10 bpm, respectively) and mean blood pressure (87 ± 9 to 91 ± 14 mmHg, respectively) were observed. These changes were significant when mental arithmetic was combined with passive HUT (all changes $p<0.05$). Conclusions: Mental challenge improves cardiovascular responses in persons subjected to orthostatic stress. Thus mental stress may be able to alleviate the symptoms of posturally related syncope in clinical practice as well as spaceflight related applications.

P4PM-12-6

ADMINISTRATION OF ADRENOMEDULLIN 2 IN THE NUCLEUS TRACTUS SOLITARIUS REGULATES ARTERIAL PRESSURE AND HEART RATE IN RATS

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Adrenomedullin 2 (AM2) is a novel member of the calcitonin gene-related peptide (CGRP) superfamily. Recent studies have shown that both central and peripheral AM2 play an important role in the regulation of the cardiovascular system in mammals. However, the target nuclei of AM2 and the role of central AM2 in cardiovascular regulation remain unknown. In the present study, we microinjected AM2 into the rat nucleus tractus solitarius (NTS). Consistent with previous reports showing the hypertensive effect of intracerebroventricular administration of AM2, the direct microinjection of the peptide into the NTS increased arterial pressure as well as heart rate. In addition, to further explore the pharmacological mechanisms underlying the effect of AM2 administered into the NTS, we examined the pretreatment effect of a CGRP receptor antagonist or an AM receptor antagonist on AM2-induced cardiovascular responses. Although a pretreatment of a CGRP antagonist did not alter the effect of AM2 on MAP and HR, an AM antagonist significantly attenuated the effect of AM2 on both MAP and HR. Our results that microinjection of AM2 into the rat NTS increases both arterial pressure and HR indicate that AM2 may play an important role in the regulation of the cardiovascular system at the NTS level.

P4PM-12-7

RENAL ADRENERGIC MECHANISMS ARE NOT ESSENTIAL FOR THE CHRONIC BLOOD PRESSURE LOWERING EFFECTS OF THE BAROREFLEX

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Suppression of renal sympathetic nerve activity and attendant increments in renal excretory function are sustained baroreflex-mediated responses in hypertensive animals. Therefore, it was surprising to find in normotensive dogs that the renal nerves were not essential for chronically lowering arterial pressure during prolonged baroreflex activation (PBA) by electrical stimulation of the carotid sinuses. However, as plasma norepinephrine (NE) decreases during PBA, we hypothesized that denervated kidneys might be exceedingly sensitive to NE and respond to reductions in NE by increasing renal excretory function sufficiently to lower MAP. This hypothesis was tested in 5 dogs subjected to bilateral renal denervation and 8 days of PBA. On days 1, 2, and 8, NE was allowed to decrease normally during PBA. On days 3-7, reductions in NE during PBA were prevented by chronic infusion of NE. During days 1, 2 and 8, MAP (from 96±4 to 74±3 mmHg) and NE (from 113±10 to 63±6pg/ml) decreased, as previously reported. On days 3-7, NE increased to or above control levels during PBA. Despite this, there were no further changes in MAP during PBA. These findings indicate that the sustained fall in MAP during PBA is not critically dependent upon renal adrenergic mechanisms.(HL-51971)

P4PM-12-8

ANGIOTENSIN II INDUCES UPREGULATION OF AT1 RECEPTORS VIA THE SEQUENTIAL ACTIVATION OF NFkB, Elk-1 AND AP-1 IN CATH.a CELLS

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It has been clearly established that increased circulating Angiotensin II (Ang II) with concurrent upregulation of brain and peripheral Angiotensin 1-receptors (AT1R) are important mediators in the pathophysiology of heart failure. In this study we determined the role of sequential activation of transcription factors NFκB, AP-1 and Elk-1 in AT1R upregulation in a neuronal cell line treated with Ang II (100nM) . Our results showed that following Ang II activation, there was a temporal increase of the p65 subunit of NFkB (11.6 fold) which was observed at 30 minutes and peaked at 1 hr and was sustained upto 24 hours. There was a concomitant decrease of IκB and increased IκK expression (6.5 fold). We also observed an increase in AT1R expression (2.7 fold) which followed the temporal increase of NFkB. The activation of NFκB was blocked by using the inhibitor Parthenolide and this led to a decrease in AT1R expression. The expression of Elk-1 was upregulated (1.9 fold) following Ang II activation and was also decreased following NFκB inhibition. Gene silencing using p65-siRNA had similar effects as Parthenolide. Therefore, our results suggest a combined role of the transcription factors NFκB, Elk-1 and AP-1 in the upregulation of AT-1R in the Cath.a cell neuronal model.

P4PM-12-9

UROCORTIN MICROINJECTED INTO THE NUCLEUS TRACTUS SOLITARII MODULATES CARDIOVASCULAR REGULATION IN RATS

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Systemic administration of urocortin I (Ucn I), a member of the corticotrophin-releasing factor (CRF) peptide family, is known to modulate hemodynamics. In the central nervous system, Ucn I is found in the nucleus tractus solitarii (NTS), which is a pivotal region for regulating the set point or arterial blood pressure (ABP). In this study, we examined the effects of Ucn I, which has a high affinity for both type 1 and type 2 CRF receptors (i.e. CRF-R1 and -R2), on cardiovascular functions at the level of the NTS. Moreover a specific agonist of CRF-R1 (i.e. CRF) and a specific agonist of CRF-R2 (i.e. Urocortin II) were also tested to identify the specific cardiovascular effects induced by individual activation of either CRF-R1 or -R2. We found that Ucn I microinjected into the rat NTS produced a significant reduction in both ABP and heart rate (HR). Both agonists for CRF-R1 and -R2 microinjected into the NTS also reduced ABP and HR. Our results suggest that Ucn I in the NTS may play an important role in cardiovascular regulation via activation of both CRF-R1 and -R2 since both receptors are known to be present in the NTS.

P4PM-12-10

VASOPRESSIN MEDIATE THE CARDIOVASCULAR EFFECTS OF GABAERGIC SYSTEM IN THE BED NUCLEUS OF THE STRIA TERMINALIS

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Chemical stimulation of BST elicited depressive and bradycardic responses. GABA is present in the BST but its role in cardiovascular control is not known. This study was performed to find the effects GABA receptor subtypes on the cardiovascular responses and to find the possible mechanisms and circuitry that mediate these responses in the urethane-anesthetized rats.The drugs were microinjected into the BST and the blood pressure and heart rate were corded. The mean of the maximum changes of the heart rate (HR) and mean arterial pressure (MAP) was compared both with those of the control group and with the reinjection values. Microinjection of muscimole, (5 mM) into the BST, produced no ignificant change in either MAP or HR. Injection of bicuculline methiodide (BMI,1mM) a caused a peak significant increase in the MAP of 41.3 ± 5.1 mm Hg and in the HR of 33.2 ± 5.6 beats/min. Injection of two doses (5 mM and 10 mM) of phaclofen, produced no significant change in either MAP or HR. We also showed that sympathetic and parasympathetic systems are not involved in these effects. On the other hand, blocking of the V1 receptors of vasopressin abolished the BMI effects indicating that GABAergic system of the BST affects the cardiovascular system by inhibition of acute vasopressin release

P4PM-12-11

EXPRESSION OF MUSCARINIC RECEPTORS ON RVLM-PROJECTING OREXIN NEURONS

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Orexin/hypocretin neurons are located in the lateral and perifornical regions of the hypothalamus. It has been demonstrated that orexin/hypocretin neurons are one of the major groups of central neurons that link the cardiosympathetic system to motor or mental stress-related cortex. The rostral ventrolateral medulla (RVLM) contains neurons that directly project to cardiosympathetic preganglionic neurons. Microinjection of orexins into RVLM elicited an pressor effect. Therefore, the projections from orexin/hypocretin neurons to RVLM may be one of the orexin/hypocretin pathways responsible for the regulation of cardiosympathetic activity. A small population of orexin/hypocretin neurons are reported to be regulated by cholinergic inputs. The purpose of the present study is to determine whether the muscarinic regulation happen in the RVLM-projecting orexin/hypocretin neurons. With neuronal track-tracing and immunohistochemisty techniques, we found that the RVLM-projecting neurons are densely located in ipsilateral paraventricular nucleus and scattered in ipsilateral lateral hypothalamic area. About 15% of RVLM-projecting lateral hypothalamic neurons are orexin/hypocretin neurons. Muscarinic receptor does located on a population of RVLM-projecting orexin/hypocretin neurons.

P4PM-12-12

SALT-SENSITIVE HYPERTENSION *PER SE*, BUT NOT HIGH-SALT LOADING, ENHANCES NEURONAL NITRIC OXIDE SYNTHASE ACTIVITY IN THE BRAINSTEM

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We have reported that brain neuronal nitric oxide synthase (nNOS) activity and its mediated sympathoinhibition are enhanced in hypertensive Dahl salt-sensitive (DSS) rats compared with normotensive DSS rats. To elucidate whether the enhanced nNOS activity is due to an increase in blood pressure or to a high-salt loading, the effects of an antihypertensive drug, nifedipine, a calcium channel blocker, on brain nNOS activity were investigated in two groups of hypertensive DSS (DSS8%) rats with or without 0.25% nifedipine (DSS8% and DSS8%-nif). Systolic blood pressure in DSS8% was gradually elevated from 1 week after the beginning of high-salt intake to reach at more than 200 mmHg at the end of 4 weeks period. On the other hand, that in DSS8%-nif was not elevated from the beginning to the end of the treatment. In normotensive DSS rats, nifedipine did not affect on SBP. Furthermore, brainstem nNOS activity was not enhanced in DSS8%-nif. The number of nNOS-positive neurons in the brainstem decreased in DSS8%-nif compared with that in DSS8%. These results indicated that salt-sensitive hypertension *per se*, but not high-salt loading, enhanced nNOS activity in the brainstem.

P4PM-12-13

OXIDATIVE IMPAIRMENT OF MITOCHONDRIAL FUNCTIONS IN NEUROGENIC HYPERTENSION

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We evaluated the hypothesis that feedforward depression of mitochondrial electron transport complex (ETC) by superoxide and hydrogen peroxide in rostral ventrolateral medulla (RVLM), a brain stem site that maintains sympathetic vasomotor tone, contributes to neural mechanism of hypertension. Compared to normotensive Wistar-Kyoto (WKY) rats, spontaneously hypertensive rats (SHR) exhibited the depressed Complex I or III activity and reduced electron coupling capacity between Complexes I/III or II/III in RVLM. Microinjection of coenzyme Q_{10} (CoQ_{10}) into RVLM of SHR reversed the depressed ETC activity, oxidative stress and hypertension. CoQ_{10} also antagonized the oxidative stress in RVLM and pressor responses to Complex 1 or III inhibitor in WKY rats. Central infusion of angiotensin II promoted mitochondrial ETC dysfunctions in WKY rats, and gene knockdown of NADPH oxidase antagonized the resultant oxidative stress in RVLM. Gene transfer of superoxide dismutase into RVLM of SHR reversed mitochondrial dysfunctions and ameliorated oxidative stress of RVLM neurons. We conclude that superoxide- and hydrogen peroxide-dependent feedforward impairment of mitochondrial ETC and a cross-talk between NADPH oxidase-derived superoxide and ETC contributes to neural mechanism of hypertension.

P4PM-12-14

VESTIBULAR AND CARDIAC MODULATION OF SKIN SYMPATHETIC NERVE ACTIVITY DURING SINUSOIDAL GALVANIC VESTIBULAR STIMULATION IN HUMAN SUBJECTS

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We have previously shown that sinusoidal galvanic vestibular stimulation (sGVS), a means a selectively activating vestibular afferents without affecting other inputs, can cause partial entrainment of muscle sympathetic nerve activity. Given that motion sickness causes sweating and pallor, we tested the hypothesis that sGVS also entrains skin sympathetic nerve activity (SSNA), but that the optimal frequencies are closer to those associated with slow postural changes (0.2 Hz). SSNA was recorded via tungsten microelectrodes inserted into the common peroneal nerve in 11 awake seated subjects. Bipolar binaural sinusoidal GVS (±2 mA, 200 cycles) was applied to the mastoid processes at frequencies of 0.2, 0.5, 0.8, 1.1, 1.4, 1.7 and 2.0 Hz. All subjects reported strong postural illusions of 'rocking in a boat' or 'swaying in a hammock'. Vestibular modulation of SSNA occurred at all frequencies but was stronger at 0.2 Hz (81.5±4.0 %) and significantly weaker at 2.0 Hz (63.2±5.4%; $p<0.01$). Interestingly, cardiac modulation of SSNA increased significantly during sGVS but was stronger at 0.8 Hz (86.2±2.0 %) than at 0.2 Hz (69.3±8.3%). We conclude that vestibular inputs can entrain the firing of cutaneous sympathetic neurones SSNA and increase their normally weak cardiac rhythmicity.

P4PM-12-15

SYNAPTIC PROCESSING BY VASOMOTOR GANGLION CELLS *IN VIVO*

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Limited information exists on how vasomotor ganglion cells handle preganglionic inputs in vivo. In 20 urethane-anaesthetised rats (1.4 g/kg, i.v.) we made sharp recordings from lumbar (L3) ganglion cells with intact spinal preganglionic inputs. Stable intracellular recordings showing spontaneous spikes and subthreshold EPSPs were recorded from 40 cells. Mean resting membrane potentials were -47 mV, and action potential amplitudes 71 mV. The activity in 38/40 cells showed strong (90-100%) cardiac rhythmicity, indicating that they were of muscle vasoconstrictor type. In line with previous work on other ganglion cells, two classes of synaptic input were identified: 'strong' (always suprathreshold) and 'weak'. Most cells received one active 'strong' input, which accounted for about one third of total spike activity. Summation of subthreshold EPSPs to threshold was rare: EPSPs either reached threshold or failed. Analysis of EPSPs in cells hyperpolarized by current injection revealed many 'weak' inputs with amplitudes slightly above or below calculated threshold. We conclude that the vasomotor ganglionic synapse behaves not as an integrator of convergent inputs, but as a variable throttle, where small changes in cell excitability can continuously modulate synaptic throughput.

P4PM-12-16

EFFECTS OF COFFEE-DRINKING ON SKIN TEMPERATURE AND HEART ACTIVITY

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To investigate effects of drinking coffee, we measured a facial skin temperature by an IR-thermography and the autonomic nervous activity (heart rate and heart rate variability) by recording ECG. Participants were healthy man (21 yrs) and woman (22 yrs). As a control drinking, plain hot water (36 degree Celsius within 0.5 degree) of about 200 ml was drunk during 2 min. Coffee was made by mixing two spoons of commercial instant coffee powder to the hot water and was kept in a heat-retention jar. Thermogram images were taken every minute for 40 min. Although temperature change on face after drinking plain hot water was small, skin temperatures on cheeks and neck were increased after drinking coffee during the later recording period for 35 min. Heart rate was slightly increased for about 5 min after drinking coffee and then gradually declined later. Fluctuation of heart rate was analyzed by high frequency (HF) and low/high frequency (LF/HF) power as the parasympathetic and sympathetic nervous activities, respectively. HF power was gradually increased 5 min after drinking coffee and LF/HF power was simultaneously decreased. These data suggested that coffee stimulated cutaneous blood circulation on face 5 min after drinking by stimulating the parasympathetic nervous activities.

P4PM-12-17

CEREBELLAR PURKINJE CELLS IN FOLIUM-P OF THE FLOCCULUS ADAPTIVELY CONTROL ARTERIAL BLOOD FLOW IN DEFENSE AND EXERCISE VIA OREXIN NEURONS

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We previously reported in rabbits that orexinergic neurons located in the hypothalamic defense area project their axons to Purkinje cells (PCs) in the folium-p of the flocculus (fp). They are involved in the cardiovascular component of defense reactions. In this study, to identify functional roles of PCs in fp, we analyzed neuronal circuit connections to and from fp and obtained the following results. 1) PCs in fp project their axons to the lateralmost area of the ipsilateral parabrachial nucleus, which is known to relay the somatosympathetic reflex (SSR). This reflex plays a role in increasing arterial blood flow to working muscles. 2) Climbing fibers projecting to the fp arise from the caudal part of the principal olive, and they discharge when 40mM K^+ solution is injected into a femoral artery that supplies muscles in a hindlimb. This observation implies that climbing fibers inform PCs in fp about a failure in adjusting arterial blood flow and so in removing K^+ secreted from contracted muscle. 3) Mossy fibers projecting to fp arise from various sources. The present results suggest that the fp adaptively controls SSR and further that orexinergic neurons switch the fp-regulated SSR between tow modes, the emergency mode for defense and the normal activity mode for exercise.

P4PM-12-18

VAGUS NERVE STIMULATION-INDUCED BRADYARRHYTHMIAS IN RATS

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The autonomic consequences of seizures can be severe. Death can result from autonomic overactivity that causes a parasympathetically mediated bradyarrhythmia. We studied the cardiovascular consequences of unilateral and bilateral stimulation of the distal segments of transected vagus nerve in male Wistar rats anesthetized with urethane. There was a consistent inverse relation between stimulus rate and heart rate with minimum HR levels occurring at 50 and 100 Hz. Bilateral vagus nerve stimulation caused more severe bradycardic episodes than unilateral stimulation. Minimum heart rates of < 30 bpm (from a starting rate of > 400 bpm) were possible and arrhythmias occurred during 50 and 100 Hz stimulation. The time to peak HR effect was shortest for the 100 Hz stimulus trains, but HR was lowest during 50 Hz stimulus trains. Small changes in body temperature (over a range of 35 to 38 C) led to consistent differences in the baseline HR and minimum rates during vagus nerve stimulation. Lower body temperatures were associated with lower heart rates. We conclude that very low heart rates (even rates incompatible with life) can be produced by high frequency vagus nerve activity. Such activity can be the final common pathway for heart failure in seizures and asphyxia.

P4PM-12-19

ROLE OF PROKINETICIN 2 IN THE NEURAL REGULATION OF THE CARDIOVASCULAR RHYTHM IN RATS

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The regulation of the cardiovascular system is subject to diurnal variation governed by the circadian system in mammals. Genetic approaches to elucidate a link between the circadian and cardiovascular systems have revealed that the molecular clock is essential to generate the cardiovascular rhythm. However, little is known about how the molecular clock transmits circadian timing signals to the cardiovascular system. Here we show that prokineticin 2 (PK2), a target molecule of the molecular clock, regulates rhythms and levels of blood pressure and heart rate in rats. We found that PK2 was abundantly expressed in the nucleus tractus solitarius (NTS), a major site of brainstem control of cardiovascular function, at both mRNA and protein levels. Interestingly, microinjection of Bv8, the amphibian homologue of the mammalian prokineticins, into the NTS of rats decreased both blood pressure and heart rate predominantly during the resting period for rodents. Moreover, in spontaneously hypertensive rats we found disrupted rhythm in PK2 expression that corresponded to altered cardiovascular rhythm. These results indicate that PK2 in the NTS coordinates the cardiovascular rhythm and that altered PK2 expression can contribute to dysregulation of blood pressure and its rhythm.

P4PM-12-20

SEROTONINERGIC CONTRIBUTION TO CORTICAL EFFECTS ACCOMPANYING RESPIRATORY RESPONSE TO ACUTE INTERMITTENT HYPOXIA

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In anaesthetized rats, vagotomized, paralyzed and artificially ventilated respiratory activity was assessed from integrated phrenic (Phr) and hypoglossal (HG) nerve activity. EEG signal recorded from the frontal cortex was subjected to spectral analysis. Acute intermittent hypoxia consisted of 5 episodes of breathing with 11% oxygen lasting 1.5 min introduced every 3 minutes. Biphasic (stimulation and decline) respiratory response to hypoxia was accompanied by changes in the EEG mostly in the low EEG frequency bands. These effects were present in each hypoxic episode. Systemic administration of 5-HT2 agonist DOI (1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane) decreased inspiratory activity of Phr and HG and suppressed power density of EEG. Following DOI the intermittent hypoxia induced an augmented respiratory response. Stimulation of respiration was faster and attained higher level comparing to the control effects. A decline of the response appeared earlier. During hypoxic respiratory response after DOI the EEG pattern changed less mainly in delta and theta EEG frequencies. Ketanserin, an antagonist of 5-HT2 receptors, caused opposite respiratory and cortical effects. The results might suggest cortical modulation of the hypoxic respiratory response via 5-HT2 receptors.

P4PM-12-21

EFFECTS OF TRP CHANNEL AGONISTS ON RESPIRATORY RHYTHM GENERATION IN THE BRAINSTEM PREPARATION ISOLATED FROM NEWBORN RAT

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It is unknown whether transient receptor potential (TRP) channels possess any functions on the medullary respiratory center. To elucidate this, we examined effects of capsaicin (a heat-sensitive TRPV1 channel agonist) and menthol (a cold-sensitive TRPM8 channel agonist) on respiratory rhythm generation in the brainstem-spinal cord preparation from newborn rat (P0-P3). The preparation was superfused by modified Krebs solution at 25-26°C, and inspiratory C4 ventral root activity was monitored. Capsaicin (1 μM) induced biphasic responses; initial decrease of C4 rate (57% of control at 1-2 min after application) and subsequent facilitation of the rhythm (204% of initial depressed rate after 10-15 min). Menthol (0.5 mM) induced gradual and partially reversible decrease of C4 rate (61% of control at 15 min after application). Effects of menthol (0.5 mM) were reversed by GABAA antagonist bicuculline (10 μM). Menthol caused pronounced reduction of driving potential of pre-inspiratory neurons. Our findings suggest that activation of TRPV1 and TRPM8 channels affect respiratory neuron activity in the medulla. Although details of the mechanism and the physiological meaning remain to be clarified, temperature, pH or unidentified ligands may be intrinsic activator of these channels.

P4PM-12-22

CHEMOSENSITIVITY OF RAT MEDULLARY RAPHE RESPIRATORY NEURONS

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The aim of this study was to determine the chemosensitivity of respiratory neurons in the medullary raphe nuclei. The experiments were performed on pentobarbitone-anesthetized (n=8) or decerebrate (n=12), paralyzed, vagotomized and artificially ventilated rats. Extracellular recordings were made from neurons showing respiratory-related activities in the midline medullary tegmentum. A total of 45 respiratory neurons were classified into Inspiratory (I) throughout, I-frequency modulated, Pre-I, Post-I, Expiratory (E) and E-frequency modulated neurons, based on their firing pattern in relation to the phase of respiration. They were located in the raphe magnus, obscurus and pallidus. These neurons were tested for responsiveness to hypercarbic ventilatory challenge, and examined for the effects of i.v. administration of 8-OH DPAT on their discharge rates. When end-tidal CO_2 concentration was raised (from 5% to 8%), 3 types of response by the raphe respiratory neurons were demonstrated in that the spike discharge either 1) increased, 2) decreased or 3) showed no response. Dose of 8-OH DPAD had little effect on firing rates of 3 types of respiratory neurons. These results suggest that some respiratory neurons in the raphe nuclei are chemosensitive, but not serotonergic.

P4PM-12-23

SHORT-TERM SYNCHRONIZATION BETWEEN PAIRS OF SINGLE INTERCOSTAL INSPIRATORY MOTOR UNIT IN MONKEY

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Effects of deafferentation on the frequency of occurrence of short-term synchronization between pairs of single inspiratory intercostal motor unit were examined in one pentobarbital-anesthetized spontaneously-breathing monkey (Macaca fascicularis). Inspiratory motor units were recorded from first to third parasternal intercostal spaces by concentric needle electrodes. To eliminate monosynaptic inputs from Ia afferents to motoneurons, T1-T5 dorsal roots on the left side were cut. Short-term synchronization was observed in 9/23 pairs on the intact side, and 19/47 pairs on the deafferentated side. Time difference between firing onset of paired units at each respiratory cycle was 111 ± 69 ms in 28 pairs showed short-term synchronization, and was significantly ($P<0.001$) shorter than the difference in 42 pairs showed no distinguishable short-term synchronization (202 ± 120 ms). Since deafferentation has no apparent effect on the occurrence of short-term synchronization, pairs of motor units having common inputs from Ia afferents could also receive common inputs from last-order neurons conveying respiratory descending commands. Furthermore, the present study suggests that the last-order respiratory neuron gives synaptic inputs to motoneurons having similar firing onset.

P4PM-12-24

THE EFFECT OF OREXIN-A AND OX_1R ON THE RESPIRATION IN NEONATAL AND ADULT RATS

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The present study is to investigate the effects of orexin-A and orexin type 1 receptor (OX_1R) on respiration in neonatal and adult SD rats. The activity of hypoglossal nerve rootlets in the transverse brainstem slice of neonatal rats and the activity of the phrenic nerve discharge in the intact adult rats were recorded. These signals and their integrations were analyzed. The changes of respiratory activities were observed by perfusing the orexin-A and the OX_1R antagonists in the slice-bathing medium of the neonatal rats and by microinjecting into pre-Botzinger complex (PBC) of the adult rats. The burst frequency and its integral area of hypoglossal nerve discharge exhibited dose-dependent increase of orexin-A (50nM, 100nM, 300nM). The OX_1R antagonists (SB-408124) attenuated almost totally the response of hypoglossal nerve activity to orexin-A (100nM). In the adult rats, SB-408124 (10 μg/ml, 0.1 μl) attenuated the integral area of phrenic nerve discharge not only in the control ($P<0.05$), but also in the response to orexin-A (100 μg/ml, 0.1 μl, $P<0.001$). These results indicate that the orexin is an important neurotransmitter via orexin 1 receptor in the respiratory regulation both in neonatal and adult rats. (NSFC30670771, STCSM07DZ19722-3)

P4PM-12-25

RESPIRATORY NEURONS IN THE CAUDAL VENTRAL MEDULLA AND THE FIRST SPINAL CORD OF THE MONKEY

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The present study was carried out to investigate the respiratory neurons in the monkey brainstem caudal to the obex and the first spinal cord. Experiment was performed on 3 adult monkeys (Macaca fascicularis) anesthetized with sodium pentobarbital, paralyzed and artifically ventilated. Phrenic nerve was dissected and cut distally to record the respiratory discharges. Spike activities of single respiratory neurons were recorded extracellularly with glass micropipettes filled with 3M NaCl solution. The spinal projection of descending axons were examined in the sacral segments by antidromic activation following stimulation of the surface of the spinal cord with a small Ag-AgCl ball electrode. Sixteen expiratory neurons were found in the region of the nucleus retroambigualis. Fourteen of 16 expiratory neurons were augmenting type and 2 neurons were unclassified. Ten of 12 expiratory neurons extended their descending stem axons in S2 spinal level. Systematic recording of respiratory neurons was performed in the caudal ventral medulla and the first spinal cord. Inspiratory and expiratory neurons were found in the region of grey matter of the ventral horn, where is more medial to the nucleus retroambigualis. Inspiratory neurons were found also in ventral horn of the first spinal cord.

P4PM-12-26

THE ROLE OF ACID SENSING ION CHANNELS (ASICs) IN THE REGULATION OF RESPIRATION BY ACIDIFYING PRE-BOTZINGER COMPLEX (PBC)

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The present study is to determine the expression of ASICs in medulla of neonatal and adult SD rats and the effect of the acidification of PBC on modulation of respiratory activity in adult SD rats. Medulla sections (n=5) were stained with anti-ASIC1 or anti-ASIC2a antibody by ABC method and the relative optical density (ROD) was analyzed. Different pH of ACSF was microinjected into PBC of anesthetized adult (n=11) rats. The phrenic nerve discharges (PND), blood pressure (BP) and heart rate (HR) were recorded. ASIC1 and ASIC2a positive cells were found in hypoglossal nucleus (XII) and ventrolateral medulla (VLM) (including PBC) of neonatal and adult rats. The ROD of ASIC1 in XII of neonatal rats was less than that of adult rats ($P<0.05$). The expression of ASIC2a positive cells was not found in XII of neonatal rats. Microinjection of acidic ACSF (pH=7.0-4.5, 0.1μl) into PBC increased integral area of PND compared to ACSF (pH7.4), especially at pH 6.5 and 6.0 ($P<0.05$), yet did not affect BP, RR and HR. The expression of ASIC1 and ASIC2a in PBC, and the low-pH-enhanced respiration indicated that ASICs may mediate the H^+-excited respiration. The different level of ASICs expression in XII indicated the role of maturation in respiratory regulation. (NSFC30670771)

P4PM-12-27

GENETIC BACKGROUNDS AFFECT PERINATAL DEATH CAUSED BY DSCAM DEFICIENCY

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Down syndrome cell adhesion molecule (DSCAM) is a neural adhesion molecule that plays diverse roles in neural development. We disrupted the *Dscam* locus in mice and found that *Dscam*$^{-/-}$ mice on C57BL/6 background died within 24 hours after birth. Whole body plethysmography of the mice showed irregular respiration and lower ventilatory response to hypercapnia. Further, a medulla-spinal cord preparation of *Dscam*$^{-/-}$ mice showed that the C4 ventral root activity, which drives diaphragm contraction for inspiration, had an irregular rhythm with frequent apnea. Optical imaging of the preparation using voltage-sensitive dye revealed that the pre-inspiratory (Pre-I) neurons in the rostral ventrolateral medulla (RVLM), the rhythm generator neurons for respiration, lost their synchroneity in *Dscam*$^{-/-}$ mice. Interestingly, these phenotypes were highly sensitive to genetic background. *Dscam*$^{-/-}$ mice with C57BL/6 and BALB/c mixed background could survive though adult, though the respiratory rhythm was not normal but moderately irregular as compared to their wild-type littermates at postnatal day 0. These results suggest that the two strains, C57BL/6 and BALB/c, may have distinct alleles of genetic modifier(s) for the DSCAM-dependent respiratory function.

Poster Session

P4PM-12-28

EFFECT OF INHALATION ALCHOLIC EXTRACT OF PEGANUM HARMALA ON INDUCTION OF ANXIETY LIKE BEHAVIOR IN ELEVATED PLUS-MAZE

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Introduction: Based on the extensive application of *Peganum harmala* (P.h) seeds in the Asian traditional medicine, we tried to investigate its possible anxiety effect.

Method: The effect of P.h. extract inhalation was evaluated in adult male rats using elevated plus-maze apparatus. The humidity of prepared ethanol extract was 37%. Animals in different groups (n=6) received 2, 4, 6, 12 or 18 gr/ml doses of the extract using Nebulizer. harmaline drug (0.13 gr/ml) was used as positive control drug.

Results: Compared with saline treated group, harmaline as the positive control significantly caused fear in rats as it was shown by increased time spent in closed arm of plus-maze ($p< 0.05$). Also, ethanol extract of P.h was able to show anxiety effect at doses 6, 12 and 18 mg/ml ($p <0.05$).

Conclusion: Our data showed effective anxiety effect of ethanol extract of *Peganum harmala*. Its effect should be considered in the context of its extensive usage in the men daily life. More studies are required to elucidate its mechanism and site of action.

Keywords: *Peganum harmala*, Fear, Harmaline, Inhalation, Elevated Plus-Maze

P4PM-12-29

QUANTITATIVE EVALUATION OF STOCHASTIC RESONANCE AS A MATHEMATICAL MODEL OF ELECTROGASTROGRAPHY DURING SUPINE POSITION

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It is clinical importance to record and analyze electrogastrography(EGG), which provide more information on the propagation and co-ordination of gastric contractions. In this study, by measuring the gastrointestinal motility, we aim to obtain a mathematical model of EGG during supine position and then speculate factors to describe the diseases resulting from constipation and erosive gastritis.

Initially, we applied the Wayland algorithm to the EGG in order to measure the degree of determinism.As a result, we could not decide whether or not a chaotic process is appropriate for the mathematical model of the EGG. On the other hand, the waveform of the electric potential in the interstitial cells of Cajal (ICCs) is similar to the graphs of numerical solutions to the van der Pol equation. Hence, we added the van der Pol equation to a periodic function and random white noises named after the intestinal motility and other biosignals. We converted the stochastic differential equations into difference equations. The EGG and numerical solutions were compared and evaluated on the basis of the translation error in the Wayland algorithm and the maximum Lyapunov exponent. The EGG was well described by the stochastic resonance in the stochastic differential equations.

P4PM-12-30

REGULATION OF GASTRIC EMPTYING RELATED TO NUCLEOTIDES AND PURINOCEPTORS IN RAT PYLORIC SPHINCTER

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Regulation of gastric emptying is coordinated with antrum (A), pyloric sphincter (PS) and duodenum (D). The relaxation of PS is one of the most important factors for promoting gastric emptying. However, little is known about the role of inhibitory neurotransmitter in the regulation of PS and gastric emptying. In this study, we investigated the effects of ATP and adenosine on the carbachol-induced contractions in A, PS and D. We also studied whether nitric oxide and purinoceptors related to gastric emptying. Methods: Isometric tension was recorded via computer-based analysis using Mac-Lab. The levels of nucleotides were measured by HPLC analysis. Levels of expression of P2 receptors were studied by Western immunoblot. Results: 0.1mM N^G-nitroarginine(NOARG) cause contraction in PS and D. 1mM ATP and 0.01 mM ADPβS caused relaxation in PS but not in A and D. These relaxation were inhibited by the P2Y(1)-selective antagonist, 0.01 mM MRS2179. The level of nucleotides contents gradually decreased along the gastrointestinal junction, but adenosine content in PS was the same as in A. The level of P2X(4) were most expressive in gastrointestinal junction. P2Y(1) receptors were visualized in PS. P2Y(1) receptors mediate relaxation, largely through NO in gastrointestinal junction.

P4PM-12-31

DISTRIBUTION AND ORIGIN OF GABAergic NERVE TERMINALS IN THE SUPERIOR SALIVATORY NUCLEUS; IMMUNOHISTOCHEMICAL AND RETROGRADE TRACING STUDY

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The superior salivatory nucleus (SSN), the primary parasympathetic center for the submandibular salivary secretion, is located in the lateral reticular formation of the medulla oblongata. Our recent electrophysiological studies have demonstrated that inhibitory control of the activity in rat SSN neurons is exerted by GABA. However, little is known about the distribution of GABAergic neurons which innervate the SSN. In this study, we examined the distribution of GAD-containing nerve fibers and $GABA_A$ receptors immunohistochemically, in combination with identification of the SSN neurons with FluoroGold (FG) tracing method. As the result, the SSN neurons made contact with many GAD-positive nerve terminals, and contained $GABA_A$ receptors. In the next step, FG was injected into the SSN to identify the origin of GAD-positive nerve terminals. We found numerous FG-positive neurons in the forebrain and brainstem. In the lateral hypothalamus and central nucleus of the amygdala, FG-positive neurons rarely contained GAD. However, FG- and GAD-positive neurons were occasionally observed in the reticular formation of the brainstem. These findings suggest that preganglionic parasympathetic neurons in the SSN mainly receive GABAergic projection from the reticular formation.

P4PM-12-32

ENHANCEMENT OF COLORECTAL MOTILITY BY GHRELIN, BUT NOT DES-ACYL GHRELIN, THROUGH AN ACTIVATION OF LUMBO-SACRAL DEFECATION CENTER IN RATS

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Ghrelin is a 28-amino acid octanoylated peptide hormone that produced mainly in the stomach. Its best-documented effects are to increase food intake and to stimulate growth hormone release. We have previousely reported that a centrally penetrant ghrelin receptor agonist causes strong propulsive contractions on colorectum in rats. The response is generated though an activation of defecation center at the lumbo-sacral cord (L6-S1). In the present study, we examined the role of acylation of the ghrelin peptide in the stimulatory effect on colorectal motility. Rats were anaesthetised with α-chloralose and ketamine, and colorectal intraluminal pressure and propelled intraluminal liquid volume were recorded in vivo. Intrathecal application of acylated ghrelin at L6-S1 region of spinal cord, but not intravenous application, elicited propulsive contractions of the colorectum in a dose-dependent manner. In contrast, des-acyl ghrelin applied at L6-S1 failed to enhance colorectal motility. Des-acyl ghrelin showed a transient antagonistic effect on acylated ghrelin. It is concluded that acylation of the ghrelin peptide is essential to promote propulsive contractions of the colorectum.

P4PM-12-33

INHIBITORY ACTION OF HERBAL MEDICINE "DAI-KENCHU-TO" TO THE CONSTIPATION INDUCED BY MORPHINE IN RATS

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Morphine is thought to inhibit gastrointestinal transit and makes constipation.

The colon motility of male Wistar rats was examined *in vivo* and *in vitro*.

Intravenous administration of morphine significantly depressed the colonic motility *in vivo*, and the inhibitory effect lasted for about 4 hours. On the contrary, the colonic motility *in vitro* restarted after the dissection and did not show significant depression. The direct administration of morphine (750 μg/ml) and naloxone (100 μg/ml) in Kreb's solution did not affect colonic motility *in vitro*. These results suggest that the mechanism of the inhibitory effect of morphine (i.v.) on the colonic motility involves indirect action of the central or autonomic nervous system, and possibly by the activation of the sympathetic nervous system.

In the chronic morphine administration study, morphine hydrochloride was injected subcutaneously once a day for 2 weeks in male Wistar rats. Their body weights and amounts of feces were less than those of intact rats.

Administration of Herbal medicine "Dai-Kenchu-To (TJ-100)" improved the reduction of body weight and feces.

Morphine inhibits gastrointestinal transit and "Dai-Kenchu-To (TJ-100)" might restrain the suppression.

Poster Session

P4PM-12-34

IMMUNOHISTOCHEMICAL STUDY OF CALCITONIN GENE-RELATED PEPTIDE (CGRP)-CONTAINING NEURONS IN THE MYENTERIC PLEXUS OF RAT COLON

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Recent studies show that mechanical or chemical stimulus applied to the mucosa initiate motility reflexes in rat colon and suggest that intrinsic sensory neurons containing CGRP mediate some of these reflexes. The present study intends to clarify chemical coding of CGRP-containing myenteric neurons and mucosal projection of myenteric neurons by immunohistochemistry. The rat colon was pretreated with colchicine to enhance CGRP-immunoreactivity in cell bodies and longitudinal muscle-myenteric plexus whole mounts were prepared. CGRP-immunoreactive cell bodies were observed in the myenteric plexus. They were large oval or round cell bodies and immunoreactive for the calcium-binding protein calretinin. Mucosal application of the neural tracer DiI demonstrated that DiI-positive myenteric neurons also had an oval or round cell body which was immunoreactive for calretinin. Neurofilament 200-immunoreactivity showed that the CGRP neurons were Dogiel type II neurons characterized by several long processes. In conclusion, the present study suggests that CGRP-containig myenteric neurons in rat colon project to the mucosa and mediate the mucosal stimuli-evoked motility reflexes.

P4PM-12-35

SYMPATHETIC EFFECT ON STRETCH REFLEX OF THE RELAXED MUSCLE IS INHIBITORY, NOT FACILITATORY, IN HUMANS

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We determined how soleus stretch reflex is modulated by sympathetic outflow induced by cold stimulation in spinal cord-injured (SCI) individuals (ASIA grade: A or B). After baseline measurement, cold stimulation was applied to the thigh using icepacks. Peak-to-peak amplitude of the stretch reflex (PP) increased significantly during cold stimulation up to 127% of baseline ($p < 0.01$) in able-bodied individuals (AB), whereas PP decreased significantly during cold stimulation down to 79% of baseline in SCI individuals at or above thoracic (T) 6 (cervical 6-T6, HSCI) ($p < 0.05$). There was no significant change in PP in SCI individuals at or below T10 (T10-T12, LSCI). Since sympathetic pre-ganglionic neurons projecting to lower extremities reside in thoracic cord 10 to lumbar cord 2, LSCI had complete or partial disruption of sympathetic pre-ganglionic neurons projecting to the soleus muscle, suggesting that the decrease in PP in HSCI was caused by intact spinal sympathetic activity in response to cold stimulation. On the other hand, the increase in PP in AB might have been related to activation of the supraspinal central nervous system provoked by cold stimulation considering the intact conduction from the supraspinal center to the leg musculature.

P4PM-12-36

DISPROPORTIONAL BRAINSTEM CONTRIBUTION TO SYMPATHETIC NERVE DISCHARGE IN NEONATAL RATS AND MICE

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Isolated spinal cords of Sprague-Dawley neonatal rats spontaneously generate sympathetic nerve discharge (SND) in vitro. We explored whether this spinally-originated SND was also obtainable in different strains of rats or mice. In vitro brainstem-spinal cord preparations were obtained from the neonates of Long-Evan rats, Sprague-Dawley rats, B6 mice, and 129 mice. The brainstem was trimmed in the mid of the levels caudal to the trigeminal nerves and rostral to the superior cerebellar artery. Histological examinations confirmed that the preparations contained the neural structures caudal to the junction of facial nuclei and superior olivary complex, including an extension of the spinal cord to T12 segment. Contributions of the brainstem to SND genesis were evaluated by transection at the levels of C1 or C8 spinal cord segment. In both strains of rats, transections at C1 or C8 did not significantly alter SND. In contrast, in both strains of mice, transections at C1 or C8 reduced SND to ~40% of their control activities, indicating that ~60% SND genesis was attributed to the brainstem. Besides, the cervical spinal cords largely did not contribute to SND genesis. We conclude that the brainstem contributes significantly to SND genesis in the neonates of mice but not rats.

P4PM-12-37

GENTLE MECHANICAL SKIN STIMULATION INHIBITS THE SOMATOCARDIAC SYMPATHETIC C-REFLEX ELICITED BY EXCITATION OF UNMYELINATED C-AFFERENT FIBERS

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The effects of gentle mechanical skin stimulation on reflex discharges in cardiac sympathetic nerve were studied in anesthetized rats. Mass discharges were recorded from cardiac sympathetic nerve while somatocardiac sympathetic A- and C-reflexes were elicited by single electrical stimuli to myelinated A- and unmyelinated C-afferent fibers of the tibial nerve. Continuous touch was applied to inner thigh skin with a force of 0.1 N for 10 min periods by a soft elastomer "brush" (1.1 cm in diameter with approx. 400 microspikes). When touch was applied ipsilateral to the stimulated tibial nerve, the C-reflex was inhibited by up to 50% of its pre-touch amplitude, whereas the A-reflex was unaffected. Inhibition of the C-reflex started during the touch period and lasted for more than 10 min after cessation of touching. Contralateral touch stimulation did not inhibit the C-reflex. The opioid receptor antagonist naloxone shortened the duration of the period of C-reflex inhibition to 10 min, but the degree of early C-reflex inhibition was unchanged. These results suggest that gentle mechanical skin stimulation inhibits nociceptive transmission conveyed by C-primary-afferents, probably via the release of both opioid and non-opioid inhibitory mediators in the spinal segmental neural pathway.

P4PM-12-38

INTERACTIVE SIGNALING BETWEEN SPINAL AFFERENTS, ENTERIC MAST CELLS AND THE ENTERIC NERVOUS SYSTEM IN GUINEA PIG INTESTINE

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Our results include: 1) synaptic responses in enteric nervous system (ENS) neurons during electrical stimulation of spinal afferents in the mesentery of isolated intestinal segments; 2) ELISA measurement of release of CGRP, substance P, mast cell proteases and histamine into the incubation medium in response to electrical stimulation of mesenteric afferents; 3) immunohistochemical localization of mast cell tryptase and expression of receptors for VR1, CGRP and substance P. Results suggest that spinal afferent collaterals innervate enteric mast cells and neurons in the ENS. Afferent input to the mast cells evokes the release of mast cell mediators (e.g., histamine and proteases), which become signals to the ENS. The signaling pathways between mast cells and sensory terminals form a positive feed-forward loop, which acts to amplify the sensitivity of the sensory terminal to nociceptive or other kinds of stimuli. Firing of the afferent stimulates the release of mast cell mediators, which act at receptors on the sensory terminal to render it more responsive to stimulation. Sensitization of the terminal and consequent elevation of afferent firing elevates release of mast cell mediators, which feeds-forward to continue a progressive build-up of activity in the feed-forward loop.

P4PM-12-39

THE SPINAL REFLEX ARC OF THE MICTURITION PATHWAY

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The micturition reflex is caused almost exclusively via Barrington's nucleus that is located in the rostral part of the pons. The present study aims to elucidate a possible role of the spinal reflex. (1) Electrical stimulation of a bladder branch of the pelvic nerve evoked reflex firings in the other bladder branch of the pelvic nerve. Reflex firings with a latency of about 10 ms were still observed after splitting the major pelvic plexus between the two bladder branches, indicating the existence of the spinal reflex other than the ganglion reflex. (2) Intracellular or extracellular recordings were performed from the sacral preganglionic neurons. Electrical stimulation of the bladder branch evoked IPSPs in most of the preganglionic neurons. EPSPs preceding the IPSPs were observed in a few preganglionic neurons. Electrical stimulation of the branch also evoked firings in a few extracellularly-recorded preganglionic neurons. The latencies of the evoked EPSPs or the evoked firings were short (13-14 ms), indicating that they were evoked by myelinated afferent fibers. The results suggest that the bladder-to-bladder spinal reflex comprise both excitatory and inhibitory pathways, and that myelinated peripheral afferents participate in the reflex.

P4PM-12-40

THE ACUPUNCTURE STIMULATION PROMOTES BLOOD FLUIDITY BY THE INHIBITION OF PLATELET ADHESION WITH THE ADRENERGIC MECHANISM

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It is known that the stress applied to rats represent quick decrease of the blood fluidity by the increase of platelet adhesion. In the present study, the effect of electrical acupuncture stimulation (ACU) on the blood fluidity and the platelet adhesion were examined using Micro Channel Array Flow Analyzer (MC-FAN) and PA-20.
Wistar male rats were used in the study. ACU which causes slight muscle twitch was applied to the acupoints. Stimulated acupoints were as follows: ZuSanli (S36), Sanyinjiao (SP6), Hegu (LI4), Neiguan (P6) in the limbs and Shenshu (BL23) at the trunk. In the additional study, we examined the effects of adrenergic agents on the blood fluidity and the platelet adhesion. Blood samples were collected under pentobarbital anesthesia.
ACU applied to S36, SP6 and L14 revealed significant increase in the blood fluidity and decreased platelet adhesion tendency. However no significant change was observed when ACU was applied to P6 and BL23. Application of α agonist decreased, while β agonist increased blood fluidity. Application of α agonist increased, while β agonist decreased platelet adhesion tendency.
The results indicate that ACU affects blood fluidity depending on acupoints and the effect might be through the endogenous adrenergic mechanism.

P4PM-12-41

VISUALIZATION OF VAGAL AFFERENT NEURONS INVOLVED IN ENERGY EXPENDITURE CONTROL BY ACTIVATION OF TRPV1 IN DIGESTIVE TRACT

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Capsiate (CST), a TRPV1 activator identified in non-pungent cultivar of chili pepper, increases NE turnover rate in the brown adipose tissue, but not that in heart and pancreas, through enhancing sympathetic outflow when administered orally. To identify the mechanism underlying these specific effects, we analyzed how CST activates TRPV1 channels located in the digestive tract using a novel method enabling direct visualization of neurons showing TRPV1 channel opening at their terminals (Meyers et al., 2003). Fluorescent signals in cells in the nodose ganglia (NG) were analyzed 4 days after intragastric application of FM1-43 (FM) in C57BL mice. A significant number of cells were found to be FM-positive in the NG. Co-administration of CST resulted in significantly stronger fluorescent signals, an effect suppressed by a TRP channel blocker. Vagotomy abolished FM-labeling in the NG. Somatic labeling was absent in the dorsal vagal complex. These results indicate that intragastric administration of CST directly activates TRPV1 channels on terminals of specific vagal afferents, a possibility strongly supporting a notion that orally administered CST excites a specific set of visceral afferents predominantly linked to energy expenditure.

P4PM-12-42

GASTROINTESTINAL TRPV1 ACTIVATION ENHANCES SYMPATHETIC OUTFLOW TO BROWN ADIPOSE TISSUE

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Capsinoids (CSNs) are almost equipotent to capsaicin (CAP) in activating TRPV1 channels. The oral administration of CSN enhances oxygen consumption in a similar manner to that of CAP. However, due to their high vulnerability, CSNs do not distribute, unlike CAP, in the systemic circulation after their oral ingestion. This led us to postulate that the effect of CSNs should involve specific activation of TRPV1 channels within gastrointestinal tract linked to selective enhancement of sympathetic outflow to organs involved in energy consumption. To directly examine this hypothesis, we measured efferent activity of the postganglionic sympathetic nerve innervating the brown adipose tissue (BAT). Intragastric administration of CSNs resulted in a dose-dependent increase in the integrated BAT-SNA, which was characterized with an emergence of sporadic high-activity phases composed of low-frequency bursts. This increase in BAT-SNA was abolished by blockade of TRP channel and that of sympathetic ganglion, and was inhibited by section of gastrointestinal vagus nerve. These results point to a neural pathway enabling selective activation of the central network regulating the BAT-SNA in response to a specific stimulation of gastrointestinal TRPV1 channels.

P4PM-12-43

EFFECT OF LEPTIN ICV ON BAROREFLEX CONTROL OF RENAL SYMPATHETIC NERVE ACTIVITY (RSNA) IN CONSCIOUS NORMAL AND FAT FED WISTAR RATS

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The study investigated the role of brain leptin in the baroreflex control of RSNA in conscious normal and fat fed rats.
Male Wistar rats (200-225g) fed normal or a fat (45% fat) diet for 4 weeks were anaesthetised, the right femoral artery and jugular vein cannulated, an electrode sealed onto the left renal nerve and a cannula implanted into the right lateral cerebroventricle. 3 days later, blood pressure (BP), heart Rate (HR) and RSNA were measured and baroreflex gain curves for RSNA were generated before and following leptin icv (5μg+10μg/h). Data (means ±SEM) were subjected to Student's t test and significance taken as $P<0.05$.
In normal rats (n=7), BP was 105±2mmHg, HR 407±10bpm and RSNA 336± 105μV/s. Leptin given icv increased RSNA by 23% after 3h at which time the sensitivity of the RSNA baroreflex curve increased by 47% (both $P<0.05$). In fat fed rats (n=7), BP, HR, RSNA, and the baroreflex curve parameters were comparable to normal rats. Neither RSNA nor the RSNA baroreflex gain were altered by leptin icv.
The data showed that leptin within the brain enhanced the baroreflex control of RSNA but that this action was suppressed following a high dietary intake of fat.
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P4PM-12-44

REGIONAL TEMPERATURE CHANGES IN THE RAT BRAIN ASSOCIATED WITH NEURONAL ACTIVATION

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The brain temperatures seem to be correlated with the neuronal activity. However, the relationships between the regional brain temperatures (rBT) and the several neuronal activities remain unclear. To elucidate the stimulus-induced changes in rBT, we simultaneously recorded the brain temperature and electrophysiological neuronal activities in rats. Since we focused on the relationships with thermoregulation and feeding behavior, the rats received feeding-related stimuli. Under anesthesia, a set of a bipolar electrode and a thermocouple was inserted into the insular cortex. The thermocouple was bonded 0.2 mm rear to the tip of the bipolar electrode. We used four taste stimuli (sweet, salty, sour, bitter) and two somatosensory stimuli (capsaicin, L-menthol). In results, rat brain temperatures tended to begin to rise 2~5 sec after the neuronal activation by comparatively stronger stimuli, regardless of kind of stimulation. The temperature rises were 0.02~0.08 ˚C and transient. These results indicated the possibility that there were relations in the temperature change and the nervous activity.

P4PM-12-45

ROLE OF THE MEDIAN PREOPTIC NUCLEUS IN THERMOREGULATION DURING LOCAL BRAIN HEATING WITH OSMOTIC STRESS

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Dehydration attenuates autonomic heat loss responses in the heat. In the present study, we hypothesized that hyperosmolality would play an important role in the responses and the hypothalamic areas would be involved in the mechanism. In anesthetized rats, isotonic (154 mM, IS) or hypertonic (1500 mM, HS) saline was injected to the internal carotid artery, and the preoptic area (POA) was locally heated at ~40 °C using a thermode. We also assessed neural connections between the POA and median preoptic area (MnPO). The POA heating increased tail skin temperature (Ttail) by 3.4 ± 0.2 °C (means ± SE, n=11) in IS injection. HS injection suppressed ($P<0.05$) the increase in Ttail during the heating of the POA. In the MnPO-lesioned rats, an increase in Ttail was similar between the IS and HS trials during the heating of the POA (3.3 ± 0.4 and 3.2 ± 0.3 °C during IS and HS infusion, respectively, n=7). Local injection of cholera toxin B shows an appearance of the substance in the MnPO 3 d after the injection. These results may suggest that hypermosmolality in the brain attenuated the neural response to the heat in the POA. Moreover, the MnPO is necessary for the response, sending signals to the POA.

P4PM-12-46

INVOLVEMENT OF OREXIN-A ON MICTURITION REFLEX IN NORMAL BLADDER BUT NOT CYCLOPHOSPHAMIDE-INDUCED CYSTITIS IN RATS

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Purpose: The purpose of the present study is to investigate the effect of orexin-A in the spinal cord on the bladder function in normal and cyclophosphamide (CYP)-induced cystitis rats.
Methods: The effects of intrathecal (i.t.) injection of orexin-A on bladder function were examined during continuous infusion cystometrogram in urethane anesthetized normal and CYP-induced cystitis rats. The effects of i.t. injection of selective orexin-1 receptor (OXR1) antagonist SB334867 on orexin-A induced bladder overactivity in normal rats were examined. Furthermore, the effects of i.t. injection of SB334867 on changes in bladder function were investigated in normal and CYP-induced cystisis.
Results and Conclusion: I.t. injection of orexin-A significantly decreased the intercontraction intervals (ICI) in normal and CYP-induced cystitis rats. Orexin-A induced bladder overactivity was suppressed by intrathecal application of SB334867 in normal rats. I.t. injection of SB334867 caused significant increase of ICI in normal rats. In CYP-injected cystitis rat models, i.t. injection of SB334867 did not change the bladder function. These results indicate that orexin-A in the spinal cord activates the micturition reflex via OXR1 in normal rats but not CYP-induced cystitis rat models.

P4PM-12-47

14-3-3η PROTEIN AFFECTS INTRACELLULAR STABILITY OF TYROSINE HYDROXYLASE

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We investigated the mechanism by which the N-terminus of tyrosine hydroxylase (TH) affects the stability of the enzyme. The results obtained by using N-terminus-deleted human TH type 1 (hTH1) mutants identified the sequence up to Ala^{23} as mediating the stability. We considered the possible involvement of a cytoplasmic chaperone protein, 14-3-3η, because 14-3-3η form a complex with TH to activate the enzyme, once Ser^{19} of the enzyme has been phosphorylated. The down-regulation of 14-3-3η proteins in PC12D cells exogenously expressing hTH1 enhanced the stability of the wild-type enzyme and that of the mutant lacking the N-terminus up to Ala^{23}. However, the stability of the mutant was reduced compared to the wild-type enzyme. The down-regulation of 14-3-3η proteins in PC12D cells exogenously expressing hTH1 mutants with the replacement of Ser^{19} by Ala^{19} or Glu^{19}, which are the mimics of the non-phosphorylated or phosphorylated form, enhanced the stabilities of the mutant enzymes as that of the wild-type enzyme. These results suggest that the 14-3-3η protein is one of intracellular proteins regulating hTH1 stability by acting on the N-terminus of the enzyme.

P4PM-12-48

SPONTANEOUS UTERUS CONTRACTIONS IN CONSCIOUS NON-PREGNANT RATS

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[Objective] It is important to evaluate the spontaneous uterus contractions, because the disorders of uterus contractions were induced gynecological diseases. We studied whether the spontaneous uterus contractions were measured by methods of a strain gauge force transducer (STG), and we evaluated effects of several pharmacological agents on the spontaneous uterus contractions in conscious non-pregnant rats. [Methods] Two STG were implanted on serosal surfaces of uteri. The uterus contractions were continuously monitored throughout the experiment in conscious. To investigate the effects of several pharmacological agents on the spontaneous uterus contractions, atropine, propranolol and prostaglandin F2α were administered as a bolus injection into the jugular vain. [Results] The spontaneous uterus contractions were recorded by methods of STG. These contractions were enhanced by propranolol and prostaglandin administration and were suppressed by atropine administration. [Discussion] Our results suggested that the spontaneous uterus contractions were regulated by cholinergic and adrenergic neurons via muscarine and beta-adrenalin receptor, respectively. Additionally, we confirmed that exogenous prostaglandin F2α could stimulate the spontaneous uterus contractions.

P4PM-12-49

CHANGES OF DPPIV ACTIVITY IN THE HYPOTHALAMUS AND HIPPOCAMPUS OF MONOSODIUM GLUTAMATE OBESE AND FOOD DEPRIVED RATS

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The proline-specific dipeptidyl peptidases (DPPs) are emerging as a protease family with important roles in the regulation of signaling by peptide hormones. We investigated CD26 levels and catalytic activity of DPPIV in soluble (SF) and solubilized membrane-bound (MF) fractions from hypothalamus (HT) and hippocampus (HC) of fasted and non-fasted monosodium glutamate obese (MSG) and normal rats. Compared to controls, MSG and/or food deprivation (FD) induced the reduction of diprotin A insensitive (DI) DPPIV activity in SF and MF from HT, as well as in diprotin A sensitive (DS) DPPIV activity in MF from HC. Paradoxically, MSG and/or FD induced opposite response of DPPIV-DI activity in MF from HC. The monoclonal protein expression of CD26 in MF by ELISA decreased in HT and increased in HC of MSG and/or FD relative to controls. The existence of DDPIV-like activity with different sensitivities to diprotin A and the identity of the less sensitive as CD26 were demonstrated for the first time in the central nervous system. Overall, data suggest the involvement of DPPIV-DI/CD26 activity in the endocrine regulation of energy balance and anxiety, respectively in the HT and HC.

P4PM-12-50

TWO PREOPTIC CELL GROUPS IN TONIC AND FEBRILE CONTROL OF RAT TAIL SYMPATHETIC FIBRES

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Mammalian cold-defence mechanisms (e.g. thermogenesis, cutaneous vasoconstriction) are tonically inhibited by preoptic neurons. That tonic inhibition is reduced by cold and fever, suggesting that a) warm-sensitive preoptic neurons are responsible, and b) the febrile mediator, prostaglandin E_2 (PGE_2) acts by inhibiting those preoptic neurons. The exact location of the preoptic neurons that inhibit cutaneous vasoconstriction, however, is unknown. Preoptic neurons in different sites were briefly inhibited by microinjections of GABA (300 mM, 15-30 nl), while cutaneous vasoconstrictor (CVC) fibres were recorded from the tail in urethane-anaesthetised rats (1.5 g/kg, i.v.). Two distinct GABA-sensitive preoptic regions were identified: a rostromedial locus (RMPO) around the OVLT, and a region centred 1 mm caudolaterally (CLPO). Injecting PGE_2 (0.2 or 1 ng in 15 nl) into these two regions caused disparate effects. In GABA-sensitive sites of the RMPO, PGE_2 injections caused a prompt rise in CVC activity and raised core temperature. In GABA-sensitive sites of the central and caudal parts of the CLPO, PGE_2 was ineffective. These results suggest that neurons in both preoptic regions tonically inhibit CVC activity but only the RMPO neurons mediate vasoconstriction in fever.

P4PM-12-51

EXCITABILITY OF SINGLE CELLS IN THE MEDULLA OBLONGATA RELATED TO INDUCING NAUSEA AND VOMITING

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The area postrema (AP) is one of the circumventricular organs that lack a blood-brain barrier, and is well known to be a chemoreceptor trigger zone for vomiting. It has been clarified the sensitivity of AP neurons to a lot of chemical substances that can be found in circulating blood. The nucleus gelatinosus solitarius (SolG) is a structure just adjacent to the AP. The SolG is considered to be involved in the vomiting center in the medulla. In this study, we focused on the single cell's excitability related to nausea and/or vomiting in the area postrema and the nucleus gelatinosus solitarius. We found c-Fos expression in the area postema and its adjacent regions during LiCl-induced nausea. The study using a patch-clamp recording technique revealed the mechanisms of cholinergic and serotonergic modulation of neuronal excitability of AP and SolG neurons. We discuss the presynaptic and postsynaptic effects of chemical substances inducing nausea and vomiting.

P4PM-12-52

ANALYSIS OF FETAL HEART RATE VARIABILITY IN FETAL CONGENITAL HEART DISEASES USING MAGNETOCARDIOGRAPHY

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The aim of this study was to measure heart rate variability (HRV) in normal fetuses and fetuses with congenital heart disease (CHD) using fetal magnetocardiography (FMCG) showing good resolution within time domain, and to assess any differences. Fourteen normal fetuses and 22 CHD fetuses were included in this study. We recorded 2-min FMCG using the 64ch SQUID system. We detected R peaks in FMCG, measured R-R intervals, and performed a spectral analysis of the intervals. We obtained the total power of the values of the spectrums between 0.04 Hz and 0.15 Hz components (LF), and between 0.15 Hz and 0.4 Hz components (HF). We also measured the ratio of LF to HF (LF/HF). With the advance of gestation, LF/HF in normal fetuses decreased slightly, whereas HF in normal fetuses increased. In 2 cases of CHD, LF/HF values deviated greatly from those of normal fetuses. In a case of CHD, HF values markedly deviated from those of normal fetuses. All cases showing LF/HF or HF deviation died soon after birth in spite of intensive care. These results suggested that parasympathetic nervous activity increased with fetal growth, and that deviations from normal parasympathetic or sympathetic nerve activities were associated with a poor prognosis.

P4PM-12-53

EMETIC RESPONSES ARE MEDIATED VIA NMDA AND/OR NK_1 RECEPTORS IN DECEREBRATE RATS

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It is known that rodents do not have vomiting ability. However, we have reported that autonomic responses corresponding to nausea such as distinctive gastric contraction, bradypnea, bradycardia and hypotension were observed after the intravenous administration of emetic drug. Recently, we have succeeded in induction of body movement similar to gagging by high frequency stimulation of superior laryngeal nerve in decerebrate rats. The purpose of this study was to clarify the role of NMDA and NK_1 receptors in the central mechanism of these autonomic and somatic responses of emesis. Receptor antagonists were administered by microinjection into medulla oblongata or intravenously. Administration of NMDA receptor antagonist MK-801 significantly diminished distinctive autonomic responses elicited by emetic drug. MK-801 also significantly inhibited the induction of gag-like movement. Administration of NK_1 receptor antagonist WIN51,708 diminished distinctive gastric contraction elicited by emetic drug and significantly inhibited the induction of gag-like movement. These results suggest that rodents show emetic responses corresponding to nausea and gagging, and that these emetic responses are mediated via both NMDA and NK_1 receptors.

P4PM-12-54

ALTERED SYMPATHETIC CONTROL OF THE HEART AND KIDNEY DURING SEPTIC SHOCK

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Sepsis is an important risk factor for acute renal failure and the associated mortality is high. In spite of this, our understanding of the pathogenesis of septic renal failure remains limited. We have shown that sepsis induced by E.coli is accompanied by hypotension, increased cardiac output (hyperdynamic state) and renal vasodilatation. The role of the sympathetic nervous system in mediating the increased cardiac output and the renal vasodilatation is unclear. To elucidate the changes in sympathetic nerve activity during septic shock, we have made direct recordings of renal and cardiac sympathetic nerve activity (SNA) in conscious sheep with sepsis induced by intravenous administration of E.coli. During sepsis there were decreases in arterial pressure, urine output, creatinine clearance and fractional excretion of sodium. Renal SNA decreased transiently, before increasing above control. In contrast, cardiac SNA increased gradually over 8 h. During sepsis the range of the heart rate baroreflex curve decreased, there was no change in the range of the renal SNA baroreflex curve and the range of the cardiac SNA baroreflex curve increased. In conclusion, we have shown that induction of a hyperdynamic model of sepsis results in differential responses of cardiac and renal SNA.

P4PM-12-55

A 5-HT_4 RECEPTOR AGONIST PROMOTES REGENERATION OF THE REFLEX PATHWAYS IN THE ENTERIC NERVOUS SYSTEM

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Moderate rectal distension elicits rectal (R-R) reflex contractions and simultaneous internal anal sphincter (R-IAS) reflex relaxations comprise the defecation reflex. Both reflexes are controlled by extrinsic nerves and enteric nervous system. The aim of the present study was to explore a novel approach to repairing the defecation reflex dysfunction using the plasticity of enteric nervous pathways in guinea pigs anesthetized with ethyl carbamate. Eight weeks after the rectal transection and subsequent end-to-end one layer anastomosis, the defecation reflex recovered to the control with regeneration of reflex pathways. Two weeks after the local treatment with a 5-HT_4 receptor agonist (10-100 μM)[applied for a patent] at the rectal anastomotic site, the R-IAS reflex relaxations recovered and some bundles of fine nerve fibers interconnected the oral and anal ends of the myenteric plexus. In the anastomotic granulation tissue, we found NF- and HuD-positive cells those were a cell proliferating marker, PCNA-negative but an enteric neural stem cell marker, DLX2-positive. These results suggested a possibility for repairing the anal dysfunction by promoting regeneration of the enteric reflex pathways with local application of the 5-HT_4 receptor agonist.

P4PM-12-56

ANTAGONISM AND INHIBITION VERSUS SYNERGY AND COOPERATION

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Since 1846 the Weber brothers used tetanic excitation on frog to induce the standstill of heart, Sherrington then proposed the concept of reciprocal inhibition on volitional flexion and stretch reflex of skeletal muscles in1897. Along the same avenue of reasoning, Eccles proposed the cholinergic and inhibitory synapses in a pathway from motor- axon collaterals to motoneurones in 1951. Despite the detailed information generated in the 1950s and 1960s, there remained controversy over the existence of Renshaw cells and their role in recurrent inhibition. So far, it has been difficult to directly test these hypotheses because of the lack of experimental tools to selectively antagonize/knockout Renshaw cells or monitor their behavior in freely moving non-anaesthetized animals.

Recently, instead of antagonism or inhibition, we have observed that the physiological systems are synergic, and cooperative in rhythms for freely moving animals. Examples will be taken from the micturition, urine storage, and drug treatment of lower urinary tract in normal and spinal cord injured rats. As to human beings, examples of elbow flexion and forearm pronation with patients of radial nerve palsy will be given. It is believed that these ideas are important in treating the so-called dynamical diseases.

P4PM-13-1

DISSOCIATION OF VISION AND ACTION: VISUALLY ESTIMATED GRASPING POSITIONS ARE DIFFERENT WHEN LIFTING UP GRASPED OBJECTS

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In this study, from a psychophysical approach, we investigated the independence of vision and action for grasping movements. Several researchers have reported that action may not be affected by visual illusions. From this point of view, we focused on grasping movement in daily life and investigated the independence. In the first experiment, we measured grasping positions with three tasks: a visual-estimation task of each grasping position, a pinch task that only grasps an object and a lift-up task that grasps an object and lifts it up. As a result, even after iterative trials of LT, we found that the grasping positions of VT are different from those of LT. This result shows the independence and the grasping positions of PT are the same as those of VT. Moreover, although both of LT and PT are considered as an action task, those of LT are amazingly different from those of PT. In the second experiment, for PT and LT, we examined the contribution of the visual feedback during prehension movement, the reaction times, and the initial movement directions. As a result, we found that the grasping positions and movements are determined before movement onset. These findings are quite important for investigating an integrated neural mechanism of vision and action in the human brain.

P4PM-13-2

PROPERTIES OF EYE MOVEMENTS FOR BETTER PURSUIT OF A HIGH-SPEED MOVING OBJECT DURING ACTIVE LINEAR HEAD MOTION

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For pursuing a high-speed moving object, pursuit with head motion is observed in natural condition. But vestibulo-ocular reflex (VOR) induced by the head motion should conflict with smooth pursuit eye movement (SP) and head motion itself. The detail of the VOR-SP interaction has been unknown, especially during pursuing a moving object with linear head motion. To determine the interaction between SP and linear VOR, we investigated eye movements and dynamic visual acuity (DVA) for pursuing a high-speed moving object (80 deg/s) in nine subjects. In addition, the effects of anticipation and VOR were examined. In most subjects, DVA score during head motion was better than that in head stationary condition (P=0.0007). In the DVA improved subjects, eye movement in the same direction as head motion, i.e. opposite to the VOR, was observed. This eye movement had a significant correlation with SP in head stationary condition (R=0.90, P=0.0009), but not with VOR (R=0.18, P=0.64). Thus, this eye movement resulted from dominance of SP over VOR. In addition, this eye movement was observed even when the visual target disappeared unexpectedly. We concluded that the voluntary linear head motion is advantageous for DVA because anticipatory SP is dominant over VOR.

P4PM-13-3

INTERNAL MODEL OF THE HUMAN HAND INFLUENCES OBJECT RECOGNITION

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In this study, from a psychophysical approach, we investigated a relation between an internal model of the human hand and object recognition of a graspable object. For human object recognition, it seems that recognition for graspable objects such as a tool becomes possible with an internal representation acquired through motor experience. From this point of view, we examined whether an internal hand model affects object recognition or not. In the measurement experiment, firstly, participants acquired a novel internal hand model by repeating a grasping task of an object displayed on a monitor, under the condition that geometrically transforms a participant's hand shape displayed on a monitor. After the training, we measured whether participant recognized a displayed object (cylinder) as a cup or not. As a result, we ascertained that the cognitive judgment depends on the amplitude of the geometrical transformation of the participant's hand. This result indicates that the trained internal hand model affects object recognition such a cup. While, the visual image of a favorite cup in daily life is independent for the internal models. Finally, we conclude that an internal model of the human hand plays an important role for object recognition of graspable tools.

P4PM-13-4

CHARACTERISTICS AND ROLES OF THETA-WAVE RESPONSES TO THE N-BACK TEST IN YOUNG PEOPLE

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The purpose of this study is to elucidate the role of the theta-wave during solving the n-back working memory task in young volunteers. Subjects wearing on the EEG-cap which possesses electrodes of international 10/20 system attended to the experimental protocol that consisted of resting on the chair and then solving the 1- to 3-back working memory tasks presented on a PC monitor in front, with both eyes open. The spontaneous EEG was recorded during both the time of resting and n-back challenging through the band pass filter of 0.5-100 Hz, and stored into the PC after passing through a 200Hz AD-converter. We also recorded the event signal to note the time of n-back presentation. Several 3 sec divisions of artifact-free EEG were taken from both before and during challenging to n-back tasks and then Fourier analyzed. Power densities of theta, alpha, beta, and gamma waves were obtained and offered for t-test analyses. Both F3 and Fz showed statistical increase of theta power during every kind of n-back test observed compared with each pre-test period. Gamma power during 1-back test was also increased significantly. These preliminary results suggest to us that the theta wave in the left frontal area plays important roles in the verbal working memory.

P4PM-13-5

INFLUENCE OF HIGH-FREQUENCY ACOUSTIC COMPONENT OF INFANT CRYING ON MOTHERS' PERCEPTION

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Ultrasonic vocalization in infant rodents is elicited to attract the mothers' attention. Although human infant crying also includes high-frequency components, the function of high-frequency components has been investigated in the previous studies. In the present study, we explored the possibility that high-frequency components of infant crying play a role in communications between the mother and child.

We presented to subjects infant crying sounds and instrumental sounds with or without ultrasound components (20 kHz to 96 kHz) and ask them whether they can discriminate sounds with ultrasounds from those without ultrasounds. Infant crying sounds and instrumental sounds were recorded by free-field microphone for high level and high frequency measurements and presented to subjects using a 192 kHz high resolution audio system.

Both groups of subjects did not discriminate instrumental sounds with high frequency components from those without high frequency components. However, the discrimination performance for infant crying sounds was higher in mothers than in nulliparous women.

This finding suggests that mothers need to recognize high-frequency components of crying to judge infant conditions.

P4PM-13-6

THE INFLUENCE OF ACTIVATION COUPLING OF CORRESPONDING MUSCLES OF FINGERS AND TOES ON THE STABILITY OF COORDINATED MOVEMENTS

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We investigated how the movement direction and the activation coupling of corresponding muscles of fingers and toes (extensors or flexors of digits) influenced the coordination of finger and toe movements. First, subjects performed cyclical extension-flexion of the right fingers and toes in the sagittal plane at 1.25 Hz with their eyes closed. Four conditions were utilized: simultaneous or alternate activations of the corresponding muscles with hand prone or supine. Both the activation coupling and the movement direction independently influenced the stability of the movements. The extent of their influence were similar with each other. Second, motor evoked potentials (MEPs) of resting right finger or toe muscles were elicited during movements of right toes or fingers at 0.75 Hz with eyes closed, respectively. MEPs was greater irrespective of hand position (prone or supine) when the corresponding muscles were activated, though MEPs of toe flexors were not influenced by finger movements when the hand was supine. It was suggested that stability of extension-flexion of ipsilateral fingers and toes was produced by coalition or conflict of the perceptual/cognitive constraint (movement direction) and the neuromuscular constraint (excitability modulation of the corticospinal pathways).

P4PM-13-7

SPATIAL DISTRIBUTION OF ZENK-IMMUNOREACTIVE NEURONS FOR SONG PERCEPTION IN THE BRAIN OF BUDGERIGARS

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Male budgerigars (*Melopsittacus undulates*, a parrot), as other songbirds and hummingbirds, develop elaborate complex songs used in social communications, through vocal learning. In songbirds, it is known that more complex male songs are more attractive and stimulating to female reproductive behaviour. We reported that male budgerigar songs induced a large increase in synthesis of ZENK protein (Zenk) of immediate early genes (IEGs) in the female NCM (caudomedial nidopallium) of the caudal telencephalic auditory areas. There was a significant effect of song complexity on the number of Zenk-immunoreactive neurons (Zenk-neurons), correlated with the number of syllable types (Eda-Fujiwara, H. *et al., Eur. J. Neurosci.*, **17**, 149-154, 2003). However, little is known about the spatial distribution of the Zenk-neurons in the NCM as well as the CMM (caudomedial mesopallium) of the caudal auditory areas. We investigated the spatial distribution of the Zenk-neurons in these areas of female budgerigars exposed to 4 male songs with different complexity. We found increased clustering neurons induced by complex songs, suggesting that the song complexity dependent neurophysiological "function (i.e. perception)" may make a spatially organized neuroanatomical "structure" in brain.

P4PM-13-8

NEURAL ORGANIZATIONS FOR VOCAL CONTROL IN THE SOCIAL RODENT, DEGU (OCTODON DEGU)

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Vocalizations of most animals are innate. In this study, we investigated the vocal control mechanism of degus which learn to modify vocal patterns by conditioning. We examined by electrical micro-stimulation under anesthesia whether the vocalization of degus is modulated by the telencephalic input. It is known that innate emotional sounds are controlled by limbic cortex and the mid brain periaqueductal gray (PAG), and that emotional vocalizations of animals can be evoked by the electrical stimulation of the PAG. Voluntary control of vocalization, in contrast to completely innate vocal reaction, needs an input from the premotor cortex to the nucleus ambiguus. In this experiment, Degus evoked emotinal sounds by the stimulations in the PAG and the anterior cingulate cortex, but the stimulations in the premotor cortex failed to evoke any sounds. Also, no courtship song with multiple song notes was emitted. Further, we found the sites in the PAG where stimulation evoked two or more kinds of sound simultaneously. These results suggested that the PAG might receive the modulation by the limbic areas in some vocalization, but most vocalizations of degus are innate and controlled by the midbrain. Courtship songs are probably controlled by a separate vocal control pathway.

P4PM-13-9

MODULATION OF THE JAW-OPENING REFLEX BY STIMULATION OF THE VESTIBULAR NUCLEI IN THE RAT

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The effects of the vestibular nuclei (VN) stimulation on the jaw-opening reflex (JOR) were studied in anesthetized rats. The JOR was evoked by electrical stimulation of the inferior alveolar nerve. The JOR were recorded as electromyographic responses of the anterior belly of the digastric muscle. The conditioning electrical stimulation of the superior (SVN), lateral (LVN) and medial (MVN) vestibular nuclei facilitated the JOR bilaterally. Facilitation started at the conditioning-test interval of 2-8 ms, and reached a maximum facilitation at 8-10 ms. Additionally, microinjection of monosodium glutamate into the SVN, LVN and MVN also elicited facilitation of the JOR bilaterally. The facilitation reached its peak at 2-5 min, and returned to the control level at 20-30 min. On the other hand, the conditioning electrical stimulation of the inferior vestibular nucleus (IVN) inhibited the JOR ipsilaterally. Inhibition started at the conditioning-test interval of 10 ms, and reached a maximum inhibition at 12 ms. Microinjection of monosodium glutamate into the IVN also elicited inhibition of the JOR ipsilaterally. Inhibition reached its peak at 5 min, and returned to the control level at 30 min. The results suggest the VN plays an important role in the JOR.

P4PM-13-10

THE EFFECT OF MOTOR IMAGERY ON GAIN MODULATION OF THE SPINAL REFLEX

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The purpose of this study was to clarify the effect of motor imagery on gain modulation of the stretch reflex and H-reflex. The subjects sat in a comfortable chair with their left leg on the footplate of the experimental device. They were then instructed to practice motor imagery tasks, which consisted of ankle planter flexion (PF) and dorsiflexion (DF), and two kinds of motor imagery effort (MVC, 50%MVC). Bipolar surface electrodes were mounted on the soleus. The H-reflex was induced by electrical stimulation applied to the tibial nerve, and the stretch reflex was evoked by the custom-built experimental device. There was no significant difference in H-reflex amplitude between the conditions. On the other hand, stretch reflex amplitude increased significantly during the PF MVC imagery condition compared to the resting condition, but not during PF 50% MVC imagery condition nor DF imagery conditions. The stretch reflex amplitude increased selectively in this study, indicating the possibility that motor imagery modulated the stretch reflex gain via the gamma motor neurons, although α motoneuron pool activation against Ia afferent was not changed.

P4PM-13-11

EFFECTS OF VIBRATORY STIMULATION ON MOTOR UNIT ACTIVITY DURING VOLUNTARY CONTRACTION

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Discharge of motor units (MU) is well known to show elongating trends in the spike interval during voluntary constant-force isometric contraction, but neural mechanisms underlying those trends remain unclear. This study examined effects of peripheral afferent stimulation on MU activity during voluntary contraction. Subjects performed voluntarily isometric knee extension with a knee angle of 90 deg at slightly above the recruitment threshold force (F_{th}) of the single MU. Vibratory stimulation was applied perpendicularly to the patella tendon with 75 and 100 Hz frequency and 0.5-0.8 mm displacement. The vibration was superimposed for 30-90 s during the ramp and constant-force voluntary contraction at various timings. Action potentials of single MU were recorded in the vastus medialis muscle using non-invasive surface array electrodes. Both F_{th} and the trend in the spike interval of the single MU changed with timing to add vibration. Nevertheless, the spike interval's elongating trend did not disappear during the constant-force contraction. In conclusion, changes in neural input information to single MUs with peripheral afferent stimulation do not eliminate the spike interval's trend of elongation in the presence of central drive.

P4PM-13-12

RELATIONSHIP BETWEEN SPIKE INTERVAL AND AMPLITUDE OF MECHANOMYOGRAPHIC SIGNAL FROM SINGLE MOTOR UNITS DURING VOLUNTARY MUSCLE CONTRACTION

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Integrated value of mechanomyographic signal increased gradually during prolonged muscle contraction at low target tension levels. It considered that the iMMG increment originated from firing rate changes in active motor unit, synchronized activity between MUs and recruitment of new MUs. It is necessary to investigate the wave form of mechnomyographic signal from single motor unit (MUMS) during the activity to explain for the increment. The aim of the present study was to investigate the effect of firing rate, or inter-spike interval, on the wave form of the MUMS during prolonged isometric constant contraction at the target torque of the recruitment threshold torque of the objective motor unit.

MUAP and MUMS was simultaneously recorded from m. vastus medialis of five healthy male volunteers using surface disc electrode (5 mm φ) and condenser microphone (10 mm φ), respectively.

Inter-spike interval of single motor unit elongated gradually and then shortened during the muscle contraction. Amplitude of MUMS maintained constant for 2 min and then abruptly increased. There was not the significant relationship between the inter-spike interval and the amplitude of MUMS under the present experimental condition.

P4PM-13-13

MODULATOY ACTIONS OF OCTOPAMINE AND SEROTONIN ON A BURSTING MOTOR NEURON IN THE FEEDING NETWORK OF APLYSIA CALIFORNICA

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Neuromodulation contributes to the function of the neural circuits that control the consummatory feeding behaviors in Aplysia (Kupfermann et al. 1979). Although actions of neuromodulators have been extensively characterized in certain neuromuscular components of the feeding system, their participation in the regulation of central circuits has received less scrutiny. Recently, we showed that one modulator of this system, dopamine, had multiple direct actions on a motor neuron (B67) that enhanced its rhythmic bursting (Serrano and Miller 2006). This study examined the actions of octopamine (Oct) and serotonin (5-HT). Application of Oct produced increases in burst duration, impulses per burst, and the depolarizing sag observed in B67 in response to long hyperpolarizing current pulses. It also enhanced its post-inhibitory rebound (PIR) firing. 5-HT application had an inhibitory effect on the endogenous bursting of B67, and decreased its sag potential and PIR. Oct caused both of the bilateral B67 motor neurons to burst in a rhythmic fashion, but it did not induce synchrony. These findings are considered in the context of previous observations in which dopamine was shown to induce both rhythmicity and synchrony in the bursting of the B67 cell pair (Serrano and Miller 2006).

P4PM-13-14

EFFECT OF NUTRITIONAL STATUS ON THE SWEET TASTE SOLUTIONS INTAKE

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To assess how nutritional status alter consumptions and preferences of sweet taste solutions and analyze the possible regulatory mechanisms of nutritional status on feeding in the guide of taste, we measured the consumptions of serials concentrations of saccharin, a non-nutritive artificial sweetener and sucrose, a rich-caloric natural sweetener in the high-fat diet induced obese rats (HF) and the long-term chow restriction rats using two-bottle preference test and compared the results to those in the normal diet rats.Our results showed that HF rats decreased significantly both sucrose intakes ($P < 0.01$) and the sucrose preferences ($P < 0.05$)in higher concentration of 0.25M, 0.5M and 1.0M, Whereas the long-term chow restriction rats showed increased consumption ($n=12$, $p=0.005$) and enhanced preference ($n=12$, $p=0.001$) only for 0.04M saccharin solution.These results show that it is at higher concentrations but not lower that the nutritional status effect on sucrose acceptance (absolute intake) of HF rats, suggesting that the HF rats form preference for hedonic solutions based mostly on caloric value. In contrast, the preference of long-term chow restriction rats was largely based on the orosensory and hedonic properties of the solutions, rather than nutritional choice.

P4PM-13-15

THE ROLE OF THE AMYGDALA IN PALATABILITY-INDUCED FEEDING

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It has been suggested that two subnuclei of the amygdala, the central and basolateral nucleus, are involved in the homeostatic control of feeding. It remains unclear, however, if they are also involved in palatability-induced feeding. In the present study, we examined the effect of pharmacological inactivation of those nuclei on palatability-induced feeding in non-food deprived rats. We injected the $GABA_A$ receptor agonist muscimol (0, 5 or 20 ng, 0.25 µl / side) bilaterally into the central or basolateral nucleus and measured the consumption of a highly palatable solution, Ensure Liquid, for 10 minutes. When injected with vehicle, rats drank more than 9 g of Ensure Liquid. The highest dose of muscimol (20 ng) significantly suppressed the consumption of Ensure Liquid, when injected into the central but not into the basolateral nucleus. In addition to the suppressive effect on feeding, a defensive like "forepaw treading behavior" was observed only after the injection of the highest dose of muscimol into the central nucleus. These results suggest that an intact function of the central nucleus of the amygdala is necessary for the expression of palatability-induced feeding. The defensive behavior elicited by a GABAergic inhibition may compete with palatability-induced feeding.

P4PM-13-16

EXPRESSION OF THE HYPOTHALAMIC FEEDING-REGULATING PEPTIDES IS INDEPENDENT OF SERUM CALCIUM LEVEL IN CACHECTIC SYNDROMES

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Purpose: Parathyroid hormone-related protein (PTHrP) is a causative factor of humoral hypercalcemia of malignancy and concurrent anorexia and cachexia. We addressed whether the cachectic syndromes were induced by hypercalcemic function of PTHrP or by other PTHrP functions.
Method: By in situ hybridization histochemistry, the expression of the hypothalamic feeding-regulating peptides mRNAs was evaluated in four different groups of rats inoculated with either human lung cancer-derived LC-6 cells that secrete PTHrP to induce hypercalcemia and cachexia (group 1) or human melanoma-derived SEKI cells that induce cachexia without PTHrP secretion or hypercalcemia (group 2), rats that were hypercalcemic due to administration of a high dose of 1,25(OH)2D3 (group 3), and rats with adjuvant-induced arthritis (AA) (group 4).
Results and Conclusion: The orexigenic peptide mRNAs were significantly increased after the development of cachexia and AA, whereas the anorexigenic peptide mRNAs were significantly decreased. Administration of a high dose of 1,25(OH)2D3 caused hypercalcemia and body weight loss without affecting the peptide mRNAs. Thus, cachectic syndromes modulate the expression of the hypothalamic feeding-regulating peptides in a serum calcium-independent manner.

P4PM-13-17

LESIONS OF THE AMYGDALA INCREASE BODY WEIGHT AND SUPPRESS ESTROGEN RECEPTOR -ALPHA GENE EXPRESSION IN THE OVARY IN FEMALE RATS

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Recently the posterodorsal amygdala (PDA) has been proposed to be the most effective site to influence feeding and body weight. And there is a sexual difference with distinct effects in female rats. The present study observed the food intake and body weight after lesionsof the PDA in female rats. The estrogen receptor -alpha (ER-α) and -beta mRNA expression in the ovary, and neuropeptide Y mRNA and pro-opiomelanocortin mRNA expression in the hypothalamus were also detected. Twelve female rats got bilateral PDA lesions (control=4). Body weight and food intake have been recorded daily for 22 days. Real-Time PCR was used to detect the mRNA expression. Histology showed five rats had lesions located in the PDA. Compared with sham rats, PDA lesions significantly increased body weight at the end of the experimental period ($t_{(7)} = 4.047$, $P<0.01$, $n=5$). The food consumption didn't show any difference between groups. The expression of ER-a mRNA in ovary had a noticeable fall in lesion group compared with the control group ($P<0.05$, $n=5$). No difference was found for the expressions of other peptides between groups. These data suggest that the PDA may play an important role in the control of body weight in female rats, partly by regulating ER-α mRNA expression in the ovary.

P4PM-13-18

DISTRIBUTION OF Fos-LIKE IMMUNOREACTIVITY IN THE BRAIN AND DRINKING BEHAVIOR AFTER CENTRALLY ADMINISTERED RELAXIN-3 IN RATS

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Purpose: Relaxin-3 has recently been cloned as a new member of the insulin superfamily using human genomic databases. The expression of relaxin-3 gene was abundant in the brain but not in female reproductive tissues. Centrally administered relaxin-3 stimulates food intake in rat. In the present study, we investigated distribution of Fos-like immunoreactivity (Fos-LI) in the brain and drinking behavior after centrally administered relaxin-3 in conscious rats. Materials and methods: We used adult male Wistar rats. After the intracerebroventricular (icv) administration of 0.9% saline, relaxin-2 (180nmol) and relaxin-3 (180nmol), we examined (1) the distribution of Fos expression in the brain, using immunohistochemistry, (2) cumulative water intake during 180 min after icv administration of solutions. Results: (1) Fos-LI was distributed in various regions of the brain, including organum vasculosum of the lamina terminalis, subfornical organ, supraoptic and the paraventricular nuclei. (2) Icv administration of relaxin-3 significantly increased water intake and the effect was as strong as that of relaxin-2. Conclusion: These results suggest that centrally administered relaxin-3 activates various regions in the brain and play an important role on regulation of body fluid balance.

P4PM-13-19

ANALYSIS THE CHARACTERISTIC OF ORAL BEHAVIORS BY THE THREE DIMENSIONAL JAW-TRACKING SYSTEM IN FREELY MOVING RATS

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To analyze the characteristic of oral behaviors (e.g., mastication, licking, lapping) in rats, it is essential to analyze the jaw-movement trajectories and EMG activity of the jaw muscles. Our aim of the present study was to develop a system for simultaneous recording of the jaw movements in three dimensions (vertical, horizontal and anterior-posterior planes) and EMG activity of the jaw muscles during various oral behaviors from freely moving rats. A jaw-tracking system, which was consisted of four magnetic sensors, was implanted on the head of the rats. A small cylindrical magnet was fixed to the middle of the animal's chin. The jaw-movement trajectories were traced by the sensors as the magnet moved. The EMG activity of the jaw muscles (masseter, digastric) was recorded. The jaw-movement trajectories in three dimensions and the EMG activity of the jaw muscles were successfully recorded during mastication. The rats protruded the mandible anteriorly during the jaw-closing phase and retruded it posteriorly during the jaw-opening phase. Such jaw-movement patterns are identical to those in mice. Thus, the newly developed system is useful to record the basic parameters of oral behaviors from rats.

P4PM-13-20

CHANGES IN AMPLITUDES OF JAW OPENING REFLEX DURING NATURAL FEEDING IN THE RABBIT

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The present study was focused on the modulation of the jaw opening reflex responses in a natural sequence of mastication including intake of foods, bolus preparation and swallowing.

The experiments were carried out on free behaving rabbits. Jaw opening reflex (JOR) was evoked in the digastric muscle by the low-threshold electrical stimulation of the inferior alveolar nerve. When the animal was taking foods, the JOR was recorded. The mean peak to peak amplitude of JOR was compared among masticatory stages.

As compared with the control, which was recorded when the animal was at rest, the JOR responses were suppressed particularly during chewing and swallowing. Furthermore, the inhibition was more moderate during swallowing than chewing.

The results suggest that the central pattern generator for mastication and swallowing inhibits the jaw opening reflex pathway in order to avoid unnecessary jaw movements in a different manner.

P4PM-13-21

EFFECTS OF ELECTRICAL STIMULATION IN THE PHARYNGEAL REGION ON THE REFLEX SWALLOWING

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Effects of electrical stimulation in the pharyngeal region on triggering reflex swallowing were investigated in human subjects. In the present study, a special catheter was designed. Stimulating electrodes were attached to a manometry catheter sensor, so that the catheter can both measure the pharyngeal pressure and electrically stimulate the pharynx.

The above assembly was passed transnasally, while looking through the endoscope, until the assembly entered the upper esophageal sphincter. The endoscope tip was located in the nasopharynx, from where adequate views of the pharyngeal wall and glottis were obtained.

By increasing the intensity of stimulus amplitude, the pharyngo-glottal closure reflex and pharyngeal muscle contraction were first observed. At a higher amplitude, they were followed by the reflex swallowing. The reflex swallowing was easier elicited in the hypo-pharynx than mid- or hyper-pharynx. Furthermore, chewing task, which was required to the subjects, strongly inhibited triggering reflex swallowing, although the pattern of manometric events in the pharynx were not different between the tasks.

The possible neural circuit of swallowing and the interaction between the swallowing and chewing center in the brainstem were discussed.

P4PM-13-22

EFFECT OF MANGANESE SHORT-TERM EXPOSURE ON EMOTIONAL STATE OF YOUNG MALE AND FEMALE RATS

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The rat's brain structural and functional sex-depended difference was reported in numerous articles. But the data about any difference in effects of manganese intoxication between male and female rats practically is not described. Emotional state in 52±2 and 96±2-days old normal and Mn-treated male and female rats was tested in open field. After first testing experimental animals during 40 days received MnCl2.4H20 dissolved in drinking water (1 mg/ml). 52-days old control females in comparison with the male ones are characterized by low level of anxiety and fear and with more exploratory activity. In 96-days old control females emotional tension is increased, exploratory activity is less pronounced practically are similar to those observed in males. In case of males there was not any difference in behavior during both first and second testing. Mentioned changes in control females might be caused by the hormonal alterations taking place during pubescence.In males and females Mn exposure cause reduction in exploratory activity, increasing in anxiety and fear. At the same time the reduction of number of entrances into the center, explored burrows and increasing number of fecal boluses are more pronounced in females in comparison with the control females and Mn-treated males.

P4PM-13-23

THE NECESSITY OF OXYTOCIN IN DEVELOPMENT OF MALE MOUSE ULTRASONIC VOCALIZATION IN RESPONSE TO FEMALE ODOR

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Using oxytocin-knockout (OT-KO) mice, we investigated a role of oxytocin in the development of ultrasonic vocalization provoked by female stimuli through mating experience. Ultrasonic tests were carried out in their home cages which were divided into 2 compartments by a double wire-mesh wall. The resident was placed in one compartment, and a stimulus estrous female was in the opposite side. After the introduction of the females, the vocalization was recorded and analyzed by an ultrasonic microphone with real time FTT spectrum analyzer. After the recordings, the wire-mesh wall was removed, and the males were allowed to copulate with the estrous females. Those ultrasonic/copulatory tests were repeated weekly for 5 weeks. Through the weekly tests, wild and heterozygous males increased the vocalization in response to estrous females, whereas OT-KO mice failed to develop it. We also demonstrated that intraventricluar injections of an oxytocin antagonist, $[d(CH_2)_5^1,Tyr(Me)^2,Thr^4,Orn^8,Tyr\text{-}NH_2^9]$-vasotocin, also suppressed the ultrasonic vocalization in another cohort of mice. These results suggest the critical role of oxytocin in development of ultrasonic vocalization during sexual behavior in male mice.

P4PM-13-24

ASSESSING INTRINSIC FEEDBACK IN A SIMPLE CENTRAL PATTERN GENERATOR NETWORK

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The cardiac ganglion (CG) of decapod crustaceans is a simple central pattern generator (CPG) that lies within and drives rhythmic contractions of the heart musculature (see Fort et al. 2004; Stern et al. 2007). This simple CPG consists of 9 neurons; 4 pre-motor neurons that are intrinsic to the system and 5 motor neurons that project to the heart musculature. Intrinsic feedback in the cardiac system occurs within the cardiac ganglion, where it is manifested in the connection between motor neurons and pre-motor neurons. The topographic separation of function in the CG enabled us to eliminate this feedback in the CG of the blue crab Callinectes sapidus. We used three different techniques: (1) the CG was ligated at a point that separated the two levels of CG neurons, and (2) impulse propagation in the trunk was interrupted using TTX or (3) sucrose. These methods provided independent ways to assess the activity of the premotor neurons in the absence of motor neuron feedback. All three techniques showed that activity of the motor neurons exerts negative chronotropic feedback to the premotor pacemaker neurons.

P4PM-13-25

MOTOR IMAGERY AND ELECTRICAL STIMULATION REPRODUCE SIMILAR CORTICOSPINAL EXCITABILITY TO IT DURING ACTUAL MUSCLE CONTRACTION

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To find a clinically usable stimulus association that possibly achieves short-term potentiation for the purpose of rehabilitation, we examined whether the combination of motor imagery and electrical muscular stimulation reproduced corticospinal tract (CoST) excitability at a level similar to that achieved during actual slight muscular contraction. Transcranial magnetic stimulations were applied to healthy young males during resting condition (Rest), motor imagery (MI), actual muscular contraction (AMC), electrical stimulation (ES), and electrical stimulation with MI (ESMI). Electromyography was recorded from the first dorsal interosseous muscle (FDI). Motor evoked potential (MEP) amplitudes during AMC and ESMI were significantly larger than those during other conditions. There was no significant difference in MEP amplitude during AMC and ESMI. MEP induced during ESMI must include spinal excitation, and how much afferent input achieved the cortical level in that condition was not revealed. Regardless of unknown mechanisms, we succeeded in reproducing CoST excitability during ESMI at a level similar to that during AMC. We may conduct a future study to reveal whether sustaining this condition can cause short-term potentiation at the cortical level.

P4PM-13-26

NEURAL MECHANISM OF MIRROR MOVEMENTS INDUCED BY DYSFUNCTION OF THE PRIMARY MOTOR CORTEX IN THE MONKEY

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In humans, mirror movements (MMs) often occur after stroke. Functional brain imaging studies on patients of stroke have shown that activity of bilateral motor cortex (M1) is increased accompanying the MMs. However it is not clear whether the increased activation was the cause of MMs or the result of MMs.

To clarify the neural mechanism of MMs, we investigated kinematic and dynamic property of MMs using acute primate model of M1 lesion induced by reversible inactivation of the hand area in M1 by microinjection of muscimol, a GABAA receptor agonist (0.5-3µl).

Three monkeys were trained to retrieve a morsel of food from the tube positioned in the front with a free hand, while the other hand was restricted. EMG activity was recorded from a total of 22 muscles of both hands. After inactivation of the hand area of the right M1, MMs appeared on the right hand during the monkey grasped a food and/or released it to the mouth with the left hand. Mirror EMG activity was then observed in both flexor and extensor hand muscles. After inactivation of the left M1, MMs and mirror EMG activity disappeared. These results suggests that following the inactivation of the right M1, the activity of the left M1 was increased and led to induction of the MM that appeared in the right hand.

P4PM-13-27

SENSORY CONTROL OF MOVEMENTS IN MICROGRAVITY

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Studies of voluntary movements of varying types, organization and complexity, namely, saccadic and slow tracking eye movements, precise hand movements, posture and locomotions have been performed with human subjects and primates both in space and in onground simulated microgravity experiments expand our understanding of mechanisms of sensory and motor adaptation under conditions of unusual sensory environment.

A functional approach based on a quantitative evaluation of characteristics of system reactions which organization was well analyzed in previous studies have been used in selection of experimental models. The data obtained in experiments performed onboard of space ships and those derived from “simulated” microgravity studies discovered profound changes of sensory support of movements that were shown during the first phase of adaptation to microgravity by prominent increase of opto-, vestibular and motor excitability and later by deep suppression of vestibular-oculomotor and vestibulomotor reactions.

The results of onground simulated studies that showed analogous changes of vestibular sensitivity in the first phase of adaptation allowed to conclude that one of important source of these disorders is a deep proprioceptive deprivation caused by the loss of support stimuli.

P4PM-13-28

PLANE SPECIFIC RESPONSE OF SIMPLE-SPIKE FIRING OF PURKINJE CELLS IN THE CAT CEREBELLAR NODULUS AND UVULA DURING VERTICAL HEAD ROTATION

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The cerebellar nodulus and uvula have been suggested to play important roles in controlling the time constant of the optokinetic responses under head-tilt conditions. The present study investigated the simple-spike (SS) firing response of Purkinje cells (P-cells) in these structures during sinusoidal head rotation in vertical plane in awake cats.

In most of our sample of P-cells (170/200), SS firing activity responded during sinusoidal vertical rotation. Of the 170 cells, 56 were tested during rotation in four vertical stimulus planes; 20, 20, and 16 of these cells responded most strongly during pitch, during roll, and during diagonal plane rotation, respectively. Based on the phase and gain analysis of SS responses, we classified the responding cells into two types: head-position (HP) and head-velocity (HV) types. HP type cells also responded to the strongest response planes under the static head-tilt condition. HP and HV type cells seemed to primarily receive otolithic input and vertical semicircular canal input, respectively. Such information could be transmitted to the brainstem nuclei to control motor dynamics for the optokinetic responses under head-tilt conditions in each specific plane.

P4PM-13-29

MORPHOLOGICAL ANALYSIS OF THE EXTERNAL ANAL SPHINCTER MOTOR NERVE AND ITS MOTONEURONS IN THE CAT

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The present study was conducted to investigate the spinal neural circuitry which controls the tonus of the external anal sphincter (EAS) in cats by examining the size distribution of the EAS motor fibers and motoneurons (MN). The EAS musculature was also tested for the presence of the muscle spindle. The size distribution of EAS motor fibers was studied after degenerating the afferent fibers by performing ganglionectomy. The EAS MNs were labeled with horseradish peroxidase and examined the cell body size distribution. Both distributions were found to be unimodal. It was difficult to distinguish between alpha and gamma MNs due to unimodal distribution. It was also revealed that there was no muscle spindle detected in the musculature. These results show the spinal neural circuitry which controls EAS lack the feedback system originated from the muscle spindles and also gamma motor system which innervates the intra-fusal fibers. While the mechanism underlying the spinally controlled tonus of the EAS including the nature and role of spinal reflexes remains unclear, the results of the present study suggest that non-spindle sensory receptors in the anal canal may supply peripheral sensory information to the reflex circuit to maintain the tonus.

P4PM-13-30

ACTIVATION OF SPINAL NEURONS DURING TREADMILL STEPPING: INCLINE EFFECT

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c-fos is used as a marker in the spinal cord and brain to identify active neural circuitries. The effect of varying loading on the hindlimbs on c-fos expression in spinal neurons associated with stepping in intact rats was determined. Rats (n=5/group) were subjected to cage activity, or running on a treadmill at a 0o or 25o incline for 30 min at 13.5 cm/s. Tibialis anterior (TA) and soleus motor pools were labelled retrogradely by intramuscular injection of Fluorogold mixed with Cholera Toxin B (CTB) or Fast Blue plus CTB, respectively. Immunofluorescent double labelling was used to detect c-fos and CTB staining at L3 and L5. Preliminary data show that treadmill stepping increased the number of c-fos+ interneurons in laminae I-III and in the medial area of laminae IV to VII compared to control. Most c-fos+ interneurons were associated with CTB+ sensory afferent terminals. There were more c-fos+ motoneurons in the stepped groups, especially in the soleus motor pool. The increases were greatest in the 25o incline group, most likely reflecting increased motor unit recruitment during incline stepping. The data suggest that stepping at an incline (enhanced loading) increases the activation of sensorimotor-related neurons in the spinal cord. *Supported by Paralyzed Veterans of America*.

P4PM-13-31

CONSIDERATION BY 3-DIMENSIONAL MOTOR ANALYSIS OF AMBULATORY FUNCTION DURING THE PROCESS OF ARTIFICIAL NERVE REGENERATION IN DOGS

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The current research into longitudinally assessing a model that provided compensation with a PGA-collagen tube indicated that, regardless of in vivo macroscopic findings and histological and electrophysiological regeneration, ambulatory motor function was not completely restored. However, ambulation was performed even without complete restoration, i.e. adapting to the environment. In the future, attainment of ambulatory patterns close to normal ambulation is projected. What this projection allows is macroscopic, morphological, and electrophysiological assessment, which are results obtained by 3-dimensionally analyzing ambulatory motor function. Morphological recovery is crucial, although the original meaning of recovery is surely that the restored morphology works functionally.

In the future, various artificial organs will be morphologically restored, and restoration in terms of function in the environment inside and outside of the body will be crucial.

P4PM-13-32

BIOCHEMICAL CORRELATES OF AGGRESSIVE BEHAVIOR IN PRISONERS

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Violence and aggression are one of the most serious problems of contemporary society. Minding the importance of this problem, we aimed to study the peculiarities of neurotransmitteral and hormonal patterns in human males committed major and minor crimes.

The concentration of testosterone in aggressive prisoners was higher than in less aggressive, but in both groups it did not exceed the level of normal population and in some cases was even lower. It is known that testosterone concentration decreases during stressful events. Agonistic conflict is a stressor, and testosterone doesn't seem to be a factor, which is always responsible for the induction of aggressive behavior.

We consider that inducer of aggressive behavior probably must show: i) higher basal concentration in dominant animals/aggressive persons, and ii) synthesis and release in stressful situation and agonistic conflict.

The most probable candidate for the role of inducer of aggressive behavior is norepinephrine. Our research showed that norepinephrine concentration in urine of aggressive prisoners was higher than in non-aggressive prisoners. Norepinephrine not only initiates aggressive response, but also shows high secretion when aggressive behaviour is necessary to provide better adaptation to an environment.

P4PM-13-33

INVOLVEMENT OF NITRERGIC SYSTEM IN CAFFEINE INDUCED ANXIOGENIC EFFECT IN ELEVATED PLUS MAZE IN MICE

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In the present study the involvement of nitrergic system in caffeine induced anxiogenic effects is evaluated in the elevated plus maze in mice. As a result of anxiogenic effect shown by caffeine (30 mg /kg, i.p.) the percentage of time spent in the open arms decreased in caffeine treated mice. Anxiogenic effect produced by acute administration of caffeine (30 mg/kg, i.p.) were prevented by pretreatment with a NO synthase inhibitor, N(G)- nitro- L-arginine methly ester (L- NAME) in 100 and 200 mg/kg, i.p. Moreover, L-Arginine (100 mg/kg, i.p.) a well known NO precursor enhanced the anxiogenic effect of caffeine (30 mg /kg, i.p.). Therefore, these results suggest that nitrergic system might be involved in caffeine induced anxiety state in mice.

P4PM-13-34

THE EFFECT OF ODOR ON ANXIETY BEHAVIOR AND RELATED BRAIN REGION REVEALED BY C-Fos STAINING

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The cerebral limbic system in the brain is thought as an important region for induction of emotion by odor. Although, by researching of receptors that receive molecules which concern odor, it recently became clear how we identify the different odor, the mechanism of emotion induced by the different odor is still not clear. In this study, we examined the effect of different odor on the anxiety-like behavior assessed by hole-board assay and elevated plus maze assay.

We also examined activated neuronal populations in the cerebral limbic system by using c-Fos immunohistochemistry.

In the hole-board assay, frequency of total crossing was decreased in rats exposed to formaldehyde and frequency of inner crossing was decreased in rats exposed to TMT compare to normal rats. In the elevated plus maze assay, exposed to formaldehyde or TMT rats have a tendency to decrease the time spent in the open arms. In contrast, exposed to α-pinene rats have a tendency to increase it.

In the rats exposed to formaldehyde and TMT, c-Fos expression in amygdala or periaqueductal gray was significantly higher than that of normal rats. In the rat exposed odor, c-Fos expression in various parts of the limbic system was peculiar changed.

P4PM-13-35

EFFECTS OF EGG LAYING HORMONE ON IDENTIFIED FEEDING NEURAL ELEMENTS OF BUCCAL GANGLION IN *APLYSIA KURODAI*

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Aplysia egg laying behavior is a complex sequence of head movements and posture initiated by the release of egg laying hormone (ELH) from the neurosecretory bag cells in abdominal ganglion. This behavior is known to suppress feeding. ELH, therefore, appears to affect the feeding neural circuits. In this study, we investigated the effects of ELH on MA (multi-action) interneuron, JO (jaw-opening) motor neuron, and JC (jaw-closing) motor neuron, which are elements of the feeding neural circuit, located in the buccal ganglia of *Aplysia kurodai*. ELH applied to the buccal ganglia did not change the spontaneous firing pattern in MA and JO, but prolonged the burst duration in JC. ELH also increased the spike number of JC during patterned feeding-like response evoked by the repetitive electrical stimulation of esophageal nerve, but did not change that of MA and JO neurons. Furthermore, ELH reduced the threshold of current-induced firing in JC. ELH mRNA level is known to elevate between mature and old animals. Increased spike frequency in JC following application of ELH may induce elongation of jaw closing periods, probably contributing to the decrease of food intake during the reproductive periods animals show egg laying *in vivo*.

P4PM-13-36

NEURAL, GLIAL AND BEHAVIORAL ABNORMALITIES IN NURTURING-IMPARED FosB NULL MICE

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During mouse parental behavior, neurons in the medial preoptic area (MPOA) are activated and express transcription factors such as c-Fos and FosB. FosB mutant (-/-) mice show defective nurturing behavior toward pups. We have shown that, during parenting, FosB is induced in MPOA neurons by the extracellular signal-regulated kinase (ERK), and that FosB then upregulate Sprouty1 and Rad, the feedback regulators of ERK and calcium signaling, respectively. This ERK-FosB-Sprouty1/Rad intracellular signaling is required for the initiation of parental behavior. Our studies also suggested the general role of feedback inhibition by FosB-Sprouty/Rad-Gem-Kir (RGK) family proteins in neuroprotection against methamphetamine excitotoxicity. In addition, FosB (-/-) mice showed persistent astrocytic abnormalities such as excessive glial fibrillary acidic protein expression and decreased brain content of serine; an astroglia-derived neurotrophic agent. Collectively, these results indicate that FosB (-/-) mice have broader neural, glial and behavioral dysfunctions, with which their nurturing defect shares the common molecular mechanism.

P4PM-13-37

GALANIN MODULATES VAGALLY INDUCED CONTRACTIONS IN THE MOUSE ESOPHAGUS

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Nitrergic myenteric neurons co-innervating motor endplates were previously shown to inhibit vagally induced contractions of striated muscle in the rodent esophagus. Immunohistochemical demonstration of putative co-transmitters, e.g., galanin, in enteric neurons prompted us to study a possible role of galanin in modulating vagally mediated contractions in an in vitro vagus nerve-esophagus preparation of the mouse. Galanin (1-16) inhibited vagally induced contractions in a concentration dependent manner. The non selective galanin receptor antagonist, galantide, blocked the inhibitory effect of galanin while the selective non-galanin receptor 1 and galanin receptor 3 antagonists, M871 and SNAP37889, respectively, and the nitric oxide synthase inhibitor, L-NAME, failed to affect this galanin induced response. Simultaneous application of galantide and L-NAME significantly reduced the inhibitory effect of capsaicin on vagally induced contractions. Immunohistochemistry revealed galanin immunoreactive myenteric neurons and nerve fibers intermingling with cholinergic vagal terminals at motor endplates. These data suggest that galanin from co-innervating enteric neurons co-operates with nitric oxide in modulating vagally induced contractions in the mouse esophagus.

P4PM-13-38

PHYSIOLOGICAL EFFECTS OF THE UPRIGHT SITTING ON THE KNEES

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This study was conducted in humans to evaluate the physiological effects in relation to upright sitting on the knees (SEIZA). Subjects were forced to continue upright sitting for 30 minutes. The H-reflex was used to assess changes in excitability of the soleus alpha-motoneurons in response to a conditioning stimulus applied to the peroneal nerve. This increse was attributed to facilitation of the Ia inhibitory interneurons projecting to the soleus motoneurons. We observed a significant increase in the reciprocal inhibition after upright sitting. Using thermography, the skin temperature decreased gradually after the beginning of sitting, and increased rapidly after the end of sitting. Changes in skin temperature of the lower limbs were observed up to 4 degrees in the cutaneous surface of tarsus, instep, and planter, resulting from a rebound response of peripheral circulation. Temporary ischemia of the lower legs due to SEIZA was more rapidly recovered after relaxation with a reaction of local increase in blood flower through the muscular and peripheral vessels of lesser compressed areas. It was concluded that the SEIZA induced sensory disturbances and blocked peripheral afferent feedback loops that provide the motoneurons with less excitation as reflex compensation.

P4PM-13-39

Sema4D/CD100 DEFICIENCY LEADS TO SUPERIOR PERFORMANCE IN MOUSE MOTOR BEHAVIOR

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Sema4D/CD100 is a type of class 4 semaphorin, exhibiting crucial roles in growth cone guidance in developing neurons. However, direct evidence of the actual involvement of Sema4D in the neuronal network development crucial for neurobehavioral performance is still lacking. To examine whether Sema4D deficiency leads to abnormal behavioral development, both wild-type and Sema4D-deficient mice were subjected to behavioral analyses. Open-field tests revealed increased locomotor activity in Sema4D-deficient mice with less percentage of time spent in the center of the field. In both the adhesive tape removal and rotarod tests, which examine motor coordination and balance, Sema4D-deficient mice showed significantly superior performance, suggesting facilitated motor behavior. Sema4D-deficient mice showed normal spatial learning and memory in the water maze task. However, the swimming speed of Sema4D-deficient mice was significantly faster than that of wild-type mice, providing further evidence of their accelerated motor behavior. Thus our mouse behavioral analyses revealed enhanced motor activity in Sema4D-deficient mice, suggesting the crucial involvement of Sema4D in the neurodevelopmental processes of the central structures mediating motor behavior in mice.

P4PM-13-40

GABAergic NEURAL DISTRIBUTION AND FUNCTION IN PLANARIAN *DUGESIA JAPONICA*

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Freshwater planarians have the most primitive central nervous system (CNS) and also have high regenerative ability. Glutamic acid decarboxylase (GAD) is the enzyme that converts glutamic acid into GABA, a major inhibitory neurotransmitter. Here, we isolated a cDNA encoding GAD in the planarian *D. japonica* (*DjGAD*). Immunofluorescence analysis using anti-DjGAD antibody showed that DjGAD protein was mainly distributed in the head region and the pharynx. After amputation of planarians posterior to the pharynx, DjGAD-immunoreactivity could not be detected in the anterior region of the tail piece until the third day of regeneration. Then, newly generated DjGAD-immunopositive neurons were detected in the anterior region on the third day of regeneration. During day 5-7 of regeneration, reconstruction of the neural network of DjGAD-immunopositive cells occurred. Additionally, we examined *DjGAD*-knockdown planarians using a phototaxis assay. Compared to control animals that show negative phototactic behavior, *DjGAD*-knockdown planarians did not avoid the direction of the light source. These results suggest that expression of DjGAD as detected by anti-DjGAD antibody is a useful marker for GABAergic neurons, and GABAergic neurons play an important role in phototaxis behavior.

P4PM-13-41

DOPAMINERGIC NEURAL DISTRIBUTION AND FUNCTION IN PLANARIAN *DUGESIA JAPONICA*

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Planarian, an invertebrate flatworm, has a high capacity for regeneration when compared with other animals. We show here for the first time that the reconstructed dopamine (DA) neural network regulates locomotion and behavior in planarian regenerates. The gene encoding tyrosine hydroxylase in the planarian *Dugesia japonica* (*DjTH*) was identified. DjTH protein was coexpressed with aromatic amino acid decarboxylase-like A (DjAADCA) in the planarian central nervous system (CNS). In addition, *DjTH*-knockdown planarians lost the ability to synthesize DA, but showed no change in 5-hydroxytryptamine synthesis. When the planarian body was amputated, DjTH-positive neurons were regenerated in the brain newly rebuilt from the tail piece at Day 3, and the DjTH-positive axonal and dendritic neural network in the CNS (dopaminergic tiara) was reconstructed at Days 5-7. At that time, autonomic locomotion and methamphetamine-induced hyperkinesia were also suppressed in *DjTH*-knockdown planarians. Planarian locomotion and behavior seem to be regulated in both cilia- and muscle-dependent manners. In *DjTH*-knockdown planarians, muscle-mediated locomotion and behavior were significantly attenuated. These results suggest that DA neurons play a key role in the muscle-mediated movement in planarians.

P4PM-14-1

REGULTATION OF (PRO)RENIN RECEPTOR EXPRESSION BY MECHANICAL STRETCH IN HUMAN PODOCYTES

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Both (pro)renin receptor [(P)RR] expression and angiotensin II (AngII) production were reported to increase in the hypertensive kidneys. Since glomerular hypertension results in mechanical distention of the capillary tuft and subsequent injury of the overlying podocyte layer, we have tested the hypothesis that mechanical strain upregulates (P)RR expression in podocytes, thereby resulting in local AngII production in podocytes. Human podocytes were subjected to cyclical stretch. Nonstretched podocytes served as controls. In whole cell lysate, (P)RR levels were measured by quantitative PCR and Western blot analysis, and AngII levels were measured by competitive ELISA. The 30% stretch decreased the (P)RR mRNA levels during the 144-h study period, while the 20% stretch significantly increased the (P)RR mRNA levels at 96-, 120-, and 144-h intervals. The 10% stretch significantly increased the (P)RR mRNA levels at 24-, 48-, and 72-h intervals. The maximum increase averaged 100% and was observed at 24 h after the 10% stretch. ($P < 0.05$) The stretch increased AngII production significantly, but it was attenuated by the (P)RR knockdown with siRNA ($P < 0.05$). In conclusion, the stretch-induced upregulation of the (P)RR may contribute to the increases in local AngII production in podocytes.

P4PM-14-2

ANG II TYPE 2 RECEPTOR MEDIATES ANG II AND HIGH GLUCOSE INDUCED REDUCTION ON RENIN/ PRORENIN RECEPTOR EXPRESSION IN CULTURED MESANGIAL CELLS

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Activation of the (pro)renin receptor ((P)RR) exerts its physiological function through a non-enzymatic mechanism, and is involved in the pathogenesis of diabetic nephropathy. In the present study, we examined which angiotensin receptor subtypes were involved in the regulation of the (P)RR in cultured rat renal mesangial cells (RMCs). Treatment with angiotensin II (Ang II) reduced (P)RR mRNA and protein expression in RMCs. This inhibitory effect was abolished with the angiotensin II type 2 receptor antagonist, PD123319, while the angiotensin II type 1 receptor antagonist, losartan, had no effect. Treatment with the angiotensin II type 2 receptor agonist, CGP42112A, reduced (P)RR expression. Exposure to high concentration glucose (30 mM), which mimics the diabetic intrarenal environment in vivo, resulted in reduced (P)RR expression. This effect was abolished by PD123319. Overall, these data suggest that both Ang II and hyperglycemia can reduce (P)RR expression in RMCs, mainly through activation of the angiotensin II type 2 receptor.

P4PM-14-3

EFFECTS OF OLMESARTAN ON INTRARENAL ANGIOTENSIN II AND OXIDATIVE STRESS IN AORTIC REGURGITATION MODEL RATS

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We examined whether impaired cardiac function after aortic regurgitation (AR) induced renal injury via renal angiotensin II and oxidative stress. Rats were subjected to AR and treated with vehicle or olmesartan (Olme; 15 mg/kg/day) for 6 months. AR induced left ventricular hypertrophy and cardiac dilatation. Olme treatment attenuated AR-induced decrease in cardiac function. AR rats showed significantly increased urinary albumin excretion (sham; 0.69+/-0.06 mg/dL, AR; 3.72+/-0.34 mg/dL, Olme; 0.70+/-0.08 mg/dL) and glomerular desmin staining. Glomerular nephrin and podocin mRNA expression was down-regulated in this model. Furthermore, AR rats showed significantly higher kidney angiotensin II (AngII) levels and glomerular dihydroethidium (DHE) staining. NADPH oxidase subunits, p22phox and gp91phox mRNA expression in glomeruli were also upregulated in AR rats. Olme treatment significantly prevented albuminuria, podocyte injury, augmentation of intrarenal AngII levels, DHE staining and glomerular p22phox and gp91phox upregulation. In conclusion, these findings suggest that impaired cardiac function after AR leads to augmentation of intrarenal AngII and oxidative stress, which may contribute to the progression of podocyte injury and albuminuria.

P4PM-14-4

EFFECTS OF MINERALOCORTICOID RECEPTOR BLOCKADE ON GLUCOCORTICOID-INDUCED HYPERTENSION AND RENAL INJURY IN ADRENALECTOMIZED RATS

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In vitro studies have demonstrated that the affinity for mineralocorticoid receptor (MR) is similar between aldosterone and glucocorticoid. We hypothesized glucocorticoid is involved in the development of hypertension and renal injury through MR-dependent mechanisms. Saline-drinking uninephrectomized rats were divided into three groups: vehicle; adrenalectomy (ADX)+hydrocortisone (Hydro; 5 mg/kg/day, s.c.); ADX+Hydro+eplerenone (50 mg/kg/day). Hydro treatment in ADX rats significantly elevated the systolic blood pressure and urinary albumin excretion compared to vehicle-infused rats (vehicle; 2.13+/-0.38 mg/day, ADX+Hydro; 5.55+/-0.48 mg/day, p<0.01). Hydro treatment also induced glomerular sclerotic changes, increased desmin staining in podocyte and tubulointerstitial fibrosis. Furthermore, collagen type 1 gene expression were also upregulated in renal cortex of Hydro-treated rats. These changes were associated with the increase in the expression of MR target genes, such as Na+/H+ exchanger 1 and serum and glucocorticoid-regulated kinase 1. Treatment with eplerenone markedly attenuated these changes. Present findings indicate that chronic glucocorticoid treatment could activate the MR and in turn, induce the development of hypertension and renal injury.

P4PM-14-5

EFFECTS OF CILNIDIPINE ON INTERARENAL RENIN-ANGIOTENSIN SYSTEM, OXIDATIVE STRESS AND RENAL INJURY IN SHR/ND RATS

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Clinical studies have indicated that the beneficial effect of a dual L/N-type calcium channel blocker, cilnidipine, on the progression of proteinuria in hypertensive patients. In the present study, we examined the effects of cilnidipine and amlodipine on intrarenal renin-angiotensin system, oxidative stress and renal injury in type 2 diabetic SHR/ND rats. SHR/ND rats were treated with vehicle (n=10), cilnidipine (n=11), amlodipine (n=9) or hydralazine (n=10) for 20 weeks. SHR/ND rats developed hypertension and proteinuria (179+/-15 mg/day), and treatment with cilnidipine, amlodipine or hydralazine similarly decreased blood pressure. Cilnidipine and amlodipine showed attenuation of proteinuria, but this effect of cilnidipine was much stronger than that of amlodipine (106+/-16 vs. 141+/-12 mg/day), while hydralazine did not affect it (174+/-10 mg/day). Treatment with cilnidipine decreased glomerular desmin staining, kidney angiotensin II level, mRNA levels of p22phox and gp91phox and dihydroethidium staining. On the other hand, amlodipine and hydralazine did not affect these parameters. These data suggest that cilnidipine elicits renoprotective effects through suppressing the renal angiotensin II generation and NADPH oxidase-dependent oxidative stress in type 2 diabetes.

P4PM-14-6

ALDOSTERONE ACCELERATES THE RENAL SENESCENCE

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We investigated the contribution of aldosterone (Aldo) to renal senescence. Sprague-Dawley rats were uninephrectomized, given 1% NaCl in drinking water and divided as following; vehicle, Aldo (0.75 μg/h, s.c.) and Aldo + a mineralocorticoid receptor (MR) antagonist, eplerenone (Eple; 100 mg/kg/day, p.o.). Aldo infusion for 5 weeks significantly increased blood pressure and urinary excretion of protein or N-acetyl-beta-D-glucosaminidase. Eple significantly suppressed these changes. Senescence-associated β-galactosidase (SA-βGal) was detected in the renal cortex, especially in proximal tubule, of Aldo group. Aldo infusion also up-regulated both mRNA and protein expression of senescence markers, p53 and p21 (P21WAF1/CIP1 as gene), and down-regulated the mRNA of a longevity factor, SIRT1, in renal cortical tissues; changes were suppressed by Eple. To see the direct effect of Aldo on cell senescence, we also used human proximal tubular cells (HPTC). Aldo directly increased SA-βGal in HPTC (30+/-5.1%); effects were abolished by treatment with Eple (18+/-5.2%) or siRNA for MR (17+/-2.9%) or p21WAF1/CIP1 (8+/-0.7%). These findings indicate that Aldo accelerates the senescence in the kidney, especially in proximal tubules, through an MR-dependent pathway.

P4PM-14-7

URINARY ANGIOTENSINOGEN AS A BIOMARKER FOR INTRARENAL ANGIOTENSIN ACTIVITY IN PATIENTS WITH IgA NEPHROPATHY

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Studies were conducted to test the hypothesis that urinary angiotensinogen provides a specific index of intrarenal RAS status in patients with IgA nephropathy. Urinary angiotensinogen (AGT) levels in healthy volunteers (n=11), and patients with IgA nephropathy (n=11) and minimal change nephrotic syndrome (MCNS) or minor glomerular abnormality (MGA) (n=4) were measured by a newly developed sandwich enzyme-linked immunosorbent assay (ELISA) system. Urinary AGT levels were not different between healthy volunteers and MCNS or MGA. However, urinary AGT levels, renal AGT expression and angiotensin II (AngII) immunoreactivities were significantly higher in patients with IgA nephropathy than MCNS or MGA. In these patients, baseline urinary AGT levels were positively correlated with renal AGT gene expression and AngII immunoreactivities. In patients with IgA nephropathy, treatment with an AngII blocker, valsartan (40 mg per day), significantly increased renal plasma flow and decreased filtration fraction, which were associated with reductions in urinary AGT levels, renal tissues AGT and AngII immunoreactivites. Thus, urinary AGT should provide a simple and noninvasive diagnostic test to identify patients with activated intrarenal AngII and associated renal risk.

P4PM-15-1

THE INFLUENCE OF ELEVATED DIETARY SODIUM INTAKE ON NAD(P)H AND SOD IN THE CORTEX AND MEDULLA OF THE KIDNEY

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This study investigated the impact of raised dietary sodium (Na) on enzymes generating reactive oxygen species (ROS) in the cortex and medulla of the kidney.

Groups of 6-8 male Wistar rats, 225-275g, were fed a normal (0.3% Na, NNa) or high Na diet (3% Na, HNa) for 2 weeks. 24h urine flow, Na and 8-isoprostane excretion were determined before, 1 and 2 weeks on the diets. Rats were killed, kidneys separated into cortex and medulla and SOD and NAD(P)H proteins extracted. Data were analysed with Student's 't' test and significance taken at P<0.05.

Urine flow, Na and 8-isoprostane excretion rose some 10 fold (P<0.001) after 1 week on the HNa diet and remained at this level at week 2. NAD(P)H oxidase activity and protein expression rose some 2-fold (both P<0.05) in cortex, but not medulla in the HNa rats. SOD activity was lower in the medulla than cortex in NNa and HNa diet rats (both P<0.05) but SOD expression in cortex and medulla was similar on both diets.

HNa intake increases NAD(P)H activity and protein levels in the cortex along with elevated 8-isoprostane excretion, with no change in SOD activity, indicative of raised ROS. These observations show that this physiological challenge causes a modest oxidative stress but without raised SOD scavenging activity.

Poster Session

P4PM-15-2

EFFECTS OF HIBISCUS SABDARIFFA LINN. CALYX EXTRACT AS AN ANTIOXIDANT IN CISPLATIN-INDUCED ACUTE RENAL FAILURE RAT

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The remarkable side effect of cisplatin, a cytotoxic agent in cancer treatment, is acute renal failure (ARF). An increase in free radicals has been shown to play an important role in this renal pathogenesis. The in vitro antioxidative effect of calyces of Hibiscus sabdariffa Linn. (HS) has been previously demonstrated. This study aimed to investigate the protective role of HS water extract (HSE), as both short and long term treatments, on renal lipid peroxidation and renal functions in cisplatin-induced ARF rats using histopathalogical and renal clearance study. Short term treatment of HSE was able to alleviate cisplatin-induced ARF by reducing renal MDA and blood urea nitrogen levels and improving glomerular filtration rate and renal blood flow. Histopathological study also revealed an improvement of this glomerular damage. The anti-nephrotoxic effect of HSE short term treatment may be related to its protective effect on free radical generation in the kidney. In contrast, long term treatment with HSE did not show any significant protective effect against cisplatin-induced ARF. In addition, the long term treatment resulted in an increase in renal lipid peroxidation in normal rats suggesting its possible pro-oxidant effect.

P4PM-15-3

LOW LUMINAL pH AGGRAVATES FATTY ACID BOUD ALBUMIN INDUCED $O_2^{\bullet-}$ PRODUCTION IN RENAL PROXIMAL TUBULAR CELL

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It is known that fatty acid bound albumin induces oxidative stress in proximal tubular cells. Although luminal pH of proximal tubule decreases according to the reabsorption of bicarbonate, it is not examined well how luminal pH affects the production of superoxide, $O_2^{\bullet-}$. Therefore, we examined the role of luminal pH in the $O_2^{\bullet-}$ production.

We applied oleic acid bound albumin (OA-alb, 15g/L) to the HK-2 cells, human proximal tubule cell line, and measured the production with dihydroethidium. The $O_2^{\bullet-}$ production was evaluated as the increasing rate of ethidium intensity under real time fluorescent microscopy. When buffer pH is above 6.6, the production was not significant ($O_2^{\bullet-}$ production; 0.15 ± 0.06 unit/sec at pH 6.9 and 0.34 ± 0.15 unit/sec at pH6.6, p=0.297). However, by decreasing buffer pH to 6.4, OA-alb increased the production to 1.45 ± 0.28 unit/sec (p =0.026 versus pH 6.6). A Na/H exchanger inhibitor, dimethylamiloride blunted the production by 62%.

In summary, the luminal acidic pH, which activates Na/H exchanger through the reduction of intracellular pH, aggravates OA-alb induced $O_2^{\bullet-}$ production in proximal tubular cells.

P4PM-16-1

LOCALIZATION AND EXPRESSION OF V1aR ALONG THE MOUSE NEPHRON DURING METABOLIC ACIDOSIS

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Arginine vasopressin (AVP) may maintain blood pressure through V1a receptors (V1aR). We examined (1) localization of V1aR mRNA along the kidney nephron and (2) changes in its expression levels induced by chronic metabolic acidosis (CMA). Expression of V1aR mRNA was estimated by high sensitive in situ hybridization (ISH) in mice (C57BL/6J, male, 10 wks). Dots of V1aR mRNA staining were counted by eye under the microscope during the conditions of control (2% sucrose) and CMA (2% sucrose+0.28M NH_4Cl, free drinking). After administration of NH_4Cl, average urine pH (± SEM) decreased significantly ($P<0.0001$) from 6.52±0.04 (n=14) to 5.8±0.03 (day 1, n=5) and was unchanged (day 6). Plasma pH once decreased from 7.32±0.02 (n=6) to 7.17±0.01 (n=5) on day 1 ($P<0.0001$), but completely recovered to 7.35±0.02 (n=9) on day 6. In control animals, normalized levels of the V1aR mRNA expression were high in macula densa and CD, low in Glm, TAL, and DCT. In CD, V1aR mRNAs expressed only in intercalated cells. Under NH_4Cl load, the level of V1aR mRNA increased at MTAL and OMCD. In conclusion, V1aR in MTAL and intercalated cells of CD may play an important role for maintaining the systemic acid-base balance, especially during CMA.

P4PM-16-2

A ROLE OF V1a RECEPTOR (V1aR) IN RENAL H^+ EXCRETION: ACID-BASE BALANCE IN MICE LACKING V1aR

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V1aR deficient ($V1aR^{-/-}$) mice (Koshimizu et al, 2006) showed both decreased plasma volume and blood pressure. We examined the body acid-base balance during chronic metabolic acidosis (CMA) in $V1aR^{-/-}$ mice.

WT mice (C57BL/6J, male) and $V1aR^{-/-}$ mice (male) were given 2% sucrose (control) and 0.28 M NH_4Cl/2% sucrose (CMA). Mice were placed in metabolic cages to assess urine pH, urine volume, osmolality, and blood samples. After administration of NH_4Cl, the WT mice plasma pH (± SEM) once decreased from 7.32±0.02 (n=6) to 7.17±0.01 (n=5) on 1 d ($P < 0.0001$), but completely recovered to 7.35±0.02 (n=9) on 6 d. In contrast, the $V1aR^{-/-}$ mice plasma pH didn't recover completely on 6 d (7.29 ± 0.02, n=5). The plasma HCO_3^- (20.8 ± 1.0) and pCO_2 (39.0±1.7) of WT mice were recovered on 6 d. However, they were opposite in $V1aR^{-/-}$ mice (HCO_3^-: 15.4±0.9, 6 d; pCO_2: 33.1±3.6, 6 d). In conclusion, V1aR may stimulate renal H^+ excretion and maintain the acid-base balance especially during the CMA.

P4PM-16-3

A ROLE OF V1a RECEPTOR (V1aR) IN WATER/ ELECTROLYTES METABOLISM: ELECTROLYTES BALANCE IN MICE LACKING V1aR

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V1aR deficient ($V1aR^{-/-}$) mice showed a slight decrease in both plasma volume and blood pressure (Koshimizu et al, 2006). In order to investigate a role of V1aR in water/electrolytes balance, we examined the body electrolytes balance in $V1aR^{-/-}$ mice, during normal and low Na^+ diet. $V1aR^{+/+}$ mice ($V1aR^{+/+}$ littermates, male) and $V1aR^{-/-}$ mice (male) were devided into two groups and were fed normal (C) and low Na^+ diets (LNa) in each group. All mice were placed in metabolic cages to assess urine pH, urine volume, osmolality, and blood samples. After LNa diet for 7 days, values of plasma electrolytes (Na^+, K^+, Cl^-) and osmolality as well as body weight and blood pressure were almost unchanged (± SEM) in $V1aR^{+/+}$ and $V1aR^{-/-}$ mice. However, in $V1aR^{-/-}$ mice PRA (plasma renin activity, R) and PAC (plasma aldosterone concentration, A) increased significantly from 44.4 ± 3.0 (n=12) to 54.2 ± 5.3 (n=10) and 531.3 ± 53 (n=12) to 839.6 ±141 (n=10), respectively. Although renal Na^+ excretion was significantly, but slightly decreased during LNa diet, renal K^+ excretion was not. In conclusion, V1aR may play an important role in electrolytes balance by changing the renal Na^+ and K^+ excretion in the R-A-A axis rather than water balance.

P4PM-16-4

SERINE PROTEASE INHIBITOR ATTENUATES HYPERTENSION AND KIDNEY INJURY IN DAHL SALT-SENSITIVE RATS

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Epithelial sodium channel (ENaC) is a key regulator of blood pressure by modulating Na reabsorption in the kidney and is involved in the development of salt-sensitive hypertension. The cleavage of γENaC by serine proteases is necessary for the activation of ENaC, and aldosterone increases the cleaved form (active form) of γENaC in rat kidney. Dahl salt-sensitive (DS) rats, but not Dahl salt-resistant (DR) rats, develop severe hypertension under high salt (HS) diet. In the current studies, we investigated the effect of HS diet and camostat mesilate (CM), an orally active serine protease inhibitor, on ENaC both in DS and DR rats. HS diet induced severe hypertension and kidney injury only in DS rats. Although plasma aldosterone was suppressed, the cleaved γENaC was more abundant in DS than in DR under HS diet. Treatment of DS rats with CM significantly decreased the abundance of cleaved γENaC and attenuated hypertension and kidney injury, indicating the blockade of ENaC activity by a serine protease inhibitor. Our results suggest that the aberrant ENaC activation under HS diet may contribute to the development of salt-sensitive hypertension in DS rats, and that serine protease inhibitors might be a new class of drugs for the treatment of salt-sensitive hypertension in humans.

P4PM-16-5

INTRA-RENAL HYPOOSMOLALITY DOWNREGULATES NATRIURETIC PEPTIDE RECEPTORS IN THE KIDNEY

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Extracellular osmolarity is known as an important factor for the regulation of natriuretic peptide receptor (NPR). We investigated the intra-renal osmoregulation of NPRs using male Sprague-Dawley rats received bromoethylamine. The administration of BEA caused a decreased food intake and body weight. Water intake was decreased on the first day and then increased from the second day. Urine volume was marked increased. Urinary excretions of sodium, chloride and potassium were decreased on the second day and then recovered to control level. Plasma level of ANP and DNP in BEA-treated rats was not different from control rats. Active renin was decreased and the inactive renin was increased. In renal cortex, the gene expression of NPR-A, and NPR-B were not changed but that of NPR-C was decreased. In renal medulla, the gene expression of NPR-A, -B, and -C were decreased. Specific 125I-ANP and 125I-DNP binding density were decreased in glomeruli and medulla of BEA-treated rat kidney. However, the binding affinity was not changed. These data suggest that disruption of intra-renal osmotic gradient may regulate the expression of NPR mRNA and its protein level. Supported by the MRC (R13-2008-005-00000-0).

P4PM-16-6

THE BROMIDE EXCRETION RATE IS DETERMINED BY SODIUM RATHER THAN BY CHLORIDE INTAKE

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We have extended the applicability of our recent, experimentally proved hypothesis that the biological half-life of bromide depends on the magnitude of sodium intake rather than on the intake of chloride, as was generally assumed. We demonstrated the parallel course of the excretion rates of sodium and bromide ions in adult rats administered simultaneously with sodium and bromide labeled with the radionuclides Na-24 and Br-82. The biological half-life of bromide excretion, as a useful substitute for the less convenient sodium half-life, determined in the present study in lactating and nonlactating female rats of the same age, as well as in breast-fed and weaned young rats of various ages, fluctuated over a very broad range (from 44 ± 4.5 h in lactating dams to 269 ± 22 h in nursed young). Evidently, the values were primarily dependent on the metabolic activity of the animals, whose physiological states markedly differed. Support from the Acad. Sci. of the Czech. Rep. (Project No. AV0Z50110509), Ministry of Educ. of CR (Project No. MSM0021622413) and from the GA CR (Grant No. 304/08/0256) is acknowledged.

P4PM-16-7

MECHANISMS OF RENAL ALBUMIN UPTAKE AND DEGRADATION

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Albuminuria is hallmark of renal disease. The conventional model of renal albumin handling is that the glomerulus is a charge selective barrier that allows only small amounts of albumin to enter the proximal tubule that are taken up and degraded by the epithelial cells. In disease this barrier is compromised and excess albumin disrupts the normal endocytic processing, leading to lysosomal dysfunction and albuminuria. Recent real time *in vivo* imaging of renal albumin handling has challenged this conventional model suggesting that large amounts of albumin cross the glomerulus. This albumin is retrieved intact to the blood by an as yet unidentified high capacity uptake pathway. The ability to distinguish between intact and degraded albumin has become a critical point of delineation in the interpretation of imaging data. We have recently developed a method using a conjugate of albumin that fluoresces only upon degradation to begin to map the uptake and degradation of albumin in the nephron. Our data indicate that the proximal tubule endocytoses and degrades significant levels of albumin. Importantly, in diabetic animals we are able to visualise a pronounced disruption of the endocytic processing and a significant reduction in degradation of albumin.

P4PM-16-8

NGAL (NEUTROPHIL GELATINASE -ASSOCIATED LIPOCALIN), IRON METABOLISM AND INFLAMMATION IN HEMODIALYZED PATIENTS: POSSIBLE RELATIONS?

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NGAL (neutrophil gelatinase-associated lipocalin) is critical in various states including bacterial infection and kidney injury. Hepcidin is a small defensin-like peptide whose production by hepatocytes is modulated in response to anemia, hypoxia or inflammation. We studied whether NGAL is related to iron metabolism and hepcidin in hemodialyzed patients and healthy volunteers. We assesed iron status , complete blood count, creatinine, albumin, serum lipids, soluble receptor of transferin-sTFR, hsCRP, TNF alpha, IL-6, prohepcidin, hepcidin, serum NGAL. Serum NGAL, prohepcidin, hepcidin were significantly higher in HD patients when compared to the healthy volunteers. Serum NGAL correlated significantly with prohepcidin, hepcidin, serum creatinine, serum urea, urea reduction ration, Kt/V, residual renal function, serum calcium, phosphate, Ca x P product, pH, serum iron, TSAT, ferritin, ESA dose, hsCRP, IL-6. In multiple regression analysis urea reduction ratio, TSAT and hsCRP were predictors of serum NGAL in HD patients.

NGAL is involved in both kidney function and iron metabolism. Taking into account anitimicrobial moieties of NGAL, further studies are needed to address the role of NGAL in the iron metabolism and inflammation in renal failure.

P4PM-17-1

INTERSUBUNIT ACTIVATION OF METABOTROPIC GLUTAMATE RECEPTOR 1A TRIGGERS Gq COUPLED SIGNALING PATHWAY, BUT NOT $G_{i/o}$ ONE

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Metabotropic glutamate receptor 1a (mGlu1a) is a key molecule for some forms of synaptic plasticity. The mGlu1a functions as a homo dimer and couples with different types of G proteins, such as G_q, G_s and $G_{i/O}$, which confers diversity in the mGlu1 signaling. A glutamate binding to one subunit has been reported to activate G_q protein through the other subunit of the dimer. We confirmed this intersubunit activation of mGlu1 dimer by the results that co-transfection of two mGlu1a mutants, R78L lacking glutamate binding and F781S lacking G protein coupling, could reproduce the glutamate-induced Ca^{2+} transient via activation of G_q protein. In contrast, in cells co-transfected with R78L and F781S, glutamate application could not evoke an increase in $G_{i/O}$-coupled GIRK current. Taken together, these results show that the intersubunit activation of mGlu1a dimer occurs in G_q signaling but not in $G_{i/O}$ signaling, suggesting a difference in the coupling events depending on the type of G proteins.

P4PM-17-2

LIGAND-INDUCED ACTIVATION OF THE $GABA_B$ RECEPTOR MONITORED BY FRET METHOD

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The $GABA_B$ receptor ($GABA_BR$), one of the family C GPCR members, exists as a heterodimer comprised of subunits GB1 and GB2. These two subunits are known to have distinct roles: GABA binds to the extracellular domain of GB1 whereas GB2 intracellularly couples to Gi/o protein. In order to clarify the activation mechanism of the $GABA_BR$, each subunit was fused with a fluorescent protein either Cerulean or EYFP at its intracellular loop and FRET changes upon GABA or Baclofen application were monitored by TIRF microscopy. As results, FRET decrease was observed between GB1 loop 2 and GB2 loop 1 or 2. This is in contrast with the findings from metabotropic glutamate receptor 1α, another family C GPCR, in which FRET increase between loops 2 and decrease between loops 1 were seen. The above FRET decrease was abolished upon $GABA_BR$ blocker CGP55845 application and was enhanced when positive allosteric modulator CGP7930 was applied, suggesting that the FRET pairs were faithfully reporting the known pharmacological properties of the original $GABA_BR$. In addition to these inter-subunit events, a possibility of movements within GB1 and/or GB2, i.e. intra-subunit changes during the receptor activation, will also be discussed.

P4PM-17-3

A TARGETED SENSOR FOR CYCLIC AMP UNCOVERS ADENYLYL CYCLASE ACTIVITY STIMULATED BY Ca^{2+}-RELEASE IN GH_3 PITUITARY CELLS

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Cyclic AMP is the prototypical second messenger, which impacts on every aspect of the life of a cell. To monitor cAMP dynamics, FRET-based sensors for cAMP have recently been developed.

We set out to study cAMP dynamics using the Epac2-camps sensor to specifically monitor changes in cAMP relying upon the activity of Ca^{2+}-stimulable adenylyl cyclase 8 (AC8). In order to target the Epac2-camps to AC8 domains, we fused the sensor to a catalytically inactive version of the whole AC8 molecule, Epac2AC8^{D416N}. Cyclic AMP dynamics measured with Epac2AC8^{D416N} in GH_3 cells suggested the expression of a Ca^{2+}-stimulable AC activity, which is susceptible to Ca^{2+}-release induced by Thyrotropin-Releasing Hormone. Remarkably, a less stringently targeted version of the Epac2-camps sensor reported a decrease in [cAMP] upon Ca^{2+}-release, which we ascribed to the presence of Ca^{2+}-inhibitable ACs.

We identified Ca^{2+}-stimulated AC activity in GH_3 cells, which most likely arose from the stimulation of endogenous AC8 by Ca^{2+}-release. Hormone-induced increases in cAMP have previously been shown to modulate the endocrine function of pituitary lactotropes. Since GH_3 cells display a strong lactotrope character, they are well suited for investigating the impact of Ca^{2+} on cAMP under physiological conditions.

P4PM-17-4

THE ROLE OF EGF RECEPTOR IN ALBUMIN-INDUCED RENAL FIBROSIS

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Chronic kidney disease (CKD) is characterized by tubulointerstitial fibrosis. During CKD, urinary albumin levels increase dramatically. Albuminuria has been shown to be a major mediator of renal fibrosis due to direct effects on proximal tubular epithelial cells (PTECs). This study examines the molecular mechanisms behind albumin induced-renal damage. Experiments were performed using the human renal proximal tubular epithelial cells (HK-2). HK-2 cells were treated with various concentrations (0.1, 1 and 5mg/ml) of albumin over 72 hours. Fibronectin protein levels, a maker of kidney fibrosis were significantly increased by 1 and 5 mg/ml albumin treatment. Phospho-EGF-receptor levels were also increased with 1 and 5mg/ml albumin treatments. Co-treatment of PTECs with albumin and an EGF-receptor antagonist, AG1478, inhibited fibronectin protein accumulation. Further experiments revealed that albumin-induced effects were mediated by the ERK1/2 mitogen activated kinase pathway, which is downstream of the EGF-receptor. This study suggests that albumin-induced fibrotic effects are mediated, in part by activation of the EGF-receptor. These findings provide novel insights into albumin-induced renal fibrosis.

P4PM-17-5

ROLE OF FUNGAL PRODUCTS IN THE INDUCTION OF ANGIOGENESIS MEDIATED BY HYPOXIA-INDUCIBLE FACTOR-1 IN MYCETOMA LESION

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Background: Eumycetoma lesion is well vascularized but the mechanisms controlling angiogenesis in the lesion are poorly understood. We investigated the role of hypoxia-inducible factor 1 (HIF-1), which regulates the transcription of vascular endothelial growth factor (VEGF), and the role of fungal products in its induction. Material and Methods: HIF-1α protein expression in the lesion was assessed in immunocytochemically stained sections. In vivo expression of VEGF mRNA was assessed by qRT-PCR. In vitro induction of HIF-1 and VEGF was assessed in HepG2 cells treated with M. mycetomatis culture supernatants using Western blot analysis, qRT-PCR analysis, and ELISA. Results: Overexpression of HIF-1α protein and VEGF mRNA was demonstrated in the mycetoma lesion ($p = 0.003$, $p = 0.001$, n=23), respectively. M. mycetomatis culture supernatants accummulated HIF-1α in HepG2 cells and increased the expression of VEGF mRNA ($p < 0.001$). VEGF secretion by HepG2 cells was increased under the effect of M. mycetomatis culture supernatants ($p = 0.003$). Conclusion: These findings denote an important role of HIF-1 in the vascularization of mycetoma lesion caused by M. mycetomatis and suggest that upregulation is partly induced by a fungal product.

P4PM-17-6

CIN85 KNOCKOUT MICE ARE HYPERACTIVE DUE TO DEFECTIVE DOPAMINE RECEPTOR ENDOCYTOSIS IN THE BRAIN

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Cbl-interacting protein of 85 kDa (CIN85) is a multiadaptor protein containing three Src homology 3 (SH3) domains, a proline-rich region and a coiled-coil domain. We have previously shown that CIN85 is involved in regulation of ligand-induced endocytosis of epidermal growth factor (EGF) receptors. To analyze the biological function of CIN85 in vivo, we created the CIN85 knockout mouse (KO), which are lacking CIN85 isoforms in the brain. CIN85 KO displayed a hyperactivity phenotype in a novel environment, characterized by an increase in forward locomotor activity, speed of movement and spontaneous entering and exploration of the unprotected area. CIN85 was localized in the postsynaptic sites of primary neurons and was shown to link activated dopamine receptors with the endocytic regulators dynamin and endophilin. CIN85 deletion in the brain results in decreased endocytosis of dopamine receptors in striatal neurons and increased dopamine levels in the striatum. We conclude that the lack of CIN85 isoforms in the brain results in defects in dopamine receptor functions involved in regulation of locomotor and exploratory behaviour.

P4PM-17-7

THREE DISTINCT MUSCARINIC PATHWAYS MEDIATING CHOLINERGIC EJPS IN MOUSE ILEUM

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Excitatory junction potentials (EJPs) evoked by transmural electrical stimulation in ileal longitudinal muscle strips from M_2 or M_3 knockout (KO) mice were intracellularly recorded and compared with those in wild-type (WT) preparations. In WT preparations, the EJPs had a mean amplitude of 6.3±0.5 mV (n=17), and they were abolished by atropine or tetrodotoxin but increased to 11.4±1.8 mV (n=8) after physostigmine treatment. Atropine-sensitive EJPs were also evoked in M_2-KO or M_3-KO preparations, but their sizes were as small as 18% and 7% of the WT size, respectively. Their respective sizes in the presence of physostigmine were 33% and 8% of the corresponding WT size (11.4 mV). No appreciable EJP was evoked in M_2/M_3 double KO preparations. Therefore, the WT EJP was considered not to be a simple mixture of EJPs mediated by M_2 and M_3 receptors. The results suggest that there are three distinct pathways mediating the cholinergic EJPs; two of these involve either M_2 or M_3 receptors, and the third one requires the presence of both receptor subtypes and may contribute to the major part of the EJPs.

P4PM-17-8

HETEROLOGOUS DOWN-REGULATION OF ANGIOTENSIN TYPE 1 RECEPTOR BY PURINERGIC $P2Y_2$ RECEPTOR STIMULATION

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Expression of Angiotensin (Ang) II type 1 receptor (AT1R) is one of major factors that determine Ang II-induced cardiovascular functions. We here demonstrated that AT1R-induced increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$ increase) is inhibited by ATP treatment in rat cardiac fibroblasts. The number of AT1R was decreased by ATP. ATP increased nuclear factor of activated T cells (NFAT) activity through $P2Y_2$ receptor. Suppression of AT1R-induced $[Ca^{2+}]_i$ increase by ATP was canceled by cyclosporine A. NFAT activation increased the expression of inducible nitric oxide synthase (iNOS), and the suppression of AT1R-induced $[Ca^{2+}]_i$ increase by ATP was canceled by an iNOS inhibitor. Expression of AT1R is regulated by NF-κB activity and NO inhibits NF-κB activity through S-nitrosylation of NF-κB. The suppression of AT1R-induced $[Ca^{2+}]_i$ increase by ATP was canceled by overexpression of p65 (C38S) mutant. Furthermore, p65 was coprecipitated with iNOS and S-nitrosylated in iNOS- and p65-expressing HEK293 cells. These results suggest that stimulation of G_q-coupled $P2Y_2$ receptor by ATP heterologously regulates another G_q-coupled AT1R expression through S-nitrosylation of NF-κB.

P4PM-17-9

CONVERTING NATURAL NEUROTOXINS TO RECEPTOR LIGANDS BY DIRECTED EVOLUTION IN VITRO

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Natural toxins have been used as invaluable molecular tools to investigate signal transduction and to probe tissue/cellular localization and structure-function studies for target proteins. Furthermore, some of the toxins such as conotoxins have been developed to antinociceptive drugs (*e.g* Ziconotide and Conantokin). We have screened and characterized peptide toxins from various venomous animals. From the Elapidae snake, *Micrurus corallinus*, twelve new proteins were identified. They share a common structural feature called a three-finger (3F) motif, however, the sequences in the three loops and their functions are extremely diverged. The nucleotide sequence analysis of the 3F proteins suggest that their genes coding for functional domains, which are critical for recognition of and affinity to target proteins, could be evolved in the accelerated rate. Thus, we have engineered one of the 3F toxins by randomizing the residues in the loops, while maintaining the scaffold to protrude the three fingers. The random peptide library was subject to *in vitro* evolution by targeting interleukin-6 receptor (IL-6R). The selected peptides were found to be agonists and antagonists for IL-6R. We have also developed *in vitro* evolution of the spider toxin directed to peptide ligands for GPCRs.

P4PM-17-10

ROSIGLITAZONE UP-REGULATES SEMAPHORIN 3G EXPRESSION AND PROMOTES ENDOTHELIAL MIGRATION

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Semaphorins (sema) play roles in angiogenesis. However, regulated expression of sema3s in vascular endothelial cells (ECs) remains largely unknown. We aimed to examine the effect of rosiglitazone on gene expression of sema3. Gene expression of sema3G in human umbilical vein ECs (HUVECs) was examined by using real-time RT-PCR and immunoblotting. Results showed that rosiglitazone significantly up-regulated sema3G expression at both mRNA and protein levels. The induction was markedly inhibited by the pretreatment with GW9662, an antagonist for PPAR-γ. adenovirus-mediated overexpression of constitutively active PPAR-γ increased expression of sema3G, suggesting a PPAR-γ-dependent mechanism. 5'-flanking region of the human sema3G gene harbors two putative PPAR-responsive elements. PPAR-γ binding was confirmed by using chromatin immunoprecipitation. Reporter assay demonstrated that rosiglitazone increased the sema3G promoter activity. Furthermore, rosiglitazone increased the secretion of sema3G into the conditioned media, which subsequently promoted EC migration. In conclusion, our results demonstrated that rosiglitazone up-regulated sema3G in ECs in PPAR-γ-dependent mechanism. It is suggested that sema3G may play an important role in mediating the pro-migratory effect of PPAR-γ.

P4PM-17-11

SECONDARY STRUCTURE AND CONFORMATION CHANGE IN POLYMORPHISM (E228G) OF FCEPSILONRI BETA CHAIN INVESTIGATED BY CD SPECTROSCOPY

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The β chain of the high affinity IgE Fc receptor (FcεRI) acts during an allergic reaction as a signal amplifier in mast cells. We have investigated the structure of the C-terminal 43 amino acids of FcεRI β chain with the mutated mouse β chain (Glu^{228}→Gly : E228G). The far-UV CD spectra of the wild type (WT) and E228G are indicative of an α-helical protein and E228G does not have any loss or collapse of α-helical content. However, we have revealed the thermal unfolding of the secondary-structural elements of the FcεRI β chain WT and E228G. The midpoint temperature (Tm) value of WT and E228G are 53.2 degree and 51.7 degree, respectively, by temperature dependence of far-UV CD spectra. The Tm for WT was approximately 1.5 degree higher than that for E228, clarifying that secondary structure of the WT protein is more stabilized than that of E228G. In addition, Gibbs free energy change of WT and E228G are 30.76 and 28.8 kJ/mol, respectively. Our data suggest that E228G affects the secondary characterizations of the FcεRI β chain protein and may play some sort of roles in allergic reactions.

P4PM-17-12

RECOGNITION OF DIETARY FAT IN THE ORAL CAVITY

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Several studies have indicated that rodents and humans recognize the presence of fat in foods not only by the texture of the food but also chemically in the mouth: this suggests that the chemical perception of fat is involved in the acquisition of a strong preference for fat. GPR120, a G-protein coupled receptor, functions as a specific unsaturated long-chain fatty acid receptor in the gastrointestinal tract, and is related to the gastrointestinal hormone release. By immunohistochemical staining of GPR120, we found that GPR120 is expressed in the taste cells of the tongue, similar to the enteroendocrine cells of the gastrotintestinal tract. We next investigated the palatability of various kinds of long-chain fatty acids by assessing licking behavior. Mice showed a higher licking response for unsaturated long-chain fatty acid but not for saturated fatty acid. The palatability of fatty acids for mice is very similar to the ligand specificity for GPR120. These results raise the possibility that GPR120 expressed in the taste cells may be involved in the chemical reception and palatability of dietary fat in the oral cavity. This study was supported by the Program for the Promotion of Basic Research Activities for Innovative Bioscience.

P4PM-17-13

THE CALCIUM-SENSING RECEPTOR IN RODENT TASTE BUDS

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The sensing mechanism for calcium, which is an essential nutrient with a distinctive taste quality, is not well understood. We propose here a system that involves the extracellular calcium-sensing receptor (CaSR), the first time that has been described in taste buds of mammals. This G protein-coupled receptor participates in the homeostasis of calcium by regulating parathyroid and kidney functions. CaSR mRNA and protein were found in a subset of taste cells from the circumvallate and foliate papillae, mainly in presynaptic type III taste cells thought to respond to salts and acids. This implies that CaSR might be involved in calcium taste. Thus, when calcium is present in food, CaSR could be activated by free amino acids as co-agonists. And the resulting taste quality will depend on the taste cell type in which CaSR is found and the ratio of free amino acids and calcium in the diet.

P4PM-17-14

FUNCTIONAL EXPRESSION STUDY OF *TAS1R1/TAS1R3* RECEPTOR VARIANTS ASSOCIATED WITH INDIVIDUAL DIFFERENCES IN HUMAN UMAMI TASTE SENSITIVITY

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Umami taste is elicited by L-glutamate, typically as its Na salt (monosodium glutamate: MSG), and is thought to be one of five basic taste qualities that plays a key role in intake of amino acids. A heterodimer of a G protein coupled receptor, *TAS1R1* and *TAS1R3*, is proposed to function as its receptor in humans. However, little is known about genetic variation of *TAS1R1* and *TAS1R3* and its potential links with individual differences in umami sensitivity. Here we investigated (1) taste recognition thresholds for MSG, IMP and MSG plus IMP in 254 Japanese subjects, (2) amino acid mutations in the coding region of umami receptor candidate genes, human *TAS1R1* and *TAS1R3*, (3) the association between these recognition thresholds and *TAS1R1*/*TAS1R3* genotypes and (4) the functions of *TAS1R1*/*TAS1R3* variants using a heterologous expression system. The results showed a strong correlation between individual differences in taste recognition thresholds for umami substances and genetic variations of *TAS1R1*/*TAS1R3*.

P4PM-17-15

IDENTIFICATION OF VESICULAR NUCLEOTIDE TRANSPORTER (vNUT) IN TASTE CELLS

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Taste receptor cells are chemosensory epithelial cells that sense distinct taste quality such as umami, sweet, bitter, sour and salty. These cells could be divided morphologically into light, dark and intermediate (or type I, II, III) cell. Although several reports have suggested involvement of ATP in taste signal transduction and its release from taste cells, there is paucity of molecular information about how these signals are transduced to taste nerves which innervate taste papillae. Since vesicular nucleotide transporter (vNUT) has been recently identified, we are now able to specify the cell types where ATP is stored and characterize the molecular mechanism of how ATP is being released.

In the present study we have explored vNUT expression in the taste tissue where ATP supposed to be released for signal transduction. We found that within tongue epithelium vNUT mRNA is expressed in circumvallate papillae but not in surrounding epithelium. By using antibodies raised against vNUT we found that vNUT is selectively expressed in type II taste cells but not in type III cells. Our results, together with previous studies, strongly suggest the role of vNUT in type II taste cell signal transduction.

P4PM-18-1

NOS-MEDIATED ALTERATIONS OF INTESTINAL P-GLYCOPROTEIN IN THE DEVELOPMENT OF DIABETES

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P-glycoprotein (P-gp), one of the important drug-efflux pumps, is known to be affected by some pathological condition. We have found that intestinal P-gp expression transiently-decreased at early phase of the development of streptozotocin (STZ)-induced type1 diabetes. Here we studied the participation of nitric oxide synthase (NOS) in the alteration of intestinal P-gp expression.

Type1 diabetes was induced in male ddY mice by the i.p. injection of STZ (230 mg/kg). We analyzed ileum P-gp expression and NOS activity by western blot analysis and ELISA method, respectively. L-NAME (1 mg/mL) was added to the drinking water.

Significant decrement of P-gp expression in ileum was found at 9th day after STZ-administration. However, it increased to control levels at 15th and 30th day. On the other hand, the NOS activity in the ileum kept higher levels than control after STZ-administration. Interestingly, administration of L-NAME during the early phase (0-9 day) significantly suppressed the decrement of P-gp at 9th day. In addition, it also suppressed the recovery of P-gp expression at 15th day when administrated during the late phase (9-15 day).

These results indicate that NOS plays distinct roles in P-gp expression in the early- and late-phase development of diabetes.

P4PM-18-2

SHORT-TERM REGULATION OF INTESTINAL IRON ABSORPTION BY TRANSPORTERS RELOCALIZATION

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Intestinal iron absorption by duodenal enterocytes comprises the coordinated activity of the influx transporter DMT1 and the efflux transporter ferroportin. A fast (1-2 h) response to avoid excessive iron absorption is known as the mucosal block, described as the ability of an initial dose of ingested iron to block absorption of a second dose. Under the hypothesis that the mucosal block is determined by the topology of import (DMT1) and export (ferroportin) iron transporters, we studied their apical-basolateral membrane localization and transport activity. Addition of iron induced a striking relocalization of DMT1 and ferroportin to basolateral and intracellular domains, respectively. ^{55}Fe flux experiments revealed inward and outward iron fluxes at both membrane domains that were in agreement with the membrane distribution of the transporters. Apical and basolateral fluxes were inhibited by antisenses targeted to DMT1 or ferroportin. We conclude that the relative abundance of DMT1 and ferroportin at the apical and basolateral membranes will determine the amount and net direction of the iron flux.

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P4PM-18-3

INTERACTION OF PHOSPHATE ANALOGS WITH PALYTOXIN-BOUND Na,K-ATPase PUMP-CHANNELS

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The Na,K-ATPase pumps Na and K by behaving like an ion channel with two gates constrained to open and close alternately. Palytoxin binding disrupts this coupling, allowing both gates to sometimes be open, transforming pumps into ion channels. But the gates still respond to physiological ligands of the pump. Here we test phosphate mimics. In inside-out patches with 100 nM palytoxin in the pipette, cytoplasmic BeF_x (200 μM $BeSO_4$ plus 5 mM NaF) severely attenuated both the small pump-channel current seen without ATP and the large current increase on adding 1 mM ATP that reflects internal gate opening. The effect of BeF_x was slowed when it was added in the presence of ATP. Current recovery after BeF_x removal was slow, but was accelerated by the presence of ATP, suggesting that ATP and BeF_x can be simultaneously bound at different sites in a pump-channel. Similar, but weaker, results were obtained with AlF_x. However, MgF_x simply reversibly decreased currents without ATP, but did not affect the activation by ATP. These results suggest that, despite the presence of palytoxin, phosphate mimics BeF_x and AlF_x stabilize an Na,K pump-channel conformation with the internal gate firmly shut, like that in X-ray crystal structures of E2-BeF_3- SERCA ATPase.

[HL36783]

P4PM-18-4

MEMBRANE POTENTIAL OF XENOPUS OOCYTE GENERATED BY ELECTROGENIC Na^+/K^+-ATPase

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Xenopus oocytes are widely used for the expression system of various ion channels and transporters. To success the functional study of such channels or transporters, good oocytes with enough negative membrane potentials should be selected. Such oocytes are considered less leaky and the negative membrane potentials are believed to be generated by K^+ conductance. Recently we found that freshly enzymatically isolated oocytes showed high negative potentials below -80 mV in control NaCl solutions and did not show depolarization with high K+ solutions. Such phenomena were not observed from the oocytes over 1 day after isolation. Na^+/K^+ ATPase inhibitor ouabain effectively depolarized the membrane potentials of freshly isolated oocytes and the membrane potentials showed K^+ dependency after ouabain application. These observations indicate that the membrane potentials of fresh oocytes are mainly generated by the electrogenic Na^+/K^+ ATPase not by K^+ conductance.

P4PM-18-5

A RED FLUORESCENT PROTEIN INSERTION DOES NOT PREVENT POST-TRANSLATIONAL PROCESSING OF THE Na/K-ATPase CATALYTIC SUBUNIT

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The first five amino acids of the catalytic α1 isoform from the Na,K-ATPase are removed during or after translation. I tested whether the insertion of irrelevant sequence would have an influence on this processing. A chimeric cDNA was prepared in which mCherry was inserted into the amino terminus of the nascent polypeptide. The resulting mutant was transfected into opossum kidney cells (OK), which were examined by fluorescence microscopy and immunoblotting. Cells exhibited both surface and intracellular fluorescence, indicating that the chimeric catalytic subunit was targeted successfully to the plasmalemma. An antibody raised against the first 9 residues of the α1 nascent chain (anti-VGR) did not bind to membranes from the transfected cells. However, a band with a mobility appropriate for the chimeric α was detected with an antibody that is specific for mCherry, confirming expression of the chimeric protein. Because anti-VGR does not recognize cleaved α1, the absence of binding suggests that the chimera underwent normal processing. Consistent with earlier work, these results suggest strongly that the determinants of amino terminal processing in the catalytic subunit of the Na,K-ATPase reside within the first few residues of the nascent polypeptide.

P4PM-18-6

Na^+/K^+-ATPase EXPRESSION IN GILLS OF THE EURYHALINE SAILFIN MOLLY, *POECILIA LATIPINNA*, ALTERED IN RESPONSE TO SALINITY STRESS

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We hypothesized that branchial Na^+-K^+-ATPase (NKA) expression changed in response to salinity stress of sailfin molly (*Poecilia latipinna*) so that they were able to survive in environments of different salinities. In the long-term experiment (one month), the physiological (plasma osmolality), biochemical (activity and protein abundance of branchial NKA), and stress (plasma glucose levels and protein abundance of hepatic and branchial heart shock protein 90) indicators of osmoregulatory challenge in sailfin molly were significantly increased in seawater- (SW-) acclimatized group compared to freshwater- (FW-) acclimatized group. Elevated levels of biochemical and stress indicators revealed that more active NKA expression was necessary to match the demand of ion secretion of SW-acclimatized sailfin molly for survival in the more stressful environment. Although the plasma osmolality increased with environmental salinities within a tolerated range, the muscle water contents, another physiological indicator, were constant among different salinity groups. In summary, the sailfin molly was proved to be an efficient osmoregulator with their branchial NKA expression changing in response to salinity stress to maintain ion and water homeostasis in environments of different salinities.

P4PM-18-7

EFFECTS OF ENVIRONMENTAL SALINITY ON Na^+/K^+-ATPase EXPRESSION IN KIDNEYS OF THE EURYHALINE MILKFISH, *CHANOS CHANOS*

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Juvenile milkfish (*Chanos chanos*), a marine euryhaline teleost, was reared in fresh water (FW), seawater (SW; 35‰), or hypersaline water (HSW; 60‰) in the laboratory for at least two weeks before experiments. To investigate the mechanisms of osmoregulation in kidneys of the marine euryhaline milkfish, muscle water contents (MWC), basic renal morphology, and expression of Na^+/K^+-ATPase (NKA) in kidneys of milkfish were examined. MWC of milkfish in various environmental salinities showed no significant difference. The renal tubules were identified by staining with hematoxylin and eosin (HE) or periodic acid Schiff's reagent (PAS). Renal NKA was found to exhibit in the epithelial cells of proximal tubules, distal tubules, and collecting tubules, but not in glomeruli, of all fish groups exposed to different ambient salinities. Relative mRNA and protein abundance of kidney NKA α-subunit, however, were found to be higher in FW-acclimatized individuals than SW- and HSW-acclimatized fish. This study integrated diverse levels (i.e., gene, protein, and histology) of NKA expression in kidneys of the marine euryhaline teleost and illustrated possible mechanisms of kidneys in osmoregulation, e.g., ion re-absorption in hypotonic environment.

P4PM-18-8

FXYD PROTEINS, THE Na^+/K^+-ATPase REGULATORY PROTEINS, IN THE OSMOREGULATORY ORGANS OF THE EURYHALINE PUFFERFISH, *TETRAODON NIGROVIRIDIS*

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FXYD proteins constitute a family of small proteins with a single transmembrane domain. FXYD proteins were identified as the regulatory protein of Na^+/K^+-ATPase (NKA) in mammals. The present study revealed three types of pufferfish FXYD (pFXYDI, II, and III) in several organs of euryhaline pufferfish (*Tetraodon nigroviridis*). The full lengths of pufferfish *FXYD* genes were confirmed by RT-PCR. Three cDNA sequences of pFXYDs were deduced to amino acid sequences and the phylogenetical relationship of pFXYDs with the other vertebrate FXYDs was analyzed. *pFXYDI* and *pFXYD III* genes were exhibited in three osmoregulatory organs (gill, kidney, and gut) of both SW- and FW-acclimated pufferfish, but *pFXYD II* was expressed mainly in the gill. The mRNA abundance determined by real-time PCR showed that *pFXYD I* in gills and guts of the freshwater group was higher than seawater fish, opposite to their expression in kidney. In addition, *pFXYD III* mRNA abundance in gut was higher in freshwater fish. Patterns of pFXYDs mRNA expression were opposite to the activity of NKA in osmoregulatory organs of SW- and FW-acclimated pufferfish. Localization *pFXYD* genes in osmoregulatory organs will be detected by in situ hybridization. Future works will focus on FXYD-NKA interaction by specific antibodies.

P4PM-18-9

Ca^{2+} TRANSPORT OF DIMERIC Ca^{2+}-ATPase REQUIRES THE ABILITY OF PHOSPHORYLATION OF BOTH MONOMERS

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The issue of functional unit of Ca^{2+}-ATPase of sarcoplasmic reticulum (SR) has been discussed for almost 50 years, though many reports concerned with this issue have been published. We focused on the relation between Ca^{2+} transport activity and the interaction of Ca^{2+}-ATPase molecules, and demonstrated that the functional unit of SR Ca^{2+}-ATPase is a dimer.

In this study, we determined the reaction steps during Ca^{2+} transport cycle where the molecular interaction for Ca^{2+} transport activity occurred, by measuring the activity of the heterodimers composed of wild type Ca^{2+}-ATPase (WT) and various function-deficient mutants.

When the mutant was arrested at the step before the interaction occurred, the Ca^{2+} transport activity of the heterodimers assumed to be lost. The Ca^{2+} transport activity of heterodimers, expressed in COS-1 cell, depended on the mutant type. The heterodimer comprised of WT and the phosphorylation-deficient mutant did not have Ca^{2+} transport activity. On the other hand, the heterodimers comprised of WT and the dephosphorylation-deficient mutants could transport Ca^{2+}. These results indicated that Ca^{2+} transport of dimer required the ability of phosphoenzyme formation of both monomers.

Reference: Ushimaru, M. and Fukushima, Y., *Biochem. J.* **414**, 347-361 (2008)

P4PM-19-1

ROLES OF pH-REGULATORY ION TRANSPORT IN MOTILITY AND SURVIVAL IN MCF-7 BREAST CANCER CELLS EXPRESSING CONSTITUTIVELY ACTIVE ERBB2

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Tumors exhibit altered pH regulation, yet the role of pH regulation in cancer development is unclear. Expression of a truncated, constitutively active ErbB2 receptor (dNErbB2), which signals in part through the ERK-RSK and PI3K pathways, is common in breast cancer and associated with poor prognosis. Here, we investigate the roles of two pH regulatory transporters, the Na/H exchanger NHE1 and the Na_xHCO_3 cotransporter NBCn1, in motility and survival in MCF-7 breast cancer cells +/- dNErbB2. pHi recovery after acid loading involved both NHE1 and NBCn1, and was modestly increased in dNErbB2(+) cells, as was NHE1 and NBCn1 expression. The migration velocity of serum-starved cells on collagen IV was increased several-fold by dNErbB2 expression. The NHE1 inhibitor EIPA reduced the velocity of dNErbB2(-) cells by 50%. In contrast, the velocity of dNErbB2(+) cells was doubled by EIPA, whereas it was decreased by 50% by the RSK inhibitor SL0101, and unaffected by the PI3K inhibitor Ly294002. MCF-7 cells are cisplatin resistant, yet EIPA strongly potentiated cisplatin-induced cell death, especially in dNErbB2(+) cells. In conclusion, NHE1 and NBCn1 are novel downstream effectors of dNErbB2 in MCF-7 cells, perhaps through altered pHi regulation.

P4PM-19-2

INSULIN INHIBITS Na^+/H^+-EXCHANGE IN VASCULAR SMOOTH MUSCLE AND ENDOTHELIAL CELLS IN SITU: INVOLVEMENT OF H_2O_2 AND TYROSINE PHOSPHATASE SHP-2

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We investigated the effect of insulin on Na^+/H^+-exchange activity, intracellular pH (pH_i) and reactive oxygen species (ROS) in smooth muscle (VSMCs) and endothelial cells (ECs) of mouse mesenteric arteries using fluorescence microscopy.

In the absence of CO_2/HCO_3^-, removal of bath Na^+ produced EC acidification inhibited by cariporide. Insulin and H_2O_2 acidified ECs 0.2-0.3 units and reduced the acidification upon Na^+-removal by ~65%. Cariporide abolished the effect of insulin and H_2O_2.

VSMCs were acidified by H_2O_2 (ΔpH_i=-0.48±0.06) and insulin (ΔpH_i=-0.03±0.01). Na^+/H^+-exchange activity after an NH_4^+-prepulse was ~80% attenuated by H_2O_2 and ~40% by insulin.

NHE1 was the only NHE isoform detected by RT-PCR analyses.

In ECs and VSMCs, PEG-catalase abolished the effect of insulin on pH_i and exposure to insulin increased the concentration of ROS. NSC-87877 and PTP inhibitor IV (selective inhibitors of tyrosine phosphatase SHP-2) reduced steady-state pH_i up to 0.3 units and inhibited Na^+/H^+-exchange activity 60-80%; when applied in combination with insulin or H_2O_2, the SHP-2 inhibitors had no further effect.

We conclude that Na^+/H^+-exchange in ECs and VSMCs is inhibited by insulin and H_2O_2 and propose that insulin signaling involves H_2O_2-mediated inhibition of SHP-2.

P4PM-19-3

β-Pix-Shank2 PROTEIN COMPLEX REGULATES NHE3

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NHE3 mediates neutral Na+ transport in the mammalian epithelial cells. Shank2, a PDZ domain-based adaptor, binds to NHE3 and positively regulates membrane expression and activity of NHE3. It has been known that small GTPases play an important role in the membrane expression and regulation of NHE3. In this study, the molecular mechanism of NHE3 regulation by Shank2 was investigated in relation to β-Pix, because β-Pix can activate small GTPases as a guanine nucleotide exchange factor and is known to associate with Shank2. When expressed in PS120/NHE3 cells, β-Pix increased the membrane expression and basal activity of NHE3. However, this was abolished by the dominant-negative Shank2 mutant that has a defect in the PDZ domain or SAM domain. Knock-down of natively expressed β-Pix also decreased Shank2-induced upregulation of NHE3. Notably, the small GTPase inhibitor Toxin B abolished β-Pix- and Shank2-induced increases of NHE3 membrane expression. Formation of β-Pix-Shank2-NHE3 complex was also demonstrated in rat epithelial tissues. These results indicate that β-Pix and small GTPases are involved in the Shank2-induced upregulation of NHE3.

P4PM-19-4

ATP REQUIREMENT OF THE Na^+-DEPENDENT Mg^{2+} EFFLUX IN RAT VENTRICULAR MYOCYTES

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We measured intracellular Mg^{2+} concentration ($[Mg^{2+}]_i$) in rat ventricular myocytes using the fluorescent indicator furaptra (25ºC). In Mg^{2+}-loaded intact cells, introduction of extracellular Na^+ induced a rapid decrease in $[Mg^{2+}]_i$ (Na^+-dependent Mg^{2+} efflux). When the cellular ATP was depleted by treatment with either 1 μM FCCP or 5 mM KCN, a rise in $[Mg^{2+}]_i$ and cell shortening to ~50% of the initial length (probably due to rigor cross-bridge formation) was coincided with a marked inhibition of Mg^{2+} efflux. A steep relation between cell length and the rate of Mg^{2+} efflux suggests absolute requirement of ATP for transport. We estimated ATP concentration at the onset of rigor cell shortening (and therefore the onset of inhibition of Mg^{2+} efflux) using a mathematical model, based on the measured rise in $[Mg^{2+}]_i$ during rigor cell shortening, on average from 2.44 mM at the onset to 2.80 mM in complete rigor. The model calculated changes in concentrations of phosphate compounds and their Mg^{2+} binding that are associated with hydrolysis of ATP (and the resultant rise in $[Mg^{2+}]_i$), and indicated that the mean rise in $[Mg^{2+}]_i$ (0.36 mM) is most consistent with breakdown of ~0.4 mM ATP. Thus, full activation of Mg^{2+} efflux appears to require ~0.4 mM ATP.

P4PM-19-5

REGULATION OF DOWNREGULATED IN ADENOMAS (DRA) BY NHERF4

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The downregulated in adenomas (DRA), also known as a solute linked carrier (SLC) 26A3, functions as a chloride/bicarbonate anion exchanger and is expressed at the apical membrane of secretory epithelial cells in intestine, pancreas and salivary glands. We found that sodium/proton exchanger regulatory factor (NHERF) 4, a PDZ-containing scaffold protein also known as intestinal and kidney-enriched PDZ protein (IKEPP), binds to DRA and modulates its activity. NHERF4 has four PDZ domains. A direct protein-protein interaction was occurred at the carboxy-terminal PDZ binding motif of DRA and the third PDZ domain of NHERF4. Interestingly, co-expression of NHERF4 decreased the surface expression of DRA and consequently reduced its anion exchange activities. NHERF4 gene knockdown using small interfering RNA increased a DIDS-insensitive anion exchange activity of DRA in the HT-29 human colon cells. In addition, phosphorylation of NHERF4 decreased its binding to DRA. These results imply that DRA is dynamically regulated by association with NHERF4 in physiological state.

P4PM-19-6

JAPANESE MEDAKA PROVIDE NEW EVIDENCE FOR OSMOREGULATORY FUNCTIONS OF Na^+, K^+, $2Cl^-$ COTRANSPORTER IN GILL MITOCHONDRION-RICH CELLS

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Previous studies focused on the protein expression and distribution of Na^+, K^+, $2Cl^-$ cotransporter (NKCC) in mitochondrion-rich (MR) cells of euryhaline teleosts when exposed to hyperosmotic environments. In the MR cell, NKCC protein was proposed to participate in Cl- secretion. Hence this study used the model animal, Japanese medaka (*Oryzias latipes*), to focus on NKCC expression from genetic to functional levels in gills. Three isoform sequences of NKCC gene including nkcc1a, 1b, and 2 were found in medaka database. Using RT-PCR to survey tissue distribution revealed that gill prominently expressed nkcc1a in medaka. *In situ* hybridization indicated that nkcc1a was localized in MR cells. Higher mRNA abundance of nkcc1a was found in gills of seawater (SW) medaka rather than freshwater (FW) fish. On the other hand, detected by the monoclonal antibody (T4) on the gills indicated that in the SW medaka higher protein amounts of NKCC localized in MR cells was found. In addition, transfer of medaka from either SW to FW or FW to 50% SW revealed that dynamic NKCC expression in gills affected establishment or destruction of hyposmoregulatory abilities of medaka. Future work of functional assay will use the morpholino oligonucleotide for gene knockdown of nkcc1a expression in medaka embryo.

P4PM-19-7

RESTING POTENTIAL OF RAT MUSCLE FIBERS IN ANISOSMOTIC MEDIUM AT LOW K^+ IS SUBJECT TO COUNTERACTION OF CATION-CI COTRANSPORTERS

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The resting membrane potential (V_m) of rat skeletal muscle fibers in Krebs medium at 5.9 mM K^+ is known to be -80 ~ -67 mV. We found that V_m was -55 mV not only in 190 mM NaCl-hyperosmotic medium but also in 150 mM NaCl-isosmotic medium (290 mOsm, IsoM) at 0.625 mM K^+. On the contrary, V_m was -115 mV in 110 mM NaCl-hyposmotic medium (215 mOsm, HypoM) at the same K^+. According to the Cl^- equilibrium potential equation, an intracellular Cl^- concentration (Cl_i) was estimated to be 18 mM in IsoM and 1.3 mM in HypoM. Now, we examined the effect of cation-Cl cotransporters (CCCs) modulators on V_m in the low K^+ anisosmotic medium (LKAM). Bumetanide, a Na-K-2Cl cotransporter (NKCC) inhibitor, at 1 μM in IsoM completely improved V_m to -117 mV by HypoM preequilibration. The half-maximal effective concentration was 0.1 μM. Meanwhile, by IsoM preequilibration, 80 μM bumetanide did not improve V_m. *N*-ethylmaleimide, a K-Cl cotransporter (KCC) activator, at 1 mM in IsoM partially improved V_m to -80 mV. These results suggest that V_m in LKAM depends on chenge in Cl_i by counteraction of Cl^--intruding NKCC and Cl^--extruding KCC. The possibility was also demonstrated that function of electrically silent and volume-sensitive CCCs may be quantified by V_m measured under LKAM equilibrium.

P4PM-19-8

EFFECTS OF AMBROXOL DERIVATIVES ON ION TRANSPORT IN HUMAN AIRWAY Calu-3 EPITHELIA

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<Background and Purpose> Ambroxol is a clinically proven mucolytic agent used in treatments for respiratory disorders, however the mechanism of ambroxol on ion transport in human airway epithelia was still unclear. In this study we examined effects of ambroxol and its derivatives on ion transport in human airway epithelial cells .
<Methods> Confluent monolayers of Calu-3 cells, a human submucosal serous cell line, were mounted into the Ussing chambers and the transepithelial short-circuit current (Isc) and conductance (Gt) were measured across monolayers in 140mM NaCl solution without bicarboniate. Initially we added benzamil (ENaC blocker), and secondly NPPB (nonspecific chloride channel blocker). Then, we estimated the $Na^+/K^+/2Cl^-$ cotransporter (NKCC1)-mediated NPPB-sensitive Isc and the NPPB-sensitive Gt under a hyper-secreting condition caused by a beta-adrenergic agonist, terbutaline.
<Conclusion>Ambroxol of 100 μM had no effect on the NKCC1, while some of ambroxol derivatives dose-dependently diminished the NKCC1-mediated Cl-secretion. We also report the relationship between the inhibitory effect and kinds of lateral chain added to ambroxol.

P4PM-19-9

MYOGENIC TONE IN MOUSE MESENRERIC ARTERIES: EVIDENCE FOR P2Y RECEPTOR-MEDIATED, Na^+, K^+, $2Cl^-$ COTRANSPORT-DEPENDENT SIGNALING

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In spite of obvious physiological and pathophysiological significance of myogenic tone (MT), upstream mechanisms that translate pressure changes to vascular smooth muscle cell contraction are poorly understood. This study examine the role of purinoceptors and Na,K,2Cl- cotransport (NKCC) in mouse mesenteric artery contractions and possible implication of these signalling pathways in MT evoked by elevated intraluminal pressure. Both ATP and its non-hydrolyzed analogue trigger transient contractions that were sharply decreased in the presence of potent antagonists of P2X1 receptors. In contrast, UTP and UDP evoked sustained contractions. These contractions were suppressed by MRS2567, a selective antagonist of P2Y6 receptors. NKCC inhibition of with bumetanide led to 2-fold attenuation of contractions in UTP-treated arteries but did not affect contractions evoked by ATP. Both UTP-induced contractions and MT were suppressed by MRS2567 and bumetanide but were insensitive to NF023. These data suggest that MT in mesenteric arteries involves P2Y6-mediated NKCC-dependent mechanism. The action of elevated intraluminal pressure on UTP release from mesenteric arteries and its role in the triggering of P2Y6-mediated signaling should be examined further.

P4PM-19-10

THE FUNCTIONAL IMPORTANCE OF DICISTRONIC TRANSCRIPTION OF A BICARBONATE TRANSPORTER AND A KINASE IN THE MOUSE

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Dicistronic or polycistronic transcription is common in prokaryotes but in eukaryotes it is considered a relatively rare phenomenon except in C. elegans where up to 15 % of the genome may be transcribed in polycistronic units. In mammals there are few reports indicating that dicistronic transcription also occurs. In prokaryotes the polycistronic genes code for functionally interacting proteins. In mammals it is not known whether the genes in dicistronic transcripts are also functionally related. In this study we report on two genes (NBCn1 and NEK10) which are closely mapped on the genome across a number of eukaryote species and which are transcribed as a dicistronic mRNA. It was not possible with RT-PCR or Norhern blot to detect mono-cistronic mRNA for the two genes. The two gene products have similar tissue distribution with prominent expression in the kidney medulla, in blood vessels and in the brain. Homozygous mutation in the promoter region for the NBCn1 causes the disappearance of both NBCn1 and NEK at the mRNA and protein level.The two gene products are regulated in parallel in potassium-depleted mice. We conclude that dicistronic transcription in the mammalian genome can have functional importance as it has in prokaryotes.

P4PM-20-1

CHOLAPODS MEDIATE ANION TRANSPORT ACROSS PLANAR LIPID BILAYERS AND POLARISED EPITHELIA

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Small molecules that mimic the action of cation transporters are well known. However, until recently chemicals that transport anions were unavailable. In previous work, we demonstrated that a family of small molecules derived from cholic acid termed "cholapods" bind anions with high affinity and promote Cl^- efflux from liposomes. To understand better how cholapods mediate Cl^- transport, we used excised inside-out membrane patches from giant liposomes. Addition of cholapods caused a concentration-dependent increase in Cl^- current. However, the lack of unitary events suggested that cholapods might transport anions by a carrier-like instead of a channel-like mechanism. Using planar lipid bilayers, we tested this possibility. Addition of cholapod in DMSO to the cis side of a voltage-clamped membrane increased the observed current. As the ion concentration increased, the magnitude of cholapod-induced current saturated. Moreover, these currents were selective for anions over cations, but exhibited little selectivity among anions. Finally, when added to the apical membrane of MDCK epithelia, cholapods induced transepithelial Cl^- transport. Thus, our data suggest that cholapods mediate anion transport across artificial and cellular membranes by a carrier-like mechanism.

P4PM-20-2

THE SALINITY-DEPENDENT CHLORIDE CHANNEL, CIC-3 IN GILLS OF THE SPOTTED GREEN PUFFERFISH, *TETRAODON NIGROVIRIDIS*

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Chloride channels (CLCs) play an important role in transepithelial transport involved in maintaining ionic homeostasis and acid-base balance. CLC-3 is broadly expressed in different organs of the mammals, including the osmoregulatory tissue, kidney. In fish, the gill is one of the major organs of ionoregulation and osmoregulation. Our previous study revealed that the protein abundance of CLC-3 was higher in freshwater (FW)-acclimatized pufferfish than in seawater (SW)-acclimatized individuals. In this study, CLC-3 has been identified from the euryhaline spotted green pufferfish (*Tetraodon nigroviridis*) and demonstrated to exist in various organs including gill. Pufferfish CLC-3 consisted of the eleven transmembrane domains, similar to CLC-3 of the other vertebrates. The levels of CLC-3 protein abundance in gills of *T. nigroviridis* will be examined by long-term and time-course acclimatized experiments to illustrate the possible Cl^- absorption mechanism.

P4PM-20-3

FUNCTIONAL CHARACTERIZATION OF A NOVEL URATE TRANSPORTER URATv1 (*SLC2A9*) AND ITS RELATION TO RENAL HYPOURICEMIA

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Reanl urate handling is clinically important because underexcretion of urate causes hyperuricemia. Here, we functionally characterized *SLC2A9* gene product GLUT9 in search of urate excreting transporter. GLUT9 expressed *Xenopus* oocytes transported [^{14}C]urate time- and concentration-dependent manner (Km, 365 μM). We found that its transport was Na^+-independent and enhanced at high concentrations of extracellular potassium, indicating that GLUT9-mediated urate transport is affected by the changes in membrane potential. GLUT9-expressing oocytes preloaded with radiolabelled urate showed a time-dependent efflux of radioactivity when incubated in the standard uptake solution. Thus, we renamed this novel urate transporter as voltage-driven urate transporter (URATv1). Urate transport via URATv1 was affected by uricosuric agents benzbromarone (IC_{50}: 36 μM). *In vivo* role of URATv1 is supported by the fact that a renal hypouricemia patient without any mutations in *SLC22A12* was found to have a missense mutation P412R in *SLC2A9*, which reduced urate transport activity *in vitro*. Considering its basolateral expression in proximal tubules in human kidney, URATv1 is proposed to be a basolateral exit pathway of urate, which is likely to act in tandem with URAT1 for urate reabsorption.

P4PM-20-4

CHOLESTEROL DEPLETION MODULATES THE TAURINE HOMEOSTASIS IN EHRLICH LETTRE FIBROBLASTS

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The organic osmolyte taurine is in mammalian cells accumulated by the Na-dependent taurine transporter TauT and released through the volume-sensitive taurine leak pathway VSOAC (volume sensitive organic anion channel). Incubating Ehrlich Lettre cells under serum-free conditions for 1 hour with 5 mM methyl-β-cyclodextrine reduces the cholesterol pool to 60 ± 5 % of the control value. Concomitantly the active taurine uptake and the cellular taurine content are reduced to 47 ± 12 % and 77 ± 12% of control values, respectively. Kinetic analysis reveals that cholesterol depletion reduces the maximal taurine transport capacity but has no impact on short term regulation of the TauT transport activity through modulation by protein kinase A and C. Addition of melittin under isotonic conditions induces phospholipase A2 mediated mobilization of arachidonic acid (AA) and taurine release via VSOAC. Cholesterol depletion potentiates melittin-induced AA mobilization and taurine release via VSOAC. Cholesterol also potentiates taurine release via VSOAC following a mild hypotonic exposure (<15% reduction in tonicity). Hence, cholesterol depletion leads to net taurine loss due to impairment of active uptake and enhancement of passive release.

P4PM-20-5

REGULATION OF THE RAT GLUTAMINE TRANSPORTER SNAT3

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Glutamine is the most abundant amino acid in the blood plasma and cerebrospinal fluid. It plays an essential role in neurotransmitter recycling in the brain, ammonia detoxification in the liver, and the compensation of metabolic acidosis in the kidney. In these organs, the uptake and release of glutamine is primarily carried out by the Sodium Neutral Amino Acid Transporter 3 (SNAT3). Due to this pivotal role played by SNAT3, an understanding of its regulation has high physiological relevance. In this study, the regulation of the rat SNAT3 transporter by Protein Kinase C (PKC) was investigated. Activation of PKC by the treatment of oocytes expressing rSNAT3 with the phorbol ester PMA resulted in the rapid down-regulation of rSNAT3 activity. Mutational analysis of putative PKC phosphorylation sites showed that this down-regulation was not due to the phosphorylation of rSNAT3 at PKC specific sites. In order to investigate the cause of the down-regulation of rSNAT3 activity, confocal microscopy on oocytes expressing eGFP-rSNAT3 was performed. These studies revealed that PKC activation led to a retrieval of the fusion protein from the oocyte plasma membrane. Preliminary data indicates that this retrieval occurs through a dynamin-independent pathway.

P4PM-20-6

A PROTEIN-PROTEIN INTERACTION IN *C. ELEGANS*; AMINO ACID TRANSPORTER-6 REQUIRES THE INTERACTION WITH GLYCOPROTEIN FOR FUNCTIONAL EXPRESSION

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C. elegans amino acid transporters-6 (AAT-6) was identified as a homologue of catalytic subunits of mammalian heterodimeric amino acid transporters (HATs). Homologues of HATs glycoprotein subunits are found in *C. elegans*; ATG-1 and -2 (amino acid transporter glycoprotein subunit-1 and -2). AAT-6 does not have a conserved cysteine which forms a covalent bond with a glycoprotein subunit in mammalian HATs family. This dimer formation is essential for plasma membrane targeting of the catalytic subunits. We found that AAT-6 exhibited amino acid transport activity when expressed together with ATG-1. AAT-6 showed broad substrate selectivity covering neutral and basic amino acids. The interaction of AAT-6 and ATG-1 was demonstrated by co-immunoprecipitration analysis. AAT-6 was sorted to the plasma membrane of Xenopus oocytes only in the presence of ATG-1. Both AAT-6 and ATG-1 were expressed in intestine of *C. elegans*. *aat-6* or *atg-1* knockout *C. elegans* showed decrease in the body length, the diameter of intestine and the brood size. Since, AAT-6 contains a PDZ binding motif at its C-terminus, suggesting that there are PDZ protein(s) interact with AAT-6. To identify the PDZ protein(s) binding to AAT-6, a yeast two hybrid system was performed. Recently, we obtained a candidate protein.

P4PM-20-7

CHARACTERIZATION OF SUBSTRATE RECOGNITION SITE IN L-TYPE AMINO ACID TRANSPORTER 1 (LAT1)

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Amino acids are important nutrients for living organisms and have to be transported into the cells via transporters. System L is a main transporter system to transport large neutral amino acids. Importantly, L-type amino acid transporter 1 (LAT1), one of the system L isoforms, is highly expressed in various cancer cells. It has been suggested that LAT1 is a key transporter to supply nutrients for cancer cell growth. Therefore, LAT1 is one of the appealing targets for cancer therapy. Since LAT1 structure is not available, the study of substrate recognition is a straightforward strategy to link the cancer therapeutic study. To characterize LAT1 substrate recognition, we have established a stable cell line (S2-hLAT1) originally from a second segment of a proximal tubule cell from mouse which stably expressed human LAT1. The substrate recognition of LAT1 was characterized by inhibition assay and efflux assay of leucine transport using leucine derivatives including amino-group, carboxyl-group and side-chain modified leucines. In addition, we applied this study to elucidate the LAT1 transport mechanism in a cancer cell model, HeLa S3. We will show the crucial moieties of the substrate for its recognition by hLAT1 and the application directing toward the cancer related study.

P4PM-20-8

THE PATHOPHYSIOLOGIC MECHANISM OF BAT1 P482L MUTATION, SPECIFIC IN JAPANESE CYSTINURIA CASES

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The heterodimeric complex of rBAT, a single-spanning glycosylated heavy chain, and BAT1, putative 12-membrane-spanning non-glycosylated light chain, is an amino acid transport system for cystine and dibasic amino acids. Their mutations result in cystinuria. We reported BAT1 P482L mutation which is specific in Japanese cause cytinuria (Shigeta, et al. Kidney Int. 2006).

P482L protein was expressed and sorted to plasma membranes as well as wild type. Pro482 is located in BAT1 C terminal cytosolic region, thus we consider that P482L suppress the transport function by interfering with intermolecular interaction.

To identify BAT1 C terminal interacting protein, we made GST fusion proteins which have BAT1 C terminal of 31 amino acid, and that of P482L mutation. We performed GST pull-down assays to lysates of HEK 293 cells or brush border membrane vesicles (BBMV). The pull-down products were subjected to SDS-PAGE, and followed by fluorescent staining. There were 5 protein bands observed as interact factors to BAT1 C-terminal and/or P482L. We will report several proteins that identified by LC-MS/MS.

P4PM-20-9

BLOOD-BRAIN BARRIER AMINO ACID TRANSPORT BY THE HUMAN IN VITRO MODEL hCMEC/D3 CULTURED UNDER PHYSIOLOGIC SHEAR STRESS FLOW

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The Blood-brain barrier (BBB), which selectively controls brain access of plasma solutes, is a specialized endothelium expressing contiguous tight junctions (TJ) restricting paracellular diffusion. Due to critical central nervous system roles, the BBB tightly regulates most interstitial amino acids (AA) levels to a fraction of blood concentrations. An impediment to the use of human in vitro BBB cell models for functional studies has been the lack of TJ formation by cultured brain endothelial cells. Recently physiologic shear stress was shown to induce the human brain microvascular hCMEC/D3 cells to form sufficiently tight barriers for small molecule transport studies. In this study we combine in silico modeling of AA transport with in vitro studies using hCMEC/D3 cells to examine the role of the known BBB transporter, the large neutral amino acid transporter LAT1 (SLC7A5). Lat1 is of particular interest since it transports some radiolabelled diagnostic tracers and CNS therapeutics. LAT1 transendothelial transport of L-leucine was examined using hCMEC (D3) cultured using an in vitro flow-based (BBB-DIV, Flocel) system. With this approach the relative contributions of molecular transporters in the establishment and maintenance of AA gradients across the human BBB can be assessed.

P4PM-20-10

CHARACTERIZATION OF AN ORPHAN TRANSPORTER IN SLC 7 FAMILY

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Solute carrier (SLC) 7 family is a large group of amino acids transporters. The family consists of two subfamilies, the cationic amino acid transporters (CATs) and the heterodimeric amino acid transporters (HATs). These transporters show wide variety of physiological roles, and some of members in the family are also clinically important. SLC7A14 is an orphan transporter that was found in SLC 7 family from human and mouse genomes by computational search. SLC7A14, Slc7a14 in mouse, is predicted to possess 14 transmembrane domains and belong to the CAT family. mRNA of the orphan transporter was detected predominantly in mouse brain by Northern Blotting. It was also confirmed by Western Blotting with anti Slc7a14 antibody that the orphan transporter was specifically expressed in mouse brain. The protein band observed in wild type mouse was not observed in brain tissue from KO mouse of Slc7a14. We will report the localization of Slc7a14 in mouse brain and the function of the protein.

P4PM-21-1

TARGETED DISRUPTION OF Wnk4 GENE DECREASES PHOSPHORYLATION OF Na-Cl COTRANSPORTER AND BLOOD PRESSURE

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We recently generated Wnk4D561A/+ knockin mice and found that a major pathogenesis of the hereditary hypertensive disease, pseudohypoaldosteronism type II, was the activation of the OSR1/SPAK kinase-NaCl cotransporter (NCC) phosphorylation cascade by the mutant WNK4. However, the physiological roles of wild-type WNK4 and whether wild-type WNK4 functions positively or negatively in this cascade remained to be determined. In the present study, we generated WNK4 hypomorphic mice by deleting exon 7 of the Wnk4 gene. These mice did not show abnormalities in blood tests, but they did exhibit low blood pressure and increased Na excretion after acute loading of NaCl. Phosphorylation of OSR1/SPAK and NCC was significantly reduced in the mutant mice as compared with their wild-type littermates. Protein levels of renal K channels, ROMK and Maxi K, were not changed, but epithelial Na channel appeared to be activated as a compensatory mechanism for the reduced NCC function. Thus, wild-type WNK4 is a positive regulator for the WNK-OSR1/SPAK-NCC cascade, and WNK4 is a promising target of anti-hypertensive drugs.

P4PM-21-2

WNK1 ACTIVITY TO OSR1 IN COS CELLS IS REGULATED NOT BY HYPOTONICITY BUT BY LOW CHLORIDE

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We recently showed by generating *Wnk4*$^{D561A/+}$ knockin mice that the activation of WNK-OSR1/SPAK-NCC phosphorylation cascade was the main cause of pseudohypoaldosteronism type II (PHAII). Although WNK1 and WNK4 were shown to phosphorylate OSR1/SPAK in vitro and the kinase activity was increased by both hypertonic and hypotonic low chloride media, little is known about the mechanisms of activation of WNK. We investigated the details of WNK activation under anisosmotic conditions. Activity of WNK was monitored with the phospho-specific antibody to OSR1. We showed increased phosphorylation of OSR1 by anisosmotic media was abolished by the siRNA to WNK1, indicating that the WNK activity in COS7 cells was that from WNK1. When the cells were switched to hypotonic low Cl media, or hypertonic media, increased phosphorylation of OSR1 was detected at 10 minutes after the switch. The phosphorylation by the former media lasted for 24 hours, but that by the latter media returned to the baseline at 24 hours after the switch. Furthermore, we found that isosmotic media with low Cl could increase OSR1 phsphorylation as well as the original hypotonic media. These results suggest that both hypertonicity and low Cl are the stimulating factors for WNK1.

P4PM-21-3

VITAMIN E TRANSPORT BY A CHOLESTEROL IMPORTER NPC1L1

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Cumulative evidence suggests that Niemann-Pick C1-like 1 (NPC1L1) is essential for intestinal cholesterol absorption and is the target of ezetimibe, a novel cholesterol-lowering drug. Based on an assumption that cholesterol and other fat-soluble molecules share a number of transport mechanisms, we hypothesized that some lipophilic compounds may be transported by NPC1L1. As we examined the cellular uptake and inhibitory effect of ezetimibe for some putative substrates using rat NPC1L1-overexpressing Caco-2 cells, it was revealed that NPC1L1 mediates the ezetimibe-sensitive uptake of alpha-tocopherol (a major form of vitamin E) but does not mediate the uptake of retinol (vitamin A) or cyclosporin A. To confirm the ezetimibe-sensitive transport of alpha-tocopherol in vivo, we performed an in vivo absorption study using rats and the results suggested a physiologically significant role of NPC1L1-mediated vitamin E absorption. Furthermore, we demonstrated that the uptake of all vitamin E tested (alpha-, gamma- and delta-forms of tocopherol and tocotrienol) was significantly increased by the overexpression of human NPC1L1 and ezetimibe inhibited their uptake. The present data suggest that NPC1L1 is involved in the ezetimibe-sensitive absorption of vitamin E.

P4PM-21-4

ANNEXIN A2-P11 COMPLEX INTERACTS WITH CD98HC

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CD98, a heterodimeric protein, was originally identified as an early T cell activation antigen. CD98 is consisting of two subunits: a 85 kDa glycosylated type II membrane protein with a single transmembrane domain (CD98 heavy chain; CD98hc/4F2hc) and a ~45 kDa multi-spanning transmembrane protein (CD98 light chains) with amino acid transport activity. CD98hc is expressed ubiquitously and particularly at high level in many tumor cells. It is well known that protein-protein interaction is important for physiological system. Importantly, it has been suggested that CD98hc has other functions beside amino acid transporter. Therefore, we have searched for CD98hc interacting proteins. Using GST protein fused with the N-terminus of CD98hc as bait to pull down, mass spectrometric protein analysis revealed that annexin A2, a Ca^{2+}-dependent and phospholipid-binding protein, binds with CD98hc. The physiological interaction between endogenous CD98hc and annexin A2 was observed by co-immunoprecipitation. Moreover, p11, forming a heterotetramer with annexin A2 to translocate the complex to the cell surface, was co-immunoprecipitated with CD98hc and annexin A2. Furthermore, the specific binding site of CD98hc to annexin A2 was studied and the effect of annexin A2 knockdown will be reported.

P4PM-21-5

POST TRANSLATIONAL REGULATION OF THE NUCLEOSIDE TRANSPORTER hCNT3 BY ALL-TRANS-RETINOIC ACID IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

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Fludarabine (Flu) is the nucleoside analog employed in the treatment of chronic lymphocytic leukaemia (CLL). It exerts its proapoptotic action once it has entered the cell mostly by equilibrative nucleoside transporters (hENTs). Flu is also known to be a good substrate of the human concentrative nucleoside transporter (hCNT3). CLL cells lack this transport activity. However, it has been reported a correlation between Flu-resistant populations of CLL and high levels of cytosolic hCNT3 protein, without detectable hCNT3-related plasma membrane transport activity. All-trans retinoic acid (ATRA) is known to have good benefit in acute promyelocytic leukemia (APL) and in acute myeloid leukaemia (AML) blasts by increasing the sensitivity to the nucleoside analog cytarabine. Our study aimed to determine the role of ATRA in the expression and activity of hCNT3 in CLL cells. We found that incubation for 5-6 hours of CLL cells with ATRA led to a 3-5-fold increase in hCNT3-related activity, by a mechanism that involves trafficking of already synthesized hCNT3 proteins to the plasma membrane. This effect was partially mediated by TGF beta 1 (known to be produced by CLL cells) by a mechanism that was dependent on the activation of the mitogen-activated protein (MAP) kinases p38 and ERK1/2.

P4PM-21-6

EVIDENCE FOR DIFFERENTIAL REGULATION OF LACTATE METABOLIC PROPERTIES IN AGED AND UNLOADED RAT SKELETAL MUSCLE

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Purpose: Skeletal muscles of elderly individuals show fatigue resistance and reduced lactate accumulation compared with those of young subjects during activities that recruit a small amount of muscle mass. The purpose of this study was to examine the changes in lactate metabolic properties of skeletal muscle with aging.
Methods: We quantified the expression of monocarboxylate transporter (MCT) 1 and MCT4 and the enzyme activities of lactate dehydrogenase (LDH) and citrate synthase (CS) in the soleus (SOL) and extensor digitorum longus (EDL) muscles from old rats (OLD), young control rats (CON), and hindlimb-suspended young rats (SUS).
Results: MCT1 expression was lower in SOL from OLD than in SOL from CON, but was similar between SOL from CON and SUS. MCT4 expression was lower in EDL from OLD than in EDL from CON, but did not differ between CON and SUS. The LDH-to-CS ratio was higher in SOL from SUS and OLD than in SOL from CON, and was lower in EDL from OLD than in EDL from the other two groups.
Conclusion: Aging causes metabolic changes that can reduce lactate accumulation during exercise and increase fatigue resistance in skeletal muscle. These changes may result from aging rather than inactivity.

P4PM-21-7

THE PHYSIOLOGICAL ROLE OF A NOVEL PROSTAGLANDIN SPECIFIC TRANSPORTER OAT-PG IN MOUSE KIDNEY

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We have identified a novel prostaglandin specific transporter as a member of SLC22 family and designated it as OAT-PG (Prostaglandin specific Organic Anion Transporter). Immunohistochemistry demonstrated that OAT-PG was localized at basolateral membrane of proximal tubules in mouse kidney. Interestingly, 15-hydroxyprostaglandin dehydrogenase which is a prostaglandin metabolizing enzyme was also localized in proximal tubules. Therefore, we hypothesize that OAT-PG plays a role in prostaglandin metabolic clearance from interstitial fluid in renal cortex. To elucidate the physiological role of OAT-PG, we generated the OAT-PG knockout mice. OAT-PG$^{-/-}$ mice did not show any apparent phenotypes. However, when PGE_2 synthesis in macula densa was stimulated, tissue prostaglandin E_2 (PGE_2) contents was significantly increased in OAT-PG$^{-/-}$ mice compared to wild type mice. Furthermore, plasma renin activity and plasma angiotensin 2 concentration were also significantly increased in OAT-PG$^{-/-}$ mice. These results suggest that OAT-PG has a role in PGE_2 clearance from interstitial fluid in renal cortex and the clearance is important for the regulation of renin release in kidney.

P4PM-21-8

HETEROGENEOUS EXPRESSION OF NUCLEOSIDE TRANSPORTERS IN HUMAN SYNCYTIOTROPHOBLAST MEMBRANES

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Vectorial flux of nucleosides across epithelia is mediated by the heterogeneous expression of concentrative (CNT) and equilibrative (ENT) transporters at the apical and basal domains of cells, respectively. Although this is the case for intestinal and renal epithelia, the basis for a putative vectorial flux of nucleosides in placenta has not been elucidated yet. To date, only equilibrative nucleoside transporters 1 and 2 (ENT1 and ENT2) have been reported to be expressed in placenta, although their plasma membrane localization is already unclear. Moreover, the occurrence of CNT transporters needed also to be addressed. We have used a variety of approaches, including immunohistochemistry and subcellular localization (basal, heavy and light apical membranes as well as raft enriched membranes from the apical domain) to study ENT and CNT type protein plasma membrane distribution in human placenta. Conversely to other epithelia, we have identified CNT1 expression both at the apical and basal membranes. We have also found a different pattern of CNT1, ENT1 and ENT2 in lipid rafts, which suggests a complex cellular and regional expression of these transporters, which favour the view that the placenta is actually a pyrimidine-preferring nucleoside sink from both maternal and fetal sides.

P4PM-21-9

WNK4 CONTROLS THE TRANSLOCATION OF MEMBRANE TRANSPORTERS VIA INTERACTION WITH SYNTAXIN13

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Introduction: With-No-Lysine(K) kinase 4 (WNK4), a serin/threonin kinase, alters the abundance of transporters at the plasma membrane, but the mechanisms are not yet understood. Here, we report that WNK4 binds to and phosphorylates Sytaxin13 (Syn13). Syn13 is endosomal t-SNARE and it is directly involved in the recycling of plasma membrane proteins. Syn13 mediates a fundamental membrane trafficking event in all cell types. Method: Syn13 IP was performed with full length and truncated WNK4 constructs in HEK293 cells. WNK4 and Syn13 interact through the kinase domain and this binding was not changed by Q562E WNK4 mutation, the cause of PHAII(Pseudo Hypo Aldosteronism II) . But interestingly, we found that WNK4 and Syn13 binding was increased by hyperosmotic stimulation in a dose dependent manner. In addition, In vitro kinase assay showed that WNK4 directly phosphorylated Syn13.

Discussion: Our study will further focus on the effect of hyperosmotic stimulation on WNK4 kinase activity and the role of WNK4 on the formation of SNARE complex with Syn13 and Vamp-2. Eventually, the novel regulatory mechanism of membrane transporter translocation will be uncovered.

P4PM-21-10

GLUCOSE TRANSPORTER 9 (GLUT9) AS A SERUM URATE REGULATOR AND CAUSATIVE GENE FOR RENAL HYPOURICEMIA

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Renal hypouricemia is an inherited disorder characterized by impaired renal urate (uric acid) reabsorption, with severe complications such as exercise-induced acute renal failure. We previously identified *URAT1/SLC22A12*, as a causative gene of renal hypouricemia. However, hypouricemic patients without *URAT1* mutations, as well as genome-wide association studies between urate and *GLUT9/SLC2A9*, imply that *GLUT9* could be another causative gene of renal hypouricemia. With a large health examination database of Japan Maritime Self Defense Force, we identified some loss-of-function heterozygous mutations in *GLUT9*, which resulted in loss of positive charges. The oocyte expression study revealed that both GLUT9 isoforms showed high urate transport activities, whereas the mutated GLUT9 isoforms markedly reduced them. Our findings, together with previous reports on GLUT9 localization, suggest that these *GLUT9* mutations cause renal hypouricemia by their decreased urate reabsorption on both sides of the renal proximal tubules. These findings also enable us to propose a physiological model of the renal urate reabsorption in which GLUT9 regulates serum urate levels in humans and can be a promising therapeutic target for gout and related cardiovascular diseases.

P4PM-21-11

THE MOLECULAR MECHANISMS OF TYPE-2 GLUCOSE TRANSPORTER INHIBITOR-INDUCED APOPTOSIS IN HUMAN HEPATOCELLULAR CARCINOMA CELL

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Phloretin (Ph), a competitive inhibitor of glucose transporter (type II), is detected in apple and different kinds of fruit. In our study, Ph was demonstrated to inhibit tumor cell growth through inhibition of glucose transporter which presented specifically in the liver cancer cells. Our results show that higher concentration of Ph (>150 μM) induce significant apoptosis in the HepG2 cells through both mitochondria- and caspase-dependent pathway. Moreover, Ph-induced apoptosis was significantly enhanced by depletion of glucose in the culture medium. Furthermore, the Hep-G2 tumor cell-xenografted SCID mice were performed to evaluate the Ph-mediated inhibition of glucose transporter. Our result from Micro-PET analysis was shown that Ph-treated tumors significantly inhibit the absorption level of 18-F-FDG. In summary, glucose transporter which specifically presented in the liver cancer cells may act as a molecular target for cancer therapy. Ph is a natural product and can be valuable applied in cancer chemoprevention or therapy.

P5AM-1-1

NITRIC OXIDE BLOCKADE DECREASES CHEMOREFLEX-MEDIATED INCREASES IN SYMPATHETIC ACTIVITY IN HUMANS

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Hypoxic exposure engages chemoreflex-increases in sympathetic activity (MSNA), heart rate (HR) and ventilation (V_E). Nitric oxide (NO) has been implicated both as inhibitory in peripheral chemoreception, and excitatory in the nucleus tractus solitarius (NTS), strengthening the chemoreflex by positive modulation of glutamatergic neurotransmission. We hypothesized that NO-blockade would desensitize the chemoreflex in humans. In healthy subjects (n=10), MSNA (microneurography), intra-arterial pressure (BP), HR, V_E (Cosmed), and blood gasses were measured during graded hypoxic exposure (FiO_2 0.21, 0.13, 0.11, 0.10, 0.09, 0.08, 0.07) before and after NO-blockade (L-NAME, 4 mg*kg-1). The L-NAME-induced increase in blood pressure was counteracted by intravenous nitroprusside. pO_2 were 13,3; 7,4; 5,9; 5,1; 4,7; 4,5; 4,2 before and 13,6; 8,4; 6,6; 6,1; 4,8; 4,5; 4,1 kPa after L-NAME. MSNA were 13; 13; 16; 18; 21; 24; 34 before and 14; 14; 13; 15; 14; 19; 25 bursts*min-1 after L-NAME, $p<0.05$. HR followed the MSNA-pattern, whereas BP- and V_E-changes were similar during the two tests. Thus, NO-blockade desensitizes the sympathetic chemoreflex by increasing the threshold for responsiveness and decreasing response to severe hypoxia. We speculate, this is caused by brainstem NO-blockade in NTS.

Poster Session

P5AM-1-2

AUGMENTED RESPIRATORY-SYMPATHETIC COUPLING CONTRIBUTES TO HYPERTENSION IN THE SPONTANEOUSLY HYPERTENSIVE RAT

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Sympathetic nerve activity (SNA) is increased in hypertension, yet the underlying cause of this is not elucidated. Using the *in situ* perfused working heart brainstem preparation we examined the interaction between neural circuits generating respiratory activity and SNA in spontaneously hypertensive (SH) and normotensive Wistar-Kyoto (WKY) rats. Pulse pressure (PP), phrenic nerve activity (PNA) and thoracic (T8) SNA were recorded simultaneously in male SH and WKY rats between 9 days and 6 weeks of age. Respiratory coupling of sympathetic nerve activity (SNA) is increased in SH rats, at all ages, inducing larger Traube-Herring waves (4.6±1.8 vs 2.3±0.8mmHg in 5 week old SH vs WKY rats; n=5). Baroreceptor denervation did not alter respiratory-sympathetic coupling. Apnoea, induced by a short period of hypocapnia (2% CO_2), caused a decrease in PP and loss of respiratory-related bursts in SNA. Upon restoration of eupnoea and re-emergence of respiratory-related SNA bursting, PP increased significantly more in SH rats (14.8±4.4 vs 4.5±1.7mmHg, $p<0.05$; n=6). Enhanced respiratory SNA coupling in SH rats is reflected in greater arterial tone. We propose that increased respiratory-related bursts of SNA are a causal factor in the development of hypertension.

P5AM-1-3

POTENTIATION OF NMDA RECEPTOR FUNCTION BY AMYLOID BETA-PEPTIDES IN RAT SYMPATHETIC PREGANGLIONIC NEURONS

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Amyloid beta-peptides (Aβ) are considered to participate in the development of Alzheimer's disease. Many studies have reported the vasoactive effects of Aβ on cerebral and peripheral vessels. However, there has been little discussion about the role of Aβ on central control of cardiovascular function. We examine the effects of Aβ on the function of neurotransmitter receptors especially NMDA receptors in rat sympathetic preganglionic neurons (SPNs). In the in vitro electrophysiological study, consecutive applications of NMDA every 5 min induced reproducible membrane depolarizations in SPNs of neonatal rat spinal cord slice preparation. Superfusion of Aβ 1-40 (0.1 and 0.3 μM) for 5 min, which caused no change of membrane potentials, significantly potentiated NMDA-mediated depolarizations in a reversible manner. The potentiated effects reached the peak at 10-20 min and lasted for over 60 min. Aβ 1-40 (0.3 μM) had no significant effects on AMPA-induced depolarizations or GABA-induced hyperpolarizations. The results suggest a selective potentiation of Aβ 1-40 on NMDA receptor function in SPNs. The mechanism underlying the potentiated effects of Aβ on NMDA receptor function will be clarified.

P5AM-1-4

VISCERAL AND SOMATIC INPUTS CONVERGE ON TO THE SUPERIOR SALIVATORY NUCLEUS NEURONS IN ANAESTHETIZED RATS

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We investigated whether preganglionic superior salivatory nucleus (SSN) neurons projecting submandibular and sublingual ganglia receive visceral and somatic inputs in urethane-chloralose anaesthetized rats. Single SSN neurons were identified by their antidromic spike responses following stimulation of preganglionic fibres. In each identified SSN neurons, ECG/ABP-triggered and repspiratory cycle-triggered correlation histograms of the activity were constructed in their excitatory amino acid receptor agonist (AMPA, NMDA or DLH) induced activity. Out of 32 SSN neurons tested, about half of SSN neurons were found to exhibit a pulse-related activity in ECG/ABP-triggered correlation histogram, suggesting receiving cardiac afferent inputs. Many of them were also found to exhibit respiratory-related activity in respiratory cycle-triggered correlation histogram, suggesting respiratory afferent inputs. Some of them also increased their excitatory amino acid activity by stimulation of lingual nerve, suggesting receiving lingual somatic inputs. These findings suggest that cardiac and respiratory afferent inputs and lingual trigeminal somatic inputs converge on to single SSN neurons, and these excitatory inputs are possibly involved in spontaneous salivation.

P5AM-1-5

EFFECT OF PHASE COUPLING BETWEEN CARDIORESPIRATORY AND LOCOMOTOR RHYTHMS ON PULMONARY GAS EXCHANGE AND MUSCLE OXYGENATION IN HUMANS

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The objective of this study was to test the hypothesis that, in humans, phase coupling between cardiac, respiratory and locomotor rhythms (CRLC) during exercise may be associated with improvements in efficiency of pulmonary gas exchange and exercising muscle perfusion. To this end, we examined whether the ventilatory equivalent for O_2 (V_E/Vo_2) and total hemoglobin change in gastrocnemius muscle measured by near-infrared spectroscopy were altered with the occurrence of CRLC during walking on a treadmill in 8 healthy volunteers. For inducing CRLC, the subjects walked at a paced rhythm with listening a cue signal generated at a frequency of mean heart rate (120 beats/min) while their breathing rhythm was either synchronized to their stepping rhythm or controlled at a constant rate of 30 breaths/min. Voluntarily synchronization of breathing rhythm to stepping rhythm enhanced the rate of CRLC occurrence. The V_E/Vo_2 was significantly decreased in CRLC condition compared with that in the desynchronized state ($P<0.01$). Changes in total hemoglobin concentration in gastrocnemius muscle were greater during CRLC ($P<0.05$). These findings suggest that CRLC may contribute to maintenance of the microvascular perfusion in exercising muscles, which may enhance muscle oxidative metabolism.

P5AM-1-6

INCREASE IN RETICULOCYTE COUNT AFTER 2 WEEKS OF APNE TRAINING

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Physiological characteristics of apnea divers may be the result of training or predisposition. Haemoglobin concentration (Hb) was higher in apnea divers than in other athletes and in untrained controls (de Bruijn et al 2008a), and serial apneas increased circulating erythropoietin (de Bruijn et al 2008b). We aimed to study if apnea training increases erythropoiesis in previously untrained subjects. Four women and 6 men performed 10 apneas daily, in two series of 5 maximal effort apneas spaced by 2 min rest. Apneas were preceded by 1 min hyperventilation but interrupted if SaO_2 reached 60%. Before and after the training, Hb and reticulocyte count were measured in venous blood samples obtained after 20 min of horizontal rest. Reticulocyte count had increased from 41.7 to 48.0 x 109/L (by 15%; $P=0.05$) after training, while Hb remained unchanged at 131 g/L. We conclude that an enhanced erytropoiesis may be induced by apnea training, which could explain the previously observed high Hb in divers after long term training. The 2 week training time may be to short to elevate Hb.

P5AM-1-7

HEMOGLOBIN CONCENTRATION AND PERFORMANCE IN ELITE APNEIC DIVERS OF BOTH GENDERS

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Several features characterize apnea divers, but their influence on diving performance often remains to be established. A previous study revealed that hemoglobin concentration (Hb) in male apnea divers was higher than in untrained controls and in elite skiers (deBruijn et al 2004). The present aim was to study Hb in elite apnea divers of both genders and to reveal any correlation between Hb and results in the World Championship of Apnea in Egypt 2008. Twelve female and 11 male divers participated in this study. Hemoglobin was measured after 5 min sitting rest on capillary samples analyzed in triplicate on a Hemocue Hb analyzer. Total competition scores, i.e. the accumulated points from dives of maximal depth, time and distance, were compared with Hb. The mean (SD) Hb was 149(11) g/L among males and 130(6) among females ($P<0.001$). Pearson correlation analysis on separated genders revealed no correlation between Hb and total score obtained in the competition. The Hb was in the range reported for healthy adults. High Hb could possibly be masked by heat induced plasma expansion by the 1-2 weeks spent by most competitors in the hot Sinai desert.

P5AM-1-8

CENTRAL V1 RECEPTORS INVOLVEMENT IN REGULATION OF CARDIOVASCULAR RESPONSES TO CHRONIC AND ACUTE STRESS IN RATS WITH THE MYOCARDIAL INFARCT

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The purpose of the study was to elucidate whether chronic mild stressing influences magnitude of the cardiovascular responses to acute stress, whether this effect is mediated by central vasopressin (VP) V1 receptors, and whether it differs in the myocardial infarcted and not infarcted rats. Sprague Dawly infarcted and sham operated rats were either exposed to 4 weeks of mild stressing (small cage, strobe light, cage tilt, water deprivation, empty water bottle, presentation of another rat) or stayed resting. Effects of chronic blockade of central V1 VP receptors with [deamino-Pen1,O-Me-Tyr2, Arg8]-Vasopressin was also investigated in these experimental paradigms. After 4 weeks of stressing arterial blood pressure and heart rate were determined and the rats were subjected to acute (air jet) stress. The results revealed that: 1) chronic stressing enhances cardiovascular responses to subsequent acute stress in the infarcted and not infarcted rats, 2) V1 VP receptors are engaged in regulation of resting blood pressure during chronic stressing and in intensification of cardiovascular responses to acute stress in the infarcted and not infarcted chronically stressed rats. The study was supported by the Medical University of Warsaw and Ministry of Education and Science (2P05A 182 29).

P5AM-1-9

EFFECTS OF ESTROGEN REPLACEMENT ON OXIDATIVE STRESS IN VASCULATURE RELATED TO PSYCHOLOGICAL STRESS IN OVARIECTOMIZED RATS

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We examined whether acute psychological stress enhance oxidative stress in plasma and vasculature, which is restored by estrogen replacement in ovariectomized rats. We used nitrotyrosine (NT) and 4-hydroxy-2-nonenal (HNE) as oxidative stress markers. Female rats aged 9 wk were divided randomly into three groups: sham-operated (S), OVX + placebo-treated (P) and OVX + estrogen-treated (E) groups. The rats in the P or E group were ovariectomized and implanted with pellets containing either placebo or 17β-estradiol 4 wk after ovariectomy. Rats aged 16 wk were catheterized for blood sampling. Six days post-surgery, rats were exposed to cage-switch for 30 min. Blood samples during the stress and the recovery were measured of the plasma concentrations of glucose, nitric oxide metabolites (NOx), nitrotyrosine (NT). The resting level of NOx in E group was higher than that in S or P groups, which was reduced by psychological stress. In aorta, mesentery and lower limb muscles dissected from the rats just after the stress, we detected NT or HNE-modified proteins by western blotting, which was different from each other group. These results suggest that estrogen possibly affect oxidative stress related to psychological stress.

P5AM-1-10

EFFECT OF RESISTIN ON BLOOD PRESSURE REGULATION IN RAT

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Resistin has been proposed as a link between obesity and insulin resistance and impaired insulin stimulated eNOS activation in vitro. However, the biological function on cardiovascular system of resistin in blood pressure in vivo is not clear. We hypothesized that resistin may cause imbalance between ET-1 and NO in vivo. The SD rats would be infused with resistin for 3 hours. One hour after resistin infusion, rats would be challenged with bradykinin, SNP or ET-1. Results showed that response of bradykinin on vasorelaxation was not changed in resistin-infused group compared with the controls. However, resistin infusion accelerated cardiovascular recovery response to SNP and delayed cardiovascular recovery response to ET-1. The levels of IRβ and Akt phosphorylation were significantly decreased in skeletal muscle, adipose tissue and aorta in resistin infused rats compared to controls. After 1h-infusion, plasma glucose, insulin, resistin, and insulin resistance were significantly increased in resistin infused rats compared to controls. In conclusion, acute resistin infusion caused an imbalance on ET-1 and NO actions through interfering cardiovascular recovery and impaired insulin signaling pathway in skeletal muscle, adipose tissue and aorta.

P5AM-1-11

ADENOSINE A2a RECEPTOR ACTIVATION ELICITS A COX- AND eNOS-INDEPENDENT REDUCTION IN BLOOD PRESSURE IN CONSCIOUS MICE

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Adenosine plays an important role for the regulation of heart rate (HR) and vascular reactivity. However, the mechanisms involved in the effect on mean arterial blood pressure (MAP) are unclear. Therefore we investigated the effect of the nucleoside on blood pressure in conscious mice. Chronic indwelling catheters were placed in femoral artery and vein in C57Bl/6J (Wt) and endothelial nitric oxide knock-out ($eNOS^{-/-}$) mice and continuous measurements of MAP and HR were conducted. Bolus infusion of 0.5 mg/kg adenosine elicited a significant transient decrease in MAP (99.3±2.3 to 63.3±3.8 mmHg) and HR (603.2±18.3 to 429.1±27.1 min^{-1}). Activation of adenosine A2a receptors with CGS 21680 (0.02 mg/kg) caused a significant reduction in MAP from 99.6±1.2 to 64.6±3.2 mmHg. The reduction in MAP observed after adenosine or CGS 21680 administration were not significantly different in Wt compared to $eNOS^{-/-}$. Finally, inhibition of cyclooxygenases (COX) by indomethacin did not change the CGS 21680 elicited blood pressure decrease. We conclude that adenosine and selective stimulation of the adenosine A2a receptor result in an immediate, transient COX- and eNOS-independent MAP reduction.

P5AM-1-12

EFFECTS OF ANGIOTENSIN II ON OPEN-LOOP CAROTID SINUS BAROREFLEX FUNCTION

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Although angiotensin II (ANG II) is considered to attenuate the arterial baroreflex, experiments under baroreflex closed-loop conditions make the interpretation of experimental results difficult. To circumvent the feedback effects, we isolated the carotid sinuses and examined the effects of intravenous ANG II (10 μg kg^{-1} h^{-1}) on the input-output relationship between carotid sinus pressure (CSP) and efferent sympathetic nerve activity (SNA) and that between SNA and systemic arterial pressure (AP) in anesthetized Sprague-Dawley rats (n=8). CSP was changed stepwise from 60 to 180 mmHg with increments of 20 mmHg. The CSP-SNA relationship approximated a 4-parameter logistic function. ANG II increased the response range from 65.7±6.3 to 77.9±7.3 (normalized units, mean±SE, P=0.008) with a marginal increase in the minimum SNA (35.7±5.7 to 56.9±11.7, P=0.057). The SNA-AP relationship approximated a straight line. ANG II increased the intercept from 34.6±5.1 to 69.0±9.3 (mmHg, P=0.002) with a marginal decrease in the slope (0.89±0.07 to 0.66±0.08, mmHg/normalized units, P=0.055), In conclusion, intravenous ANG II of a pressor dose increased SNA at a given CSP but did not decrease the response range of SNA.

P5AM-1-13

THE BALANCE OF MUSCLE SYMPATHETIC NERVE ACTIVITY AND ADRENERGIC VASCULAR RESPONSIVENESS IS IMPAIRED WITH AGING

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Muscle sympathetic nerve activity (MSNA) increases with aging and is a major contributor to systemic vascular resistance. High resting MSNA in young normotensive men is balanced by reduced vascular responsiveness to norepinephrine (NE). *The goal of this study was to assess whether aging alters this balance of MSNA and vascular responsiveness*. Healthy older men (age 50-72, n=15) participated. Young men with the lowest resting MSNA had the strongest vasoconstrictor response (i.e., largest % decrease in forearm blood flow (FBF)) to infused NE and had a positive correlation of MSNA to % decrease in FBF to NE (r=0.82, p=0.001). In contrast, in older men this relationship is abolished (r=0.42, p>0.05). Similarly, in response to tyramine (6 mcg 100 ml-1 min-1) young men had a significant positive correlation (r=0.55, p=0.04) between MSNA and % decrease FBF to NE while in older men this relationship is abolished (r=0.10, p>0.05). In summary, there is a balance of MSNA to adrenergic vasoconstriction in young men that is abolished with aging. This loss of balance could contribute to increased risk for hypertension with aging since high MSNA is no longer balanced by a decrease in adrenergic responsiveness.
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P5AM-1-14

ESTIMATION METHOD OF CENTRAL SYSTOLIC PRESSURE FROM PERIPHERAL PULSE WAVES WITHOUT USING GENERALIZED TRANSFER FUNCTION IN RABBITS

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We attempted to estimate central aortic systolic pressure (cSBP) from peripheral pressure waves without applying generalized transfer function in normal and Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits aged 12 months. Two catheter-tip transducers (2Fr) were advanced to the ascending aorta (AA) and distal end of the right brachial artery (BrA) through the right common carotid and radial arteries, respectively under pentobarbital anesthesia. Changes in pressure waves in response to intravenous infusion of angiotensin II and sodium nitroprusside were simultaneously recorded in AA and BrA. Pressures at the first (SBP) and second (SBP_2) systolic peaks of brachial pressure waves, and average of SBP and SBP_2 (SBP_m) were strongly correlated with cSBP in the two rabbit groups. When the difference between SBP_m and cSBP was plotted against their average in Bland-Altman plot, instead of plotting the difference between SBP_2 and cSBP against their average, the mean difference was almost zero and its standard deviation was considerably smaller in the two rabbit groups. We conclude that SBP_m at the brachial artery could be a precise estimate of cSBP independently of the presence of atherosclerosis and of vasoactive drugs.

P5AM-1-15

THE EFFECTS OF SLEEP BRUXISM ON BAROREFLEX CONTROL OF THE CIRCULATION IN HUMANS

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We have reported in awake human subjects that isometric biting evoked pressor responses; increased heart rate (HR), arterial blood pressure (BP) and muscle sympathetic nervous activity, which was accompanied by a reduction in baroreflex sensitivity (BRS, FASEB J, 21(6), A571, 2007). We examined whether these responses occurred also during sleep bruxism (SB) and, if so, whether the responses were altered when sleep deepened. We determined sleep stage by polysomnography while measuring HR by electrocardiogram and beat-by-beat BP with Finometer in 12 young subjects who slept over night for 6 hrs in a test room. We determined BRS from HR response to spontaneous change in systolic BP and also an index of sympathetic nervous activity (SNA) from low-frequency / high-frequency component of HR variability. We found that systolic BP, HR, and SNA decreased while BRS increased as sleep stage increased ($P < 0.05$). However, whenever SB for 3.5 min on average occurred, systolic BP, HR, and SNA increased and BRS decreased to their awake levels in any stages of sleep (all, $P < 0.05$). These responses were greater than those by isometric biting in awake subjects ($P < 0.05$). Thus, pressor responses were evoked by SB, accompanied by a reduction in BRS, and enhanced as sleep deepened.

P5AM-1-16

EFFECTS OF β-ADRENERGIC BLOCKERS ON ANAPHYLACTIC HYPOTENSION IN CONSCIOUS RATS

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Anaphylactic shock is sometimes fatal in the patients treated with β-adrenergic antagonists. However, it is not known which subtype of β-adrenergic receptor (AR), β_1- or β_2-AR, is primarily responsible for the fatal outcome. Effects of β_1- and β_2-AR antagonists were determined on the survival time of the conscious Sprague-Dawley rats that suffered from the ovalbumin-induced anaphylactic shock. The control rats showed systemic hypotension along with portal hypertension, but did not die within 48 hrs after antigen. The survival time of the rats pretreated with the nonselective β-AR of propranolol (1mg/kg; n=7), selective β_1-AR of atenolol (2mg/kg; n=7), and selective β_2-AR of ICI118,551 (0.5mg/kg; n=7) were 17±2 (SE), 22±3, and 49±12 min, respectively. Injections of epinephrine (3μg/kg) at 3 and 5 min after antigen improved the survival time for the propranolol and ICI groups, and prevented death for the atenolol group. Furthermore, β_2-AR agonist, fenoterol (0.25mg/kg), inhibited fatal outcome in the ICI and atenolol groups. In conclusion, in rat anaphylactic shock, inhibition of β_2-adrenergic receptors causes more detrimental actions than that of β_1-adrenergic receptors.

P5AM-1-17

PATHOPHYSIOLOGY OF ANAPHYLACTIC HYPOTENSION IN RATS

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The hemodynamic mechanisms for anaphylactic shock remain unclear. We here presented the hemodynamic characteristics of anaphylactic shock of rats with emphasis on hepatic venoconstriction. In anesthetized rats sensitized with ovalbumin, an intravenous injection of the antigen caused not only a decrease in systemic arterial pressure from 120 to 43 mmHg but also an increase in portal venous pressure which persisted for 20 min after the antigen (the portal hypertension phase). The elimination of the splanchnic vascular beds, by the occlusions of the celiac and mesenteric arteries, combined with total hepatectomy attenuated anaphylactic hypotension during the portal hypertension phase. The isolated perfused liver experiment revealed the antigen-induced predominant presinusoidal constriction, and liver weight loss. The hepatic blood volume reduction was also confirmed in the antigen-injected anesthetized rats by measuring the hepatic volume with a use of ultrasonic crystals. Furthermore, we demonstrated that cysteinyl leukotrienes and cyclooxygenase products, but not PAF, are mainly involved in anaphylactic hepatic venoconstriction in isolated perfused rat livers. In conclusion, hepatic venoconstriction plays a significant role in rat anaphylactic hypotension.

P5AM-1-18

BLOOD PRESSURE RESPONSES TO THE ELECTRICAL STIMULATION OF THE BONE IN ANESTHETIZED RATS

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It has been shown that noxious cutaneous stimulation reflexively increased blood pressure via somatic afferent and sympathetic vasoconstrictor nerve activation. In the present study, we investigated the effects of bone stimulation on blood pressure and the neural mechanisms involved.

Male Wistar rats were anesthetized with pentobarbital and artificially ventilated. Two small holes 3-4 mm apart were manually drilled into the femur down to the bone marrow. Two stainless needles were inserted into the holes, and an electrical square wave current was passed between the needles (0.5ms, 20 Hz, 1-10 mA, for 20 s). Electrical stimulation of the femur at 5 and 10 mA produced an intensity-dependent decrease in blood pressure. This response was abolished by severance of the femoral and sciatic nerves ipsilateral to the stimulation. Furthermore, the renal sympathetic efferent nerve activities (as a representative index of vasoconstrictor activities) decreased following the stimulation. These results suggest that noxious electrical stimulation of the femur reflexively decreased blood pressure. It can be inferred that the afferent nerve pathway is the somatic afferent nerves, and the efferent nerve pathway is the sympathetic vasoconstrictor nerve.

P5AM-1-19

SYMPATHETIC NEURAL_HAEMODYNAMIC BALANCE IN AGING HUMANS: IMPLICATIONS FOR BLOOD PRESSURE REGULATION

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We tested the hypothesis that the balance among muscle sympathetic nerve activity (MSNA), cardiac output (CO) and total peripheral resistance (TPR), which is important in regulating blood pressure (BP) in young men, is altered with aging. Multi-unit recordings of MSNA were obtained in 14 young men (26 ± 1 yr) and 15 older men (61 ± 2 yr). CO was measured via acetylene rebreathing and BP was recorded via a brachial catheter. Resting MSNA was lower in the young men (41 ±3 bursts 100hb-1) compared to the older men (66 ± 4 bursts 100hb-1, $P<0.05$). However, diastolic blood pressure (DBP) was similar in the young and older men (93 ± 2 mmHg vs. 90 ± 2 mmHg). As expected, in the young men MSNA was not related DBP ($r=-0.07$), but trended towards a positive correlation in the older men ($r=0.45$, $P=0.09$). In the young men MSNA was positively related to TPR ($r=0.51$, $P<0.05$) and inversely related to CO ($r=-0.45$, $P<0.05$). Unexpectedly, MSNA was not related to TPR ($r=-0.22$) or CO ($r=0.16$) in the older men. In summary, it appears that healthy older men do not rely on TPR and CO to balance higher levels of MSNA and must rely on other integrated physiological mechanisms to maintain a normal BP.

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Poster Session

P5AM-1-20

CRITICAL CLOSING PRESSURE OF THE CEREBROVASCULAR CIRCULATION DURING DYNAMIC EXERCISE

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The aim of the present study was to calculate the critical closing pressure (CCP) of the cerebral vasculature at rest and during exercise, as a method by which the changes in cerebral vascular tone can be identified. Seven subjects were seated upright at rest for fifteen minutes and then performed fifteen minutes of right legged knee extension exercise. The middle cerebral artery blood velocity (MCA V) and arterial blood pressure in the radial artery were continuously recorded. In the right MCA the CCP was increased during heavy (75% maximal workload) exercise (17.7 ± 4.2 to 22.2 ± 4.1 mmHg, P=0.027), while CCP of the left MCA was unchanged from rest (P=0.103). In addition, the increases in CCP of the right MCA were related to the increases in plasma norepinephrine concentrations (P=0.022), but not to changes in mean arterial pressure (P=0.704) or the partial pressure of arterial carbon dioxide (P=0.353). We conclude that the exercise induced increase in cerebral vascular tone would serve to protect the blood brain barrier from the exercise induced-hypertension. In addition, the exercise induced increase in cerebral vascular tone was associated with increases in cerebral neural activity and modulated by cerebral metabolism.

P5AM-1-22

PERINATAL TAURINE DEPLETION DECREASES BAROREFLEX SENSITIVITY INDEPENDENT OF ESTROGEN RECEPTORS IN ADULT FEMALE RATS

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Perinatal taurine status influences the autonomic control of arterial pressure in adult offspring. This study tests the hypothesis that perinatal taurine depletion decreases baroreflex sensitivity via estrogen receptors in adult female rats. Female Sprague-Dawley rats were either taurine depleted (beta-alanine 3% in tap water, TD) or untreated control from conception until weaning. Their female offspring were then fed normal rat chow and tap water throughout the study. At 7-8 weeks of age, blood chemistry and cardiovascular parameters were measured in conscious and unconscious conditions with or without an estrogen receptor blockage (tamoxifen 10 mg/kg/day for 5 days). Plasma sodium, potassium, creatinine, blood urea nitrogen, hematocrit, fasting blood glucose, mean arterial pressure, and heart rate were not significantly different among groups. Compared to control, baroreflex sensitivity control of heart rate and renal nerve activity were blunted in TD without tamoxifen or control rats with tamoxifen. Tamoxifen pretreatment did not alter baroreflex sensitivity in the TD rats. These results suggest that perinatal taurine depletion impairs baroreflex sensitivty independent of estrogen receptors.

P5AM-1-23

DIFFERENTIAL SHIFTS IN BAROREFLEX CONTROL OF SYMPATHETIC OUTFLOWS DURING 2-DEOXY-D-GLUCOSE-INDUCED GLUCOPRIVATION IN CONSCIOUS RATS

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The present study examined a potential role of arterial baroreflex in causing the differential responses of renal (RSNA) and lumbar sympathetic nerve activity (LSNA) and heart rate (HR) observed during 2-deoxy-D-glucose(2DG)-induced glucoprivation in conscious rats. Male Wistar rats were chronically instrumented with electrodes for measurements of RSNA, LSNA, and electrocardiogram, and with arterial and venous catheters. At least three day after the surgery, 2DG (750mg/kg) was administrated intravenously following a 60 min control period. The baroreflex curve for RSNA, LSNA and HR was determined by changing systemic arterial pressure (Pa) using rapid intravenous infusion of vasoactive drugs. Pa-RSNA baroreflex curve was shifted rightward at 10 min after the 2DG administration and then the magnitude of shift in the Pa-RSNA curve was diminished at 120 min after the 2DG administration. The Pa-LSNA baroreflex curve did not change significantly compared with that obtained during control period. The Pa-HR baroreflex curve shifted progressively downward after the 2DG administration. These results indicate that the glucoprivation induced by 2DG administration results in acute shifts in baroreflex curve for RSNA, LSNA and HR in a regionally different and time dependent manner.

P5AM-2-1

NEURONAL NITRIC OXIDE SYNTHASE IN EPIDERMIS IS INVOLVED IN CUTANEOUS CIRCULATORY RESPONSE TO MECHANICAL STIMULATION

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The source of nitric oxide (NO) in the cutaneous circulation remains controversial. We hypothesized that epidermis might generate NO in response to mechanical stimulation. In hairless mouse (HR-1) skin organ culture, various mechanical stimulations such as temperature and weight resulted in NO release, which declined within 30 minutes after cessation. Similar NO release occurred from a reconstructed skin model containing only keratinocytes and fibroblasts, and was suppressed after detachment of the epidermal layer. Mechanical stimulation of skin organ cultures of HR-1 mice caused enlargement of cutaneous lymphatic vessels. The enlargement was significantly lower after detachment of the epidermal layer than for normal skin samples. These results are consistent with the idea that NO generated by epidermis plays a significant role in the cutaneous circulatory response to mechanical stimulation.

P5AM-2-2

EFFECT OF OF ANGIOTENSIN(1-7) ON THE EXPRESSION OF E-SELECTIN INDUCED BY ANGIOTENSIN II AND ITS MECHANISM IN VASCULAR ENDOTHELIAL CELL

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Object To observe the effect of angiotensin-(1-7)[Ang-(1-7)]on the expression of E-selectin(Es) induced by angiotensinII(AngII)in HUVECs and its mechanism on the expression of Es induced by AngII.Methods HUVECs were cultured in DMEM.The cell activity was determined by MTT assay. Es antigen was measured by ELISA. Es mRNA was examined by RT-PCR.Results A gradual increase in Es antigen and Es mRNA were observed in HUVECs stimulated with increasing concentration of AngII(10-10~10-6mol/L)(r=0.965,P<0.05),and peaked at 10-7mol/L. Ang-(1-7)(10-9~10-6mol/L) alone could not affect the expression of Es in HUVECs(P>0.05). When pretreated with Ang-(1-7)(10-9~10-6mol/L),Ang-(1-7) could inhibited the expression of Es antigen and mRNA induced by AngII in dose-dependent manner (r=-0.943,P<0.05),and 10-6mol/Lwas the strongest concentration. Ang-(1-7) at 10-6mol/L decreased Es antigen in HUVECs in a time dependent manner, reaching a maximum level after 4h. L-NAME alone,which is the inhibitor of NOS,had no marked effects on Es antigen and Es mRNA in HUVECs,but L-NAME significantly inhibited the effects of Ang-(1-7) on Es expression induced by AngII(P<0.05).Conclution AngII could induce the expression of Es in vascular endothelial cells and Ang(1-7) inhibited this effect at mRNA level and NO pathway maybe participates in the inhibitory effect of Ang (1-7) on the expression of Es induced by AngII.

P5AM-2-3

CONTRAST ANGIOGRAPHY OF THE RAT RENAL MICROCIRCULATION IN VIVO USING SYNCHROTRON RADIATION

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We have developed a new method for contrast microangiography of the rat renal circulation using synchrotron radiation. The method was applied to determine responses of the renal arterial vasculature to angiotensin II (ANG) and electrical stimulation of the renal nerves (RNS). Iodinated contrast agent was administered directly into the renal artery of pentobarbital anesthetized rats before and during (i) intravenous infusion of ANG (1.6 μg /kg/min) or (ii) its vehicle, or (iii) RNS at 2 Hz. Vascular caliber was determined using a newly developed algorithm. Up to 4 levels of branching could be observed simultaneously along the arterial tree (resting diameter of 28-400 μm). Vessel diameter was not significantly altered by vehicle infusion (+3.1 ± 3.5% change) but was significantly reduced by ANG (-24.3 ± 3.4%) and RNS (-17.1 ± 3.8%). ANG-induced vasoconstriction was independent of vessel size, but RNS-induced vasoconstriction was greatest in vessels with a resting caliber of 100-200 μm and least in vessels with a resting caliber <40-100 μm. In conclusion, the method we describe herein provides a new approach for assessing renal arterial responses to vasoactive factors along several orders of branching.

P5AM-2-4

ATRIAL NATRIURETIC PEPTIDE (ANP) BLUNTS POST-PRANDIAL VASODILATATION IN CONSCIOUS DOGS, PARTICULARLY AFTER SALTY FOOD

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A little-known action of ANP is to cause selective vasoconstriction in the upper gastrointestinal tract. The physiological significance of this is unknown. To determine whether ANP affects normal post-prandial vasodilatation, and whether ingested salt is a relevant factor, trained greyhound dogs (n=5) were instrumented with a cranial mesenteric blood flow (MBF) probe and allowed to recover fully. On each experimental day, the dog consumed one of 3 identical meals (7% protein, 6% fat and 16% carbohydrate) with no added salt (LS), 1g added salt (NS) or 21g added salt (HS), in the presence or absence of an ANP infusion (40 ng/kg/min). Post-prandially, MBF rose progressively, reaching a maximum at ~60min (e.g., 383 ± 51 to 743 ± 101 ml.min $^{-1}$ after LS meal). After HS meal, the MBF response occurred earlier than with LS or NS but the maximum increase in MBF was similar. ANP infusion always reduced resting MBF (~20%) and the maximum post-prandial MBF rise (by ~20%; $P < 0.05$). After HS meal only, ANP reduced the rate of rise in MBF (160 ± 54 *vs* 44 ± 20 ml.min $^{-1}$ /min, $P < 0.05$). These findings directly implicate, for the first time, a role for the cardiac hormone, ANP, in the physiological responses to salty food.

P5AM-2-5

BETA-2 ADRENERGIC RECEPTOR HAPLOTYPE AND FOREARM VASODILATOR RESPONSE TO SYSTEMIC TERBUTALINE DURING GANGLIONIC BLOCKADE

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Beta-2 adrenergic receptor gene (ADRB2) polymorphisms influence vasodilation, but studies comparing regional blood flow with systemic vasodilation conflict, possibly due to counter-regulatory baroreflexes during systemic B-agonist, or interactions between SNPs at positions 16 and 27. We determined the forearm blood flow (FBF) response to systemic B-2 agonist terbutaline (TRB) while baroreflexes were inhibited during ganglionic blockade (trimethaphan, TMP) in healthy adults homozygous for ADRB2 haplotypes (n=45, mean age±SE 27±1, BMI 24±0.3, 28 female). Groups were homozygous for Arg16+Gln27 (n=12), Gly16+Gln27 (n=8), and Gly16+Glu27 (n=25). Following placement of an IV catheter (drug infusion), brachial arterial catheter (BP), and forearm plethysmography (FBF), TMP (3-7 mg/min) was titrated until baroreflex control of HR was abolished. To counter the fall in BP during TMP, phenylephrine infusion was titrated to restore BP to pre-TMP levels. TRB was infused at 33 and 67 ng/kg/min for 15 min each. From pre-TRB baseline, there was no evidence to suggest that the change in FBF, forearm conductance, or resistance was different based on haplotype. We conclude that during baroreflex inhibition, ADRB2 haplotype does not influence FBF during systemic B-agonist.

P5AM-2-6

ENDOTHELIAL TRANSIENT RECEPTOR POTENTIAL VANILLOID CHANNEL 1 AND SMALL CONDUCTANCE POTASSIUM CHANNEL STRENGTHENED ADHESION OF MONOCYTE

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The process of migration of monocyte through vascular endothelial cell layer has been started from strong adhesion of monocyte to endothelial cells. In this study, effects of ionic transmittance through endothelial cell on monocyte adhesion were studied by electrophysiological technique. We focused on transient receptor potential vanilloid channel 1 (TRPV1) and small conductance potassium (SK) channels that are expressed on endothelial cells. Whole cell current was continuously recorded from human umbilical vein endothelial cell (HUVEC) by patch electrode. Monocyte (THP-1) application induced 2 phase change of current, 1st, transient inwardly, 2nd, continuous outwardly. Transient inward current was not recorded with 50nM SB366,791, selective TRPV1 channel antagonist, and continuous outward current was decreased by 100nM apamin, SK channel antagonist. Furthermore, both SB366,791 and apamin significantly decreased the number of monocyte adhered to HUVEC (control: 231±38, SB366,791: 96±16, apamin: 108±26 cells/mm^2). These results suggested that inward calcium current via TRPV1 channels and outward potassium current via SK channels of endothelial cells have facilitated adhesion strength between monocytes and endothelial cells.

P5AM-2-7

STRETCH-INDUCED UP-REGULATION OF SOC ACTIVITIES IN HUVEC

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We have studied about molecular identities of stretch-activated (SA) channels and the mechanism of Ca^{2+} influx evoked by mechanical stretch in human umbilical vein endothelial cells (HUVECs). Previously, we showed that a targeting suppression of transient receptor potential 2 (TRPV2) protein expression in HUVEC using a TRPV2-specific morphorino-oligo completely blocked a transient increase of intracellular Ca^{2+} in response to stretch through the activation of SA channels. Here, we examined the remodeling of Ca^{2+} responses evoked by uni-axial cyclic stretch in HUVEC by fura2 fluorescence imaging. Before and after the cyclic stretch for 15 min, a magnitude of single stretch-evoked Ca^{2+} transient did not change. However, the Ca^{2+} influx through the store-operated Ca^{2+} channels (SOCs) was significantly increased after the 15min- cyclic stretch. On the other hands, TRPV2-knocked down HUVECs suppressed the increased SOC activities and caveolae formation after cyclic stretch. Such the up-regulation of SOC activities through stretch-dependent TRPV2 activation might contribute to sustained intracellular Ca^{2+} increase, which is thought to be a primary etiology of the vascular remodeling, and a potent risk factor of pressure-dependent hypertrophic diseases.

P5AM-2-8

EFFECT OF TRPA1 BLOCKADE ON THE MUSCLE REFLEX RESPONSE MEDIATED BY PHOSPHATE

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Transient receptor potential A1 channels (TRPA1) are cation channels found preferentially on nociceptive sensory neurons. Our recent data suggested that TRPA1 located on muscle afferents plays a role in activating the muscle reflex, a sympathoexcitatory drive originating from contracting muscle. It remains unknown what are stimulants/mediators of TRPA1 during contraction. The present study tested the effect of TRPA1 blockade on the muscle reflex response mediated by diprotonated phosphate, hydrochloric acid, and lactic acid. In decerebrate rats, diprotonated phosphate (pH=6.0; 86 mM; 0.1 ml) was intra-arterially injected into hindlimb muscle circulation (n=9), and led to a 52% increase in renal sympathetic nerve activity (RSNA). This response was significantly ($P<0.05$) reduced by intra-arterial injection of HC-030031 (3 mg), a TRPA1 selective blocker, by 45%. TRPA1 blockade had no significant effect on the increase in RSNA seen when hydrochloric acid (12.5 mM; 0.1 ml, n=7) or lactic acid (20 μM; 0.2 ml, n=6) was injected. We suggest that diprotonated phosphate which is increased by muscle contraction may stimulate TRPA1 located on muscle afferents. Supported by AHA BGIA 0865416D (Koba).

P5AM-2-9

EFFECT OF GINSENOSIDES ON RHEOLOGICAL FUNCTIONS OF ERYTHROCYTES AGAINST OXIDATIVE STRESS

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We study to evaluate the effects of iron-induced oxidative stress and the protective effects of the extract from Panax ginseng against oxidative damage on rheological properties of erythrocytes. Using the rheological properties of erythrocytes as an index, we have screened the components of Panax ginseg extract and identified Rg2 and Rh1 as the active ingredients. These two ginsenosides prevented the oxidative stress-induced elevation of erythrocyte suspension viscosity and the impairment of erythrocyte deformability. Rg2 and Rh1 ginsenosides did not have antioxidant activity in aqueous phase and did not inhibit the peroxidation of membrane lipids, either. However, they inhibited the oxidation-induced derease of thiol-group in Band 3 (anion-exchanger-1), one of the important structural porteins of erythrocyte membrane, but not in other structural proteins: band 1 & 2 (spectrin), band 4.1 or band 5 (actin). These results suggest that ginsenosides Rg2 and Rh1 protect the rheological functions of erythrocytes against oxidative stress by preventing the oxidation of thiol-group in band 3 protein.

P5AM-2-10

EXERCISE TRAINING IMPROVES IMMUNE RESPONSE BY UPREGULATING MKP-1 IN SYSTEMIC INFLAMMATORY MOUSE MODEL

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Chronic exercise modulates immunity with unknown mechanisms. Mitogen-activated protein (MAP) kinase mediates the production of inflammatory cytokines, including TNF-α and IL-6, whereas MAP kinase phosphatase (MKP)-1 plays an essential role in intracellular homeostasis by negatively regulating macrophage MAP kinase activation under inflammatory conditions. Since our preliminary human microarray study indicated that leukocyte MKP-1 mRNA was elevated by exercise training, we hypothesized that chronic exercise might prevent excessive inflammatory responses by upregulating macrophage MKP-1. To verify this hypothesis, we examined the immune responses in sedentary or 8-wk treadmill exercise-trained male C57BL/6 mice. In comparison with sedentary, exercised mice showed i) higher basal MKP-1 mRNA in peripheral leukocytes and peritoneal macrophages, ii) lower basal p38 MAP kinase activity and enhanced MKP-1 staining in macrophages, and iii) lower leukocytes infiltration into peritoneal cavity, lower serum levels of IL-6 and TNF-α when exposed to i.p.-injected LPS. In addition, macrophages isolated from exercised mice showed higher LPS stimulated MKP-1 protein levels. Taken together, MKP-1 upregulation is partially responsible for exercise training-improved innate immune response.

P5AM-2-11

DAI-KENCHU-TO RELAXES THE GUINEA-PIG SUBMUCOSAL ARTERIOLE

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Dai-kenchu-to (DKT) is known as an herbal medicine used for postoperative ileus. It was also reported that DKT increases abdominal blood flow. However, no report exists about the effect of DKT on isolated submucosal arterioles. The aim of this study is to clarify the influence of DKT in submucosal arterioles of the guinea-pig intestine. Experiments were carried out with diameter recording and intracellular membrane potential recording. In the arterioles contracted by Ba^{2+} (0.5 mM), DKT (0.1 mg/ml - 10 mg/ml) increased the diameter (dilatation) of the arteriles. Tetrodotoxin, L-N $^{\omega}$ -nitroarginine did not inhibit the dilatation. Indomethacin partially inhibited the relaxation. In smooth muscles of the arterioles depolarized by Ba^{2+} to about -40 mV, DKT (3mg/ml) did not repolarized the membrane, while acetylcholine repolarized the membrane to about -53 mV. It is concluded that in submucosal arterioles, DKT causes the arterilear dilatation by activation of cyclooxigenases without no effects on the membrane pontetial.

P5AM-2-12

A COMPUTATIONAL MODEL FOR CEREBRAL CIRCULATION AND ITS APPLICATION FOR HAEMODYNAMIC MODELLING IN VASCULAR SURGERIES

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In this work we introduce a computational pipeline which starts from image digitization to vascular tree construction and blood flow modeling. We apply the pipeline to a 3D CTA image and extract the cerebral vasculature which includes an arterial tree and a venous tree from the ascending aorta to the superior vena cava. The vasculature has total 71 vessel segments and 34 bifurcations. The radius of the smallest vessel is 0.7mm. With a spatial step of 1mm the vasculature is discretized into 5460 grid points. We then relate a 1D formulation of the governing equations with a wall constitutive equation, and solve the system using a MacCormack finite difference scheme. Coupling with a bifurcation model and a microcirculation model we are able to compute the pressure, velocity, radius variation for each grid point during a cardiac cycle. We apply the computational model to two surgical scenarios: 1) occlusion of one of the two inner carotid arteries (ICA) in carotid angioplasty by an inflated balloon; 2) systemic circulation when a catheter system is inserted into the arterial tree. The simulation results show that the anterior cerebral circulation is compensated from the other ICA in scenario 1, and the velocity profile and pressure change only slightly (2-3%) in scenario 2.

P5AM-2-13

MECHANICAL STRETCH AUGMENTS INSULIN-INDUCED VASCULAR SMOOTH MUSCLE CELL PROLIFERATION VIA INSULIN-LIKE GROWTH FACTOR 1 RECEPTOR UPREGULATION

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Insulin resistance and hypertension are implicated in the pathogenesis of cardiovascular disease, however; little is known about effects of insulin on vascular smooth muscle cells (VSMCs). We investigated the effect of mechanical stretch on insulin-induced VSMC proliferation. VSMCs were stretched by Flexcer cell system. Insulin stimulated cell proliferation and glucose uptake in stretched VSMCs, although insulin had no effect on proliferation in non-stretched VSMCs. Mechanical stretch augmented insulin-induced ERK and Akt phosphorylation, moreover, both MEK inhibitor and PI3 kinase inhibitors attenuated insulin-induced proliferation in stretched VSMCs. EGF receptor inhibitor and Src inhibitor also attenuated insulin-induced proliferation in stretched VSMCs, whereas showed no effect on glucose uptake, suggesting that Src and EGF receptor-dependent signaling pathway was involved in VSMCs proliferation. Furthermore, mechanical stretch significantly increased IGF-1 receptor protein expression, though stretch did not affect insulin receptor and IRS-1 expression. IGF-1 receptor siRNA attenuated cell proliferation. These data provides experimental evidence that insulin directly stimulates VSMCs proliferation under a dynamic mechanical environment via downregulation of IGF-1 receptor.

P5AM-3-1

FILTERABILITY OF ERYTHROCYTES FROM PATIENTS WITH VALVULAR HEART DISEASE

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Background: Prognosis in patients with valvular heart disease is improved by prosthetic valves. However, mechanical stress of these valves on intact human erythrocytes is not fully investigated. **Methods:** We investigated erythrocyte filterability in patients with operated heart disease, using nickel mesh filtration technique. Venous blood was drawn from these patients (n = 10, valve replacement; n = 6 and valvuloplasty; n = 4) and controls (n = 5) after obtaining informed consent. Erythrocyte suspension was prepared (Ht = 3.0%) using HEPES-buffered solution and erythrocyte filterability was evaluated by pressure-flow curves. **Results:** Pressure-flow curves in these patients are superimposable with those of controls (3.07 ± 0.08 ml/min vs. 3.06 ± 0.03 ml/min at 100 mmH_2O). Flow rate was remarkably reduced (less than 1.00 ml/min) in a patient with prosthetic mitral valve failure causing perivalvular blood leakage and hemolytic anemia requiring repeated transfusion. Stomatocytes and fragmented erythrocytes were commonly observed in these patients. **Conclusion:** This study indicates that erythrocyte filterability is not reduced by mechanical stress of physiologically functioning prosthetic valves, but is severely impaired by malfunction of these valves.

P5AM-3-2

PROPORTIONAL CHANGES IN DISTRIBUTION OF THE BRONCHIAL ARTERIAL BLOOD FLOW AFTER SMOKE INHALATION

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Current investigations have demonstrated that there are multiple vascular channels connecting bronchial-to-pulmonary vasculature. We investigated the redistribution of the bronchopulmonary anastomotic flow after smoke inhalation. Twenty ewes were divided into three groups; in the smoke group (N=7), animals were subjected to cotton smoke, in the BEAO+smoke group (N=6), the bronchial artery was ligated before smoke inhalation, and the other animals received air insufflation alone (control group, N=7). The systemic blood flow to the lung parenchymal tissue and airway tissues were sepalately determined using microsphere technique, while the total bronchopulmonary anastomotic flow (pouch flow) was determined. Although microsphere data showed 15-25 times increase in blood flow into the airways, lung parenchyma tissue did not show any increase after inhalation. The smoke inhalation induced three times increase in the pouch flow, and the ligation of the bronchial artery completely blocked it. These findings suggest that large part of the bronchial blood flow is directed to the bronchial artery-to-pulmonary artery connection in normal state, whereas smoke inhalation induces proportionally high blood flow to the vascular channels connecting bronchial artery-to-bronchial capillaries.

P5AM-3-3

ANALYSIS OF THE CHEST TRANSCUTANEOUS OXYGEN PRESSURE CHANGES OBSERVED DURING WALKING TESTS IN PATIENTS WITH LOWER EXTREMITY ARTERIAL DISEASE

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Chest transcutaneous oxygen pressure ($tcPO_2$) changes over time mimic the changes in arterial PO_2 during moderate exercise. We analysed the profiles observed for chest-$tcPO_2$ during walking treadmill tests in 552 patients with claudication. We tested the reliability of $tcPO_2$-changes obtained simultaneously through two different probes in 15 patients. The test-retest reproducibility was analysed prospectively in another 31 patients to estimate whether chest-$tcPO_2$ changes are reliable & specific of each patient's response to walking. The $tcPO_2$-changes were usually characterized by a progressive increase during walking & a progressive decrease in the recovery period from exercise. In 15 percent of the patients we found an abrupt decrease at exercise onset, associated to a transient recovery overshoot, assumed to rely on exercise induced hypoxemia (EIH). High cross-correlation coefficients were found for $tcPO_2$ changes obtained from two different probes in the same patient (0.919±0.091) or in test-retest recordings (0.800±0.129). The chest $tcPO_2$-changes seem characteristic of each patient's response to exercise. Changes in chest $tcPO_2$ during walking could allow for the detection of EIH as a potential cause or worsening factor for exercise-induced limb pain.

P5AM-3-4

EVALUATION OF A SIMPLIFIED INDEX (ENTROPY) ABOUT SLEEP STATE OF ELECTROENCEPHALOGRAMS RECORDED BY A SIMPLIFIED POLYGRAPH, MemCalc-Malkin2

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Background: Polysomnography (PSG) is the gold standard for the diagnosis of sleep apnea syndrome (SAS), but it takes time to analyze the PSG and PSG cannot be performed repeatedly because of efforts and costs. Therefore simplified sleep respiratory disorder indices are needed. The Memcalc method, which is a combination of the maximum entropy method for spectral analysis and the non-linear least squares method for fitting analysis (Makin2, Suwa Trust, Tokyo, Japan), has recently been developed. Spectral entropy which is derived by the Memcalc method might be useful to expressing the trend of time-series behavior.

Aim: Spectral entropy of EEG which is calculated with the Memcalc method was evaluated by comparing to the PSG results.

Subjects: Suspected obstructive SAS patients (n=70)

Methods: EEG was recorded using Makin2 with PSG recording using Alice 3(Respironics) from 20:00 to 6:00. Spectral entropy of EEG, which was calculated every 30 seconds using the Makin2, was compared to detum from PSG recordings.

Results: Entropy had a correlation with AHI, while it tended to have inverse correlations with both stage 3+4 and stage REM.

Conclusions: Spectral entropy, which was calculated with Makin2, might be a possible index evaluating the severity of sleep respiratory disorder.

P5AM-3-5

ACUTE ADAPTATION OF DYNAMIC CEREBRAL BLOOD FLOW REGULATION TO INTERMITTENT HYPOXIC APNEAS

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The purpose of this study is to examine the effect of acute sleep apnea events on dynamic cerebral blood flow regulation in healthy subjects. Each subject performed one 30-s hypoxic apnea every one minute for 20 min (intermittent hypoxic apneas events; IHA). During each hypoxic apnea event arterial oxygen saturation reached 80-85%. The rate of regulation (RoR) was calculated as an index of dynamic cerebral autoregulation (CA) from the response data of arterial blood pressure and cerebral blood flow velocity to acute hypotension produced by the cuff release technique. The RoR was significantly attenuated following the IHA protocol (0.78 ± 0.09 to 0.47 ± 0.07 /sec; $P = 0.003$), indicating a loss of dynamic CA during the apnea event. Therefore, the loss of CA during the apnea event is a possible mechanism for the increased risk of stroke in the obstructive sleep apnea patient.

P5AM-3-6

ROLE OF SUPEROXIDE ANION IN THE CHEMOREFLEX AND CARDIOVASCULAR RESPONSES TO CHRONIC INTERMITTENT HYPOXIA IN SPONTANEOUSLY HYPERTENSIVE RATS

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In this study, we examined whether chronic intermittent hypoxia (CIH) affects in chemoreflex activation and cardiovascular responses in conscious spontaneously hypertensive rats (SHRs) and, if so, by what mechanisms. We used age-matched (8-9-wk-old) adult male SHRs exposed to repetitive 1.25-min cycles of intermittent hypoxia (IH) or room air (RA) for 30 days. Blood pressure were measured daily by the telemetry for assessing the autonomic function by heart rate variability analysis. FosB immunoreactivity in caudal region of the nucleus tractus solitarius (cNTS) was measured after IH. We found that IH markedly increased the normalized low-frequency power of pulses interval spectrogram (LF%) (an index for cardiac sympathetic outflow) and mean arterial pressure (MAP), whereas RA evoked only a mild elevation of these responses. Additionally, after 9 days of IH exposure, MAP, LF%, and FosB expression in cNTS were significantly elevated and lasted until the end of the observation period. Pretreatment with MnTMPyP, a superoxide anion scavenger, prevented CIH-induced these responses. These results suggest that CIH-induced hypertension is associated with chemoreflex activation and facilitation of sympathetic outflow, and superoxide plays an important role in CIH-induced these responses.

P5AM-3-7

ORAL MORPHINE CONSUMPTION DELAYED LATERAL VENTRICLES AND CHROID PLEXUS IN WISTAR RAT EMBRYOS

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The present study focused on the effects of maternal morphine consumption on choroids plexus development in female Wistar rats. The experimental groups after pregnancy received 0.05 mg/ml of morphine by tap water while, the control group received water. On 17th day of pregnancy, the pregnant animals were anesthetized by chloroform and the embryos were removed surgically. The embryos were fixed in formalin 10% for 4 weeks and hematoxylin and eosin were applied. The sections were examined for choroids plexus development by light microscope and MOTIC software. Severe reductions of the third as well as lateral ventricles were observed in the experimental group. In addition, an increase in the choroids plexus area in the experimental group regarding to controls was identified. The study showed that oral morphine consumption has caused to a decrease in the third and lateral ventricles and an increase in choroids plexus area. This defect may cause behavioral changes observed in the F1 generation from addicted pregnant animals.

P5AM-3-8

BLOOD FLOW INDICES IN THE PATIENTS OF SYSTEMIC SCLEROSIS RECORDED BY IMPEDANCE PLETHYSMOGRAPHY

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Systemic Sclerosis is a chronic multi system disorder of unknown etiology.Vascular abnormalities like increased vasospasm & reduced vasodilatory capacity plays a key role in the pathogenesis of systemic sclerosis .

A microprocessor based Non Invasive Impedance Plethysmography (IPG) instrument NICOMON (L&T INDIA) is used to measure the Blood Flow Index (%BFI) at Upper arm, forearm and wrist in 10 patients of Systemic Sclerosis in the age group 25-69 years. It is a case control study.

Ten patients (Male: Female 1:4) of Systemic Sclerosis the BFI was recorded in the following range Rt. Upper Arm-1.663%, Rt. Elbow-1.637%, Rt. Forearm-1.735%, Rt. Wrist-1.205%, Lt. Upper Arm- 1.535%, Lt. Elbow-1.383%, Lt. Forearm-1.208%, Lt Wrist- 0.857%. The BFI in the control subjects were in the range of1.25% to 2.82%. A statistically significant difference exist between cases and controls for Blood Flow Indices in the region of Right Wrist (p value <0.012) and Left Wrist (p value<0.001).

Patients suffering from Systemic Sclerosis have decreased blood flow along their extremities, particularly distal segment, as microvasculature is one of the first affected systems. The IPG studies can be utilized as a cost effective and practical tool to assess the progression and prognosis of Systemic Sclerosis

P5AM-3-9

PEROXYNITRITE DECOMPOSITION CATALYST REDUCES VASOPRESSIN REQUIREMENT IN SEPTIC SHEEP

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The standard therapy for methicillin-resistant Staphylococcus aureus (MRSA) sepsis is becoming less effective. We tested the hypothesis that peroxynitrite may cause hypo-responsiveness to vasopressin (VP) in septic sheep.

Method: Sheep were instrumented with multiple catheters to monitor hemodynamics for 24h. Sepsis was induced by instillation of live MRSA (2.5X10^11CFU) into lungs by bronchoscope under anesthesia. Then, sheep were awakened, placed on ventilator, and fluid resuscitated. Groups: 1) no injury, no treatment, n=6; 2) MRSA, n=4; 3) MRSA+VP titrated when mean arterial pressure (MAP) fell by 10 mmHg, n=4; 4) MRSA+INO4885 (peroxynitrite decomposition catalyst) started 6 h post-injury (0.1mg/kg bolus followed by 0.02 mg/kg/h), n=4; and 5) MRSA+VP+INO4885, n=4.

Results: MRSA induced severe hypotension (MAP: 94plusmn3 at baseline and 66plusmn1at 24h post-injury) refractory to aggressive fluid and vasopressin. Septic sheep required AVP with infusion rate 86plusmn18 U/min at 24h post-injury to keep MAP close to baseline whereas additional INO4885 treatment reduced VP dose to 10U/min.

Conclusion: Peroxynitrite inhibition largely reduces vasopressin requirement and it may be a novel treatment option against MRSA sepsis-induced cardiovascular collapse refractory to vasopressors

P5AM-3-10

HYPER-HYDROXYETHYL STARCH RESCUES RATS FROM HEATSTROKE-INDUCED DEATH

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We hypothesized that hyperhydroxyethyl starch (HyperHAES), which contains 6% HAES and 7.2% NaCl, would be superior to 6% HAES or 7.2% NaCl treatment alone during experimental heatstroke. As compared with values for normothermic controls, the 0.9% NaCl solution-treated heatstroke rats had lower mean arterial pressure, cerebral perfusion pressure, cerebral blood flow, brain partial pressure of oxygen, and plasma levels of protein C. In contrast, 0.9% NaCl solution-treated heatstroke rats had higher values of intracranial pressure, brain levels of glutamate, glycerol, lactate/pyruvate ratio, neuronal damage scores, plasma levels of prothrombin time, partial thromboplastin time, D-dimer, and tumor necrosis factor-α, blood urea nitrogen, creatinine, aspartate and alanine aminotransferase, and alkaline phosphatase. The heatstroke-induced hypotension, cerebral ischemia and hypoxia, hypercoagulable state, activated inflammation, and hepatic and renal dysfunction can be significantly reduced by HyperHAES. The order of effectiveness in resuscitation of heatstroke is: HyperHAES > 7.2% NaCl > 0.9% NaCl or 6% HAES. Our results suggest that HyperHAES improves survival during experimental heatstroke by attenuating multiorgan dysfunction.

P5AM-3-11

THE EFFECTS OF DIPICOLINIC ACID ON BLOOD CIRCULATION

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Dipicolinic acid (2,6-pyridinedicarboxylic acid; DPA) is a substance existing within spores of members of the Bacillus genus and which is involved in the heat resistance of the spores and other stabilizing characteristics. There are relatively large amounts of DPA in natto, a traditional food in Japan consisting of fermented soybeans. Japanese people have daily intakes of 0.6-4.0 mg of DPA on average through eating natto. As one of the physiological activities of DPA, we have already reported that it has inhibitory activity against platelet aggregation. In the current study, we confirmed that the addition of DPA promotes the synthesis of tissue-type plasminogen activator (t-PA), the thrombolytic enzymes secretion by human cells. The amount of t-PA secretion peaked at a level 46.7 times as much as the control, when DPA with a concentration of 5mM was added. An increase in the amount of t-PA mRNA expression was also confirmed through testing. Tests conducted by using eight varieties of DPA derivatives showed that such functions were specific only to DPA.

P5AM-3-12

THE INFLUENCE OF HYDRAZIN'S DERIVATIVES ON A FUNCTIONAL CONDITION OF LYMPHATIC SYSTEM

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Asymmetrical dimethylhydrazinum used as rocket fuel > its derivative are extremely toxic connections to various kinds of animal < vegetative organisms. The purpose was to research the influence of hydrazin's derivatives on a functional condition of lymphatic system. Experiments with use of nitrosodimethylaminum, phenilhydrazinum, hydrazin sulfate, hydrazid of izonicotinum acids have been made. By results of experiments under action hydrazines transport function of a chest lymphatic channel was oppressed more than on 40% return of proteins from tissues in lymph, hence, decreases. So, the amplitude of spontaneous constriction of a chest lymphatic channel suppressed on 46% at sharp and chronic intoxication NDMA, the force of a chest lymphatic channel's constricthion was suppressed from 20 up to 37% in other groups. About it also can approve reduction of concentration of the common protein of blood > to increase of their quantity in a lymph, > also under indications hematokrit which in our experiments have shown reduction of blood's plasma volume in relation to blood's cellular fraction. It can be because of significant reduction of the common proteinum in plasma.

P5AM-3-13

SPONTANEOUS CONTRACTILITY OF HUMAN LYMPHATIC VESSELS: POSSIBLE INVOLVEMENT OF c-KIT POSITIVE PACEMAKER CELLS

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The coordinated activity of smooth muscle cells (SMCs) in lymphatic vessels is essential for lymph propulsion. This study aims to determine how human lymphatic SMCs generate and coordinate contractility. With ethical approval and informed patient consent, thoracic ducts (TD) were obtained during oesophageal cancer surgery. Ring segments of TD (without valves) were mounted on a wire myograph and normalised to a diameter previously determined to produce optimal average active tension. Vessels were loaded with Ca^{2+}-fluorophores for confocal microscopy. In the media of the TD wall, SMCs were observed arranged in bundles with varying orientations. Some cells displayed oscillations in Ca^{2+}_i-dependent fluorescence even at basal tone levels. This basal Ca^{2+}_i activity was frequently observed in cells with bright fluorescence, which sometimes presented an atypical SMC morphology. Tone development was associated with increased number of cells with Ca^{2+}_i events and synchronisation of Ca^{2+}_i oscillations occurred. RT-PCR analysis of TD detected mRNA for c-kit and its ligand SCF (soluble and membrane forms), which was confirmed by sequencing. In conclusion, our working hypothesis is that c-kit positive (ICC Cajal-like) cells could be responsible for synchronising the activity of human TD SMCs.

P5AM-3-14

AGE-INDUCED ALTERATIONS OF LYMPHATIC CONTRACTILITY

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All functions of lymphatic system require the lymph flow, which can not exist without the driving force generated by contractions of lymphatic vessels (LV). However the mechanisms regulating the lymphatic contractility and particularly the mechanisms of age-related alterations of lymphatic pumping remain unclear. Using confocal imaging we observed the profound reduction of muscle cells in aged LV. In vivo flow measurements using fast video microscopy demonstrated decreased basal lymph flow; maximal lymphocyte velocities in aged mesenteric LV are 4-6 times lower than in the adult LV, contraction amplitude diminished by 50-60%; while the contractions are irregular with long periods of inactivity. The profound inhibition of the contractile activity has been observed also in isolated aged rat thoracic duct (TD) and mesenteric LV. The basic flow/eNOS-dependent regulation is abolished in aged TD, protein message is greatly depleted. At the same time in aged TD the substantial iNOS activation, confirmed by Western blotting and immunohistochemistry, occurs; its functional importance confirmed. We concluded that depletion of the contractile reserves in LV in elderly diminishes their ability to provide the adequate transport of lymph during the periods of the increased volumetric loads.

P5AM-3-15

SPLEEN CONTRACTION DEVELOPS PROGRESSIVELY ACROSS LONG APNEAS

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The human spleen contracts during apnea but the initiation is not fully understood. Hypoxia appears to be one, but not the sole factor involved (Richardson et al 2008). Contrastingly, it has been suggested in a study on 15 s apneas that the entire spleen contraction occurs directly after onset of apnea, thus ruling out chemoreceptor input (Palada et al 2007). We aimed to investigate the development and main contributor to spleen contraction during longer apneas. Eight male apnea divers performed breath holds of 4 min to 5 min 30 s duration. The arterial oxygen saturation (SaO_2) and spleen volume were measured every 15 s from 2 min prior to 4 min post apnea. Apnea was performed after a deep inspiration, but without other preparation. Mean(SD) pre apneic spleen volume was 226(38) ml. At the onset of apnea spleen volume transiently declined to 178(18) ml at 15 s (P=0.1), but after 1 min the spleen was restored to 208(27) ml. Thereafter it contracted progressively in relation to declining SaO_2, with a minimum volume of 120(20) ml (P=0.01) occurring with SaO_2 nadir at the end of apnea. Eight min after apnea spleen volume was fully restored. We conclude that spleen contraction during long apneas is biphasic, with maximal contraction coupled to chemoreceptor input at apneic termination.

P5AM-3-16

EFFECT OF MASSAGE ON BLOOD FLOW IN THE LOWER EXTREMITIES OF WOMEN

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Edema of the lower extremities is often observed in women due to the small pumping effect of muscles of lower extremities and secreted progesterone. In this study, we examined the physiological effects of a massage machine on blood flows in lower extremities of women. We used a massage machine which applied a pressure of 18 kpa to the lower legs intermittently. Five women were included in this study. After confirmation of each subject's menstrual phase, we measured circumference and temperature of the thighs and calves, and the velocity of blood flow in the popliteal arteries and veins by ultrasonography. Then the subject received massage for 15 min. Soon after the massage, we measured the circumference, the temperature, and the velocity of blood flow of the lower extremities again. The circumferences decreased and the velocities of blood flow in the popliteal arteries and veins increased regardless of menstrual phase. However, although the mean temperature of the lower limbs during the follicular phase and menstruation increased, the temperature of the lower limbs in the luteal phase decreased. The physiological effects of massaging lower extremities might be different in women depending on the menstrual phase.

P5AM-3-17

THERMAL SENSATION MEASUREMENT USING HUMAN PERIPHERAL SKIN TEMPERATURE

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Control of indoor thermal environments in accordance with people's preferences makes an important contribution to comfort. To keep a suitable temperature environment, we tried to estimate a subject's thermal sensation using biological signals. Human skin temperature is controlled by blood flow, and human peripherals have many blood capillaries and arteriovenous anastomoses (AVAs). AVAs adjust peripheral temperature depending on ambient temperature and human condition. Therefore, the peripheral temperature is believed to be related to one's thermal sensation. Accordingly, we focused on peripheral skin temperature to reduce the number of sensors in order to make an objective thermal sensation measurement system suitable for use in daily life. First, we performed an experiment involving alteration of environmental temperature to reveal the relation between peripheral skin temperature and thermal sensation vote. Next, we made a thermal sensation processing algorithm and evaluated the algorithm. When thermal sensation indices estimated by our algorithm were compared with the subject's vote, error of mean squares was 1 or less in most cases. As a result, the possibility of thermal sensation measurement using peripheral skin temperature was confirmed.

P5AM-3-18

VISUALIZATION OF HUMAN SKIN HEMODYNAMICS BY USE OF RGB IMAGES

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To visualize the human skin hemodynamics, we investigated a method that is specifically developed for estimating the concentrations of oxygenated and deoxygenated blood in skin tissue from RGB digital color images. Monte Carlo simulation of light transport in skin tissue specifies a relation between the chromophore concentrations and the RGB-values. The total blood concentration and oxygen saturation can also be reconstructed. In vivo imaging of the total blood concentration was performed for 20 subjects during the upper limb occlusion at 50 mmHg-pressure, together with the strain-gauge plethysmograph (SPG). The arterial inflow and the venous capacitance in skin tissue were calculated from the increase rate and the change of the total blood concentration. We confirmed that the arterial inflow and the venous capacitance in skin tissue obtained from the method correlate closely with the limb arterial inflow and venous capacitance measured by SPG.

P5AM-3-19

PROGRESSION OF HYPERFILTRATION IN AN EARLY DIABETIC RAT WAS VISUALISED BY MULTIPHOTON MICROSCOPY

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The purpose of this study was to visualise glomerular filtration under physiological conditions and to evaluate filtration quantitatively.

We used 4 to 8 week STZ diabetic rat models. We measured preglomerular vascular diameters, left renal blood flow and creatinine clearance. Glomerular filtration was visualised by multiphoton microscopy. Various sizes of dextran (3k to 70k Dal) conjugated with Texas Red in 0.5 ml were administered intravenously by bolus shot. Peak values during the time course of Texas Red intensity in Bowman's space were normalized for comparison.

In diabetic rats, both afferent and efferent diameters, left renal blood flow and creatinine clearance were larger than those in control rats ($p<0.05$). This indicates the existence of hyperfiltration. In fact, filtration of the larger dextrans (40k and 70k Dal) were clearly visualized by multiphoton microscopy in early diabetic rats indicating greater leakage even from the early stage of diabetes with a significant difference by about 20 to 30 % for the 40k and 70k Dal sieving coefficients comparing with the control group and this leakage was found progressive along the duration of diabetes.

Glomerular hyperfiltration in early diabetic rats was progressive and it was clearly visualised by multiphoton microscopy.

P5AM-4-1

COMPARATIVE STUDIES ON THE EFFECT OF CURCUMIN AND TETRAHYDROCURCUMIN ON GINGIVAL MICROCIRCULATION IN STREPTOZOTOCIN-INDUCED DIABETIC RATS

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The study was aimed to investigate the effect of daily feeding of curcumin and tetrahydrocurcumin (THC) on gingival microcirculation in diabetic rats. The rats were divided into four groups: control (CON), diabetes (iv. injection of streptozotocin 55mg/kgBW, STZ), STZ treated with curcumin (300mg/kgBW/day, STZ-CUR) STZ treated with THC (100mg/kgBW/day, STZ-THC). At 8th and 12th week, leukocyte adhesion to endothelium was evaluated in gingival postcapillary venules by counting the number of adherent cells labeled with rhodamine 6G. The gingival blood flow (GBF) was measured using laser Doppler flowmetry. Blood samples and livers were collected for biochemical and oxidative stress analyses, respectively. The results demonstrated that at both monitor time points, the number of adherent leukocytes was significantly increased in STZ-rats while GBF was decreased. In the STZ-THC rats, the GBF was significantly greater, whereas the leukocyte adhesion was significantly less than that in the STZ rats. The same result was observed for STZ-CUR group except that leukocyte adhesion seem to be decreased but not significantly at 8th week. Therefore, our findings suggested that curcumin is less active than THC in protecting the abnormality in gingival microcirculation of diabetic rats.

P5AM-4-2

CIRCULATORY RESPONSES TO ELECTRO-ACUPUNCTURE OF THE HINDLIMB IN DIABETIC RATS

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We investigated the responses of blood pressure and heart rate to electro-acupuncture in rats with streptozotocin-induced diabetes. The effects of electro-acupuncture on blood pressure being mediated by sympathetic nerves, we also investigated the responses of these variables to exogenous noradrenaline. Two weeks after injection of streptozotocin (40 - 50 mg/kg) or saline, rats were divided into 3 groups: a control group (saline injection), a mild-diabetes group (plasma glucose concentration above 400 mg/dl), and a severe-diabetes group (plasma glucose concentration above 600 mg/dl). Under general anesthesia with urethane-chloralose and artificial ventilation, the rats were given electro-acupuncture for 10 min at 10 mA, 20 Hz, to the right tibialis anterior muscle; alternatively they were injected 6 mg/kg noradrenaline. Blood pressure and heart rate were markedly increased after electro-acupuncture or injection of noradrenaline in control rats. Both responses were attenuated in the mild diabetic group, and were more markedly reduced or abolished in the severe diabetic group. The observations suggest that high level of plasma glucose impairs the circulatory responses to electro-acupuncture by affecting responsiveness to noradrenaline released from sympathetic nerve terminals.

P5AM-4-3

RESVERATROL IMPROVES ENDOTHELIAL FUNCTIONS IN HIGH-FAT DIET-INDUCED DIABETIC MICE

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Epidemiological studies suggest that red wine consumption is associated with reduced in cardiovascular mortality in diabetic patients. The aim of this study was to investigate the mechanism of resveratrol (RSV) protects against diabetes-induced endothelial dysfunctions.

Within high-fat diet (HFD) for 17 weeks, C57Bl/6 mice developed type 2 diabetes. Oral gavage fed with RSV significantly reversed the above symptoms in HFD fed mice. Furthermore, diabetic mice exhibited an increasing of leukocytes rolling, adhering, and transmigrating in the post-capillary venules of cremaster muscle. In contrast, treatment of RSV significantly attenuated diabetes-induced leukocyte rolling, adhesion, and transmigration. The phenylephrine (PE)-induced vasoconstriction was dramatically attenuated in HFD mice; whereas, RSV treatment significantly rescued the vessel responsiveness to PE. Our result also shows that the phosphorylated AMPK, Akt, and eNOS protein levels were significantly reduced in aorta of HFD mice. Consistence with the observation on increasing of blood vessel responsiveness, RSV also elevated AMPK, Akt, and eNOS protein phosphorylation levels. Overall, these results indicate that RSV attenuated diabetes-induced endothelial dysfunctions by activating AMPK/Akt/eNOS pathway.

P5AM-4-4

ALTERATIONS IN LYMPHOCYTE MEMBRANE PROTEIN CONTENT AND INCREASED LYMPHOCYTE RIGIDITY IN CATS WITH DIABETES MELLITUS

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We have previously shown that red cell deformability was decreased in cats with noninsulin dependent diabetes mellitus.In the present study we aimed to extend our findings to lymphocyte deformability and alterations in lymphocyte membrane proteins. In this regard, we analysed lymphocyte deformability in cats with non-insulin dependent diabetes mellitus. We also assessed membrane protein content by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.We found that lymphocyte rigidity was significantly increased in cats with non-insulin dependent diabetes mellitus compared to controls . sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed that the band which corresponds to the protein with a weight of 37 kDA had disappeared in cats with non-insulin dependent diabetes mellitus. We suggest that the observed abnormalities in membrane proteins may play a role in reduced lymphocyte deformability associated in diabetes mellitus and may be a co-factor in increased blood viscosity.

P5AM-4-5

IMPAIRMENT OF PRESYNAPTIC REGULATION OF NOREPINEPHRINE RELEASE FROM ADRENERGIC NERVES OF CAUDAL ARTERY IN TYPE 2 DIABETIC GOTO-KAKIZAKI RATS

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The spontaneously diabetic Goto-Kakizaki (GK) rat is a model of type 2 diabetes mellitus. In the present study, we examined endogenous norepinephrine (NE) release from caudal arteries of 12-week-old GK rats and age-matched Wistar rats. NE was quantified by an HPLC-electrochemical detection technique. Electrical field stimulation (EFS: 1 Hz, 0.5 msec duration, 50 V, for 3 min) evoked significant NE release from caudal arteries of both rats. The NE content of caudal arteries was significantly lower in GK rats than Wistar rats although the amount of NE release was almost equal in both rats. The content of NE in blood vessels is generally consistent with sympathetic innervation. Furthermore, we examined the effects of an α_1-adrenoceptor agonist methoxamine, an α_2-adrenoceptor agonist clonidine and an A_1-adenosine receptor agonist 2-chloroadenosine on the release of endogenous NE evoked by EFS. These agonists significantly reduced NE release from Wistar rats. On the other hand, these agonists did not affect NE release from GK rats. These results suggest that the dysfunction of presynaptic receptors on sympathetic nerves in GK rats may be related to the autonomic nervous system dysfunction associated with diabetic complications.

P5AM-4-6

VASOMOTOR DYSFUNCTION AND ALTERATIONS OF ADIPOKINES IN METABOLIC SYNDROME PATIENTS WITH INSULIN RESISTANCE

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Aim: To evaluate relationships between adipokines (adiponectin, resistin, leptin, interleukin-6, tumor necrosis factor-alpha) and cutaneous vasomotor responses in metabolic syndrome (MS) patients with IR. MS patients with IR were divided into two groups: 20 patients with type-2 diabetes mellitus (without insulin therapy and pronounced diabetic complications) (DM) and 20 patients without DM. 20 healthy subjects were selected as controls (C). The study groups were matched for age and sex. Adipokines were measured by xMAP technology. We recorded cutaneous laser Doppler flux (LDF) variables: postocclusive hyperemia (m1-LDF), vasoconstrictor response (v-LDF) to deep inspiration; and heat induced hyperemia (m2-LDF). Results: Only the patient group with diabetes demonstrated a significant diminution in v-LDF compared to the group of healthy subjects ($p<0.05$). m1-LDF was decreased in both patient groups in comparison with the group of controls ($p<0.05$), but only in diabetics the decrease of m2-LDF was significant ($p<0.05$). Adipokines levels were changed ($p<0.05$) in diabetic patient group. Conclusion: MS patients with insulin resistance have significant cutaneous vasomotor dysfunction, but diabetics (with insulin resistance and MS) have also changed adipokines levels.

P5AM-4-7

VASOMOTOR DYSFUNCTION AND ALTERATIONS OF CIRCULATING ADHESION MOLECULES IN PATIENTS WITH INSULIN RESISTANCE

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Aim: to evaluate relationships between intercellular adhesion molecule-1 (sICAM-1), vascular cell adhesion molecule-1 (sVCAM-1), E-selectin (sE-selectin) and cutaneous vasomotor responses in metabolic syndrome (MS) patients with insulin resistance (IR). MS patients with IR were divided into two groups: 18 patients with type-2 diabetes mellitus (DM) and 18 patients without DM. 18 healthy subjects were selected as controls (C). The study groups were matched for age and sex. sICAM-1, sVCAM-1, and sE-selectin were measured by xMAP technology. We recorded cutaneous laser Doppler flux (LDF) variables: postocclusive hyperemia (m1-LDF), vasoconstrictor response (v-LDF) to deep inspiration; and heat induced hyperemia (m2-LDF). Results: Only the patient group with diabetes demonstrated a significant diminution in v-LDF compared to the group of healthy subjects ($p<0.05$). m1-LDF was decreased in both patient groups in comparison with the group of controls ($p<0.05$), but only in diabetics the decrease of m2-LDF was significant ($p<0.05$). sICAM-1, sVCAM and sE-selectin levels were elevated ($p<0.05$) in diabetic patient group. Conclusion: MS patients with IR have significant cutaneous vasomotor dysfunction but diabetics have also elevated sICAM-1, sVCAM-1, and sE-selectin levels.

P5AM-4-8

ESTIMATION OF THE CAPABILITY OF SHAPE RECOVERY OF EACH ERYTHROCYTE BY USING A MICRO-CHANNEL TECHNIQUE

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We fabricated a micro-channel array on a silicon chip. Diluted human blood of 10% hematocrit with autologous plasma was made to flow through the micro-channel. The motoin and the shape recovery process of each erythrocyte was photographed using a high speed video camera after leaving the micro-channel. In analysis of these experimental data, the erythrocyte was modeled as a Kelvin visco-elastic solid. Temporal change in compressive strain of the erythrocyte's shape from the initial stage just after leaving the channel exit was plotted against time elapsed on a semi-logarithmic coordinate paper. From the gradient of a fitting straight line with experimental points we obtained a characteristic relaxation time for each erythrocyte which was considered to be a measure of the capability of shape recovery or deformability. Using this technique we measred the relaxation times of normal human RBC, glutaraldehyde-hardened one etc. It was found that the relaxation time of the latter was about 60% shorter than that of the former, and furthermore that the relaxation time of RBCs of diabetic person was in the middle between them in numerical value, which suggested that this method was useful for a early diagnosis of diabetes.

P5AM-4-10

STUDY OF PROTEIN BIOMARKER FOR DIABETES TYPE 2 AND ROLE OF THIAMIEN ON THEIR LEVELS

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This research project aims to characterize protein biomarkers which are specific to the various stages of diabetes mellitus type 2 and to assess their levels as a result of administration of high dose thiamine. Type 2 diabetic patients and the same number of age and sex-matched normal healthy controls were recruited from sheikh Zayed Hospital, Lahore. Clinical history and all base line biochemical parameters have been assessed by different standard referred protocols. Thiamine and placebo was given in a double-blinded design. The biochemical profiles of patients was studied over a 6 month period and results was significant decreases in albumin excretion rate in 35 % of thiamine treated patients as compared to placebo group. Protein profile of type 2 diabetes patients has been investigated and particularly the level of some marker proteins like CRP,and Apo-I were assessed. Identification and characterization of protein biomarker were done by a more recent protein mapping technology using ProteomeLab PF 2D and mass spectrometry MALDI-TOF TOF and analysis. These studies have contributed to improved and more effective treatment for type 2 diabetic patients with incipient nephropathy with expected decrease risk of kidney failure.

P5AM-5-1

PSYCHOLOGICAL STUDY OF THE EFFECT OF A SNP IN GPR7 GENE IN THE EVALUATION OF HUMAN FACIAL EXPRESSION

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G-protein coupled receptor 7 (GPR7), also named neuropeptide B/W receptor-1 (NPBWR-1) was recently identified as a receptor for neuropeptide B and W. The strong expression of GPR7 in the central nucleus of the amygdala and bed nucleus of the stria terminalis suggests a potential role in regulation of emotional response. We have studied possible function of GPR7 in human social interaction by using recognition or emotional evaluation of facial expressions (angry, fearful, happy and neutral) as indices. We studied effects of a frequent SNP in GPR7 gene located at nucleotide 404 (A/T), which produces an amino acid substitution (tyrosine to phenylalanine) at codon 135 (Tyr135Phe). This codon encodes tyrosine residue that is highly conserved throughout the G protein-coupled receptors, and constitute the highly conserved DRY motif of G protein-coupled receptors. Mutations of residues within this motif usually abolish receptor function. We compared responses of subject with/without SNP in terms of recognition (which one of 4 expressions?) and emotional evaluation (arousal, valence and dominance). Results showed statistically significant difference between 2 groups only in the evaluation of dominance for angry faces.

P5AM-5-2

NEURAL BASIS OF ASSOCIATIVE MEMORY OF FACES IN THE MONKEY ANTERIOR INFERIOR TEMPORAL CORTEX

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To investigate neural basis for memory of facial identities, we recorded neuronal activities from the ventral, anterior inferior temporal cortex (AITv) of monkeys during the performance of an asymmetrical paired association (APA) task. In the task, the associative pairs consisted of the four pairs of a picture and five faces in five different views but of a unique identity. The results show that some AITv neurons responded selectively to a particular associative pair. Responses of these associative pair -selective neurons showed a tuning to facial views, implying a view-variant representation of facial identity during encoding. On the othrer hand, the population of the AITv neurons with significant selectivity to faces well represented facial identity which is view-invariant. Some AITv neurons showed sustained activities during inter-stimulus delays which were selective to a particular facial identity. Further investigation applied to the delay activities suggested that the activities were evident only in the situation view-invariant facial identity had to be recalled, which are in favor of a view-invariant representation of facial identity during retrieval. These results indicated a functional neural organization in the AITv for naturalistic memory of facial identities.

P5AM-5-3

CHARACTERIZATION OF NEURONAL ACTIVITIES IN AREA 35 OF MACAQUE PERIRHINAL CORTEX DURING A PAIR ASSOCIATION TASK

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Area 35 (A35) is a narrow strip on the fundus of the rhinal sulcus, which belongs to the perirhinal cortex together with area 36. Several lesion studies suggest key roles of the perirhinal cortices in visual association memory. Our previous studies showed that area 36 exhibited neuronal activity related to stimulus-stimulus association memory. In the present study, we systematically investigated the neuronal properties of A35 while two macaque monkeys performed a pair-association task. To accomplish recordings from A35 efficiently and precisely, we localized the recorded sites with MRI-detectable markers *in vivo* during the experimental phase and confirmed them with electrolytic lesions through postmortem histology. For each neuron, we calculated the correlation coefficient between the response to a stimulus and that to the paired associate as an index of the pair-coding response (adaptive pair-coding index, aPCI). Because A35 neurons tended to have variable selectivity onset, the window for aPCI was defined as starting with each neuron's selectivity onset. aPCI distribution was significantly shifted toward positive values ($n = 71$, $P < 0.001$, Wilcoxon-signed rank test), suggesting that A35 represents pair-association memory.

P5AM-5-4

NEURAL CORRELATES OF *EXCLUSION* FOR RAPID FORMATION OF NOVEL-TO-NOVEL RELATION

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When one is asked to choose one alternative from a novel one and familiar ones corresponding to a novel sample object, he distinctly tends to choose the novel alternative by rejecting familiar ones. This is a noticeable cognitive bias of human cognition, called *exclusion*, which may support infants' word-learning thorough labeling a novel name onto a novel object. In this fMRI study, we investigated neural correlates of *exclusion*. Visual sample stimulus (S1 or S2) and their corresponding visual comparison stimuli (C1 and C2) were presented to the subjects, who were asked to choose either of comparisons. Subjects were trained to choose C1 in the presence of S1, and to choose C2 in the presence of S2. After the training, the subjects were exposed to novel relations. Defined comparison C1 (or C2) and novel comparison N2 were presented with novel sample N1 (exclusion test). The subjects readily chose N2 by rejecting the defined comparison. The brain activation in the exclusion test was compared with that in the trained relation. Significant activation was observed in the right prefrontal cortex and the left inferior parietal lobule. Our results suggest that these areas are involved in rejecting the defined comparison and rapidly forming novel-to-novel stimulus relation.

P5AM-5-5

WITHIN-SUBJECT INSPECTION OF TWO-DIMENSIONAL SURFACE MAPS DISSOCIATED ADJACENT REGIONS IN POSTERIOR IFG ASSOCIATED WITH DISSIMILAR FUNCTIONS

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The right posterior inferior frontal gyrus (pIFG) is associated with both response inhibition and negative feedback processing. It is unclear, however, whether these two cognitive requirements increased activity of a single common focus in the pIFG or they increased activity of two separate foci in the pIFG. The present functional magnetic resonance imaging study tested these two possibilities by employing the same subjects to perform the two tasks, one of which required response inhibition and the other required negative feedback processing. The region associated with response inhibition was calculated based on the anti-saccade task used in Chikazoe et al. (2007). The region associated with negative feedback processing was calculated based on the modified Wisconsin Card Sorting Task (Konishi et al., 2002). Because of the individual difference in sulcal structures, the pIFG activation in each individual subject was analyzed using two-dimensional surface mapping based on Caret (Van Essen et al. 2001). By comparing the coordinates of the two activations of individual subjects, it was revealed that the region associated with response inhibition was located caudal to that associated with negative feedback processing.

P5AM-5-6

NEURONAL ACTIVITY IN THE MACAQUE POSTERIOR PARIETAL CORTEX REFLECTING RECONFIGURATION OF COGNITIVE SET

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Flexible behavior requires prompt shifting of internal cognitive sets whenever external demands change. To elucidate how this dynamic process is implemented in the primate brain, activity of single neurons was recorded from the posterior parietal cortex (PPC) of two monkeys performing an analog of the Wisconsin Card Sorting Test, which is used clinically to test cognitive flexibility. In this task, subjects responded to a bivalent stimulus based on one of two dimensions (color or shape). Whenever the relevant dimension intermittently changed without any notice, the monkeys had to shift their cognitive set and respond based on the new dimension. We trained the monkeys to promptly perform set shifting, mostly within a single trial, and found shift-related activity: PPC neurons were activated transiently when the monkeys shifted from one cognitive set to another competing set, but not when they shifted in the opposite direction. This dynamic shift-related activity emerged about 4 s before the actual behavioral response that was based on the new cognitive set, and was predictive of whether the monkeys would successfully shift their cognitive set. These results suggest that PPC neurons could signal the new cognitive set to be reconfigured and contribute to successful set shifting.

P5AM-5-7

SELF-ORDERED DIMENSIONS IN THE WCST THAT EFFICIENTLY DETECT LATERAL PREFRONTAL ACTIVATION ASSOCIATED WITH SHIFTING UNDER NOVEL SITUATIONS

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In this fMRI study, we devised "self-ordered dimensions" applied to the Wisconsin Card Sorting Test (WCST). In the modified WCST, the order of the dimensions was determined based on the subjects' own choice of the dimensions made during performance of the WCST. This procedure may keep the overall level of cognitive load matched between the initial novel shifts and the subsequent less novel shifts by decreasing the load in the initial shifts, and may keep the subjects naive to the initial shifts because it avoids advanced attempt of novel dimensions. The self-ordered dimensions revealed prominent dorsolateral prefrontal activation associated with shifting under novel situations, and the dorsolateral prefrontal activation was 1.8 times greater than that in the original WCST using fixed-order dimensions. We also detected the medial prefrontal activation associated with the novelty of shifting, consistent with prior neuropsychological studies of the WCST. These results indicate that the dorsolateral and medial prefrontal activations reflect shifting under novel situations, and suggest the potential usefulness of the self-ordered dimensions in neuroimaging and neuropsychological investigations of the WCST.

P5AM-5-8

BRAIN ACTIVITIES ASSOCIATED WITH PREPARATION AND EXECUTION OF STOPPING

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Response inhibition is one of the most important executive functions. The go/no-go and stop tasks have been most often used to test response inhibition. In the go/no-go task, a go or no-go cue is given in each trial, and subjects respond only to a go cue. In the stop task, a go cue is given in all trials, and sometimes a stop signal is given following a go cue after a delay period. In the stop task, subjects press a button while they are preparing for stopping even in the go trials whereas subjects just respond to a go cue without preparation for stopping in the go/no-go task. In the present fMRI study, the stop task was modified to investigate the preparatory process. Go trials without preparation for stopping as in the go/no-go task were introduced and were compared with go trials with preparation for stopping as in the stop task. The imaging results revealed multiple fronto-parietal activations during execution of stopping, consistent with previous response inhibition studies. Moreover, a part of these regions were also activated during preparation for stopping. These results demonstrate that preparation for stopping recruits a part of the neural correlates of response inhibition and suggest that inhibitory networks are partially required before stopping during the stop task.

P5AM-5-9

DORSAL, BUT NOT VENTRAL, AREA OF MEDIAL PREFRONTAL CORTEX IS INVOLVED IN THE CHOICE RESPONSE ON A RUN-CLIMB-RUN BEHAVIORAL TASK

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The brain dopamine systems are suggested to mediate behavioral choice based on cost/benefit analysis. The present study was designed to investigate the lesion effects of the dorsal and ventral subareas of medial prefrontal cortex (mPFC) on a choice behavior of run-climb-run (RCR) task. Rats were trained to traverse an uncovered floor alleyway (150 cm), climb a vertical rope (35 or 140 cm represented as the short or long rope), and run across an upper board (100 cm) to access chocolate for the reinforcement. All subjects were trained to climb the short rope for obtaining 1 piece of chocolate for reward and further trained to climb the longer rope for receiving 4 pieces of chocolate. They were then introduced to a concurrent choice test before lesion, in which the subjects significantly chose the long rope to obtain a larger reward rather than the short one to obtain a smaller reward. Subsequently, they received excitotoxic lesion in either dorsal or ventral mPFC. The post-lesion data showed that dorsal mPFC lesion significantly shifted the choosing from the long rope into the short one. These data suggest that the dorsal mPFC is critically involved in RCR behavior and essential for the choice made between high-cost-high-reward and low-cost-low-reward options.

P5AM-5-10

"DOING MORE AND GET MORE" TASK PERFORMANCE: THE IMPORTANCE OF THE ORBITOFRONTAL CORTEX IN RATS

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Humans as well as animals understand the living principle of doing more in order to get more. However, the underlying mechanism is not well addressed. Here, we show evidence that the orbitofrontal cortex (OFC) is essentially involved. We trained rats on a behavioral paradigm called "Doing more and get more" (Dm-Gm) task. In this task, rats were required to nose-poke in a hole on one side of a rectangular box. The animals decided by themselves how long they keep on nose-poking in the hole. After nose-poking out, the animals were required to return back to the opposite side of the box to get water reward. The longer the nose-poking in was, the bigger the reward was. We found that reversible and bilateral inactivation of the OFC with local infusion of the GABA-A agonist muscimol significantly impaired the performance of rats on the "Dm-Gm" task: the animals were not able to pay more efforts to get more reward. In some cases, rats with muscimol infusion were not able to complete the task at all. The present result strongly suggests that the OFC is essential for the "Doing more and get more" task performance.

P5AM-5-11

"DOING MORE AND GET MORE" TASK PERFORMANCE: THE CONTRIBUTION OF THE MEDIAL PREFRONTAL CORTEX IN RATS

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Humans as well as animals understand such a living principle as doing more in order to get more. However, the underlying mechanism is poorly understood. We trained rats on a behavioral paradigm called "more effort, more reward" (mE-mR) task. In this task, rats were required to nose-poke in a hole on one side of a rectangular box. The longer the nose-poking in was, the bigger the reward was. We found that reversible inactivation of the mPFC by intra-mPFC infusion of the GABAa-receptor agonist muscimol significantly impaired the performance of rats on the task. Intra-mPFC blockade of NMDA receptors had no effect on the task performance. However, intra-mPFC blockade of D1/D5 receptors by local injection of SCH 39166 significantly reduced the animal's motivation of paying more effort to get bigger reward. We also investigated activities of neuron ensemble in the mPFC when rats were performing the behavioral task. We identified three types of neurons which were related to the task performance: Type I neurons showed change in activity related to the nose-poking in, Type II and Type III neurons related to the nose-poking out and reward respectively. The present results strongly suggest that the mPFC is an essential for the planning or organization of the mE-mR task performance.

P5AM-5-12

NEURAL SIGNALS IN THE ROSTRAL PART OF THE ANTERIOR CINGULATE CORTEX MODULATED BY THE REWARD PROXIMITY AND AMOUNT

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Anterior cingulate cortex (ACC) is known to be related to reward expectancy or motivation. We previously reported that single neurons in the caudal ACC (cACC) of monkeys change their firing according to reward proximity (Shidara & Richmond, 2002). However, 1) to what extent do different sub-regions of the ACC have distinct functions and 2) whether the neuronal activity of the rostral ACC (rACC) is modulated by the reward amount and proximity is unknown. To investigate these questions, we recorded single-unit activity from the rACC of a monkey while it performed a task with different reward amount and schedule length. Most of recorded neurons showed task-related activity (64/71). Activity of 42/64 neurons were modulated by the schedule progress. 38/42 neurons showed decreasing activity along with schedule progress, which contrasts with the cACC data (Shidara and Richmond, 2002) in which 27/36 neuronal responses with gradual changes showed increasing activity. There were only a few neurons that were modulated by the reward amount (7/64). These results suggest that (1) there might be a functional difference between the rostral and caudal part of the ACC and (2) the ACC might play an important role in the representation of the reward proximity rather than the reward amount.

P5AM-5-13

SINGLE NEURONS IN MONKEY DORSAL RAPHE NUCLEUS DURING REWARD SCHEDULES

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Previous works pointed out that dorsal raphe nucleus (DRn) controls cortical excitability needed to regulate sleep-wake cycle, pain and mood. Recently, Doya's reinforcement learning model hypothesized that serotonergic activity in DRn regulates how farther the future reward is taken into account, that is, discounting factor. The aim of our study is to clarify whether the single neuronal activities in DRn are related with reward during performing multi-trial reward schedules. This task requires the monkey to perform 1, 2 or 3 repeats of visual discriminations to obtain reward. Here we recorded from 88 single neurons in/around DRn. 66/88 neurons showed 134 phasic responses in various task events. 55/134 responses were reward contingent in terms of responsive schedule states, and 48/134 were evoked after reward onset in terms of responsive task event. These results, that the substantial number of neuron carried information of reward, suggest that the DRn might play an important role in regulating reward seeking behavior.

P5AM-5-14

AN EVENT RELATED POTENTIAL STUDY OF SYNTACTIC PROCESSING IN STUTTERERS ANF FLUENT SPEAKERS

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Though causes of stuttering are still under heavy debate, some recent neuroimaging studies support the idea that basal ganglia (BG) dysfunction might be involved in developmental stuttering. The P600 event-related potential (ERP) component is considered a specific marker of syntactic aspects of language processing, and it has been demonstrated that patients with BG dysfunction show no or reduced P600. Considering the fact that linguistic factors such as syntactic complexity and length of utterance play a role in stuttering frequency, possible involvement of the BG in production of disfluent speech may be predicted to result in abnormal P600 effects among stutterers.

In the current study, experiments were performed with adult stutterers and fluent speakers. Syntactically correct and incorrect Japanese sentences were presented randomly in visual or auditory form to subjects, and ERP (P600) data were recorded from scalp electrodes on both cortices. In this paper, we will use our results to consider functional differences in language processing activities in the stutterers' brains with a focus on hemispherical differences. We will also present a hypothesized etiology of developmental stuttering.

P5AM-5-15

THE RELATIONSHIP BETWEEN A FREQUENCY COMPONENT OF ELECTROENCEPHALOGRAM AND THE CEREBRAL OXYGENATED HEMOGLOBIN DURING A COMPUTER GAME

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We examined the relationship between frequency components of an electroencephalogram and cerebral oxygenated hemoglobin.

The subjects were 8 healthy adult males. The left and right prefrontal areas, oxy-hemoglobin and deoxy-hemoglobin were analyzed using near infrared spectroscopy. An electroencephalogram was recorded from the left and the right prefrontal areas on the forehead scalp by a bipolar method. The frequency band of the alpha (8-13Hz) and the beta (13-30Hz) components were recorded continuously as an integral value with 3 seconds. The occurrence pattern of oxygenated hemoglobin and the frequency component of the electroencephalogram in the prefrontal area were monitored by simultaneous recording.

During a computer game, oxy-hemoglobin in the left prefrontal area more significantly degreased than during the time of rest. In the right prefrontal area, oxy-hemoglobin decreased when the game started, but the decrease was not significant. On the other hand, the occurrence pattern of frequency of the alpha band did not correspond to oxy-hemoglobin.

Our data shows that the occurrence pattern of oxy-hemoglobin corresponded to the frequency of the beta band. The decrease of oxy-hemoglobin suggests the possibility that neuronal activity in the cerebral cortex is reflected.

P5AM-5-16

THE EFFECT OF A MAXIMUM EXERCISE LOAD ON CEREBRAL ACTIVITY

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We examined the occurrence patterns of the reaction time and accuracy of reaction following treadmill running load. In addition, the type of effect shows the activity patterns of the electroencephalogram (EEG). The subjects were eight males. Measurements of the EEG and at the choice reaction task were conducted before and after the exercise. The choice reaction task was done by visual stimulation which randomly showed stars with 6, 7 and 8 points (will be described as star6, star7 and star8) and by conducting 3 types of task with each star as the targeted stimulation. The recorded EEG was FFT processed and separated into each frequency range, beta1 (13-20Hz), beta2 (20-30Hz) seeking amplitude spectrum. Comparison was done on activity patterns of EEG before and after the exercise by creating a grand average topography of the region. As a result, the reaction time of the choice reaction task (star6 and star7 as the targeted stimulation) was significantly abbreviated after exercise. In terms of EEG, the beta 1 band in the choice reaction task (star6 and star7 as the targeted stimulation) increased significantly after exercise in the left temporal resion. In this study, the left temporal region is thought to be activated by the exercise.

P5AM-5-17
EEG STUDY OF CREATIVITY PARAMETERS

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Distinguished are 3 psychological parameters of creativity: fluency, flexibility and originality. Most of psychophysiological studies of creativity were performed using the creativity tasks with no separation of these parameters (Martindale, 1975; Petsche, 1996: Molle, 1999: Carlsson, 2000; Razoumnikova, 2000; Bechtereva et al., 2000, 2004; Howard-Jones, 2005).
In order to investigate separate creativity parameters three types of original tasks were developed: Fluency task, Flexibility task, Originality task.
Healthy volunteers undergone computer EEG registration while being tested. EEG was recorded from 19 sites (10-20 system). Mean values of EEG power and coherence for each subject in each state were calculated in frequency bands delta, theta, alfa1, alfa2, beta1, beta2, gamma.
Results indicates that fluency and flexibility are characterized by general increasing of EEG power in gamma and beta bands, especially in frontal and temporal areas, while originality is characterized by decreasing in the same bands.
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P5AM-5-18
CENTRAL AMPK CONTRIBUTES TO SLEEP REGULATION

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AMP-activated protein kinase (AMPK) is an energy sensor that controls glucose and lipid metabolism, and hypothalamic AMPK pathway regulate food intake. Sleep is also known to affect energy metabolism. We hypothesized that AMPK may play a role in sleep regulation. To test this hypothesis, we investigated the effect of compound C (CC), a nonspecific inhibitor of AMPK, on sleep regulation. Central administration of CC decreased amount of non-rapid eye movement (NREM) sleep and suppressed EEG delta oscillation during NREM sleep. Furthermore, CC decreased mRNA expression of inducible cyclooxygenase (Cox2) and neural and inducible nitric oxide synthases (Nos1 and Nos2) in the hypothalamus. In addition, six-hr sleep deprivation (SD) increased mRNA expression of hypothalamic Cox2 and Ca^{2+}/calmodulin (CaM)-dependent protein kinase kinase β (CaMKK2) which is an activator of AMPK. After 6-hr recovery of SD, the increased expression of Cox2 and CaMKK2 were returned to baseline level, while carnitine palmitoyltransferase 1c (Cpt1c) mRNA expression was increased. These data suggest that the central AMPK may play an important role in the regulation of NREM sleep, and hypothalamic lipid metabolism might be involved in sleep homeostasis.

P5AM-5-19
THE EFFECTS OF THE SLEEP QUALITY ON THE ATTENTIONAL CONTROL IN PRESCHOOLERS

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A number of the previous researches have indicated that the poor sleep quality impairs the various cognitive functions, e.g. working memory, in adults, but, there has been little direct evidence regarding this issue for child population. The control of attention is an indispensable component of the various cognitive functions. Thus, the present study aimed at identifying the parameter of the sleep quality, if any, that influences the children's ability of the attention control. To achieve this goal, we monitored the wake-sleep activities of 20 preschoolers for four consecutive days by actigraph, and calculated the parameters defining their sleep quality. The preschoolers' abilities of attention control were assessed by a standardized test (Attentional Network Test , ANT) while recording PFC (prefrontal cortex) activation by near-infrared spectroscopy. The analysis revealed that the children with longer total sleep time show inferior ability to focus the selective attention to designated location, but, no other correlations tested reached significance. These results indicate that the sleep quality does affect the activities of the subcomponents of the broad attention system in preschoolers' developing brain.

P5AM-5-20
PHYSIOLOGICAL ANALYSIS OF FOOT-BATHING IN SEAWATER

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To investigate effects of foot-bathing in seawater, we measured EEG and the autonomic activity (respiratory rate, heart rate, heart rate variability and blood flow in skin). Participants were healthy 4 students. A control bating water was used by warmed tap water (40 degree Celsius within 1 degree) and artificial sweater was contained 3% NaCl. EEG on 25 positions was measured by the 10-20 electrode system and recorded for 20 min during bating after 5 min resting condition. Increase in amplitude of delta band and in slope of power spectral density on the occipital region was observed. Increase in blood flow on cheek was observed in 3 subjects. Fluctuation of heart rate was analyzed by high frequency (HF) and low/high frequency (LF/HF) as the parasympathetic and sympathetic nervous activities, respectively. After 15 min bathing, all the subjects were increased in LF/HF and decreased of HF. These results suggested that foot-bathing induced sleepiness and however after 15 min or later the sympathetic nervous activity was stimulated. Comparing to just warmed water, bating in the salt containing water enhanced amplitude of delta or alpha band and less increased in LF/HF. These data indicated that foot-bathing in seawater enhanced more sleepiness and relax than in salt-free water.

P5AM-5-21
CHANGE IN FEEDING BEHAVIOR BY siRNA FOR NPY-Y1 RECEPTOR INTO MOUSE PARAVENTRICULAR NUCLEUS

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NPY is the most potent peptidergic neurotransmitter for feeding regulation in the hypothalamus, among orexigenic peptides such as AgRP and anorexigenic ones such as alpha-MSH. Although blockade of NPY Y1 receptor in the paraventricular nucleus (PVN) suppresses feeing, the feeding behavior is increased in NPY Y1 receptor-knockout mice, probably due to compensatory mechanism. To investigate the compensation, we first studied the effect of in vivo knockdown of Y1 receptor by a plasmid-based small interference RNA (siRNA). siRNA against Y1 receptors was stereotaxically injected into the arcuate nucleus (Are) and PVN. Acute knockdown of Y1 receptor gene expression in PVN resulted in decreased feeding clearly for 2 days, but not in Arc. Based on the anatomical structure of NPY neurons (which originate from Arc and terminate to PVN), knockdown in gene expression of Y1 receptor might suppress the activity of anorexigenic neurons in PVN. There was no compensatory increase in feeding on short-term knockdown. Thus siRNA plasmid-induced knockdown of NPY gene expression serves as a powerful tool for regulation of endogenous feeding-regulating genes in the brain.

P5AM-5-22
THE ROLE OF HIPPOCAMPUS IN SOCIAL COMMUNICATION OF OCTODON DEGU

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Hippocampus (HPC) has been thought to play an important role in processing the contextual information. However, the function of hippocampus in the social communication is not clearly understood. We examined the effects of HPC neurotoxic lesion on communication of social rodent Octodon Degu in three contexts: Familiar male-male interaction, novel male-male interaction and novel female-male interaction. We compared the frequency and duration of social behavior between before and after HPC lesion. In familiar male-male interaction and novel male-male interaction, greeting and fighting increased, whereas grooming and huddling decreased after HPC lesion. In novel female-male interaction, courtship behavior of HPC lesioned degus was deteriorated. HPC lesioned degus repeated to contact to female regardless their refuge. And their song structure changed. In the object-recognition test, HPC lesioned degu showed a normal habituation to familiar object and a normal recognition of the novel object, while the spatial recognition was impaired. These results were same as those reported in previous study with other rodents. Taken together, hippocampus might play the important role not only in the spatial recognition, but also in social communication.

P5AM-5-23

A NOVEL MECHANISM FOR THE ACTION OF METHYLPHENIDATE IN MEDIAL PREFRONTAL CORTEX OF RAT

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Methylphenidate (MPH), a reuptake inhibitor of dopamine (DA) and norepinephrine (NE), is widely used as a therapeutic drug for the attention deficit hyperactivity disorder (ADHD). However, the role of MPH in regulating excitatory synaptic transmission on the prefrontal cortex (PFC) neurons is poorly understood. In the present study, we examined the effect of MPH on excitatory synaptic transmission in layer V/VI pyramidal cells of rat medial prefrontal cortex (mPFC) by using whole-cell patch-clamp recording. Our results show that, 1) bath application of MPH (50 μM) produced a significant enhancement in the eEPSC mediated by ionotropic glutamate receptors; 2) MPH significantly enhanced both NMDA-receptor and non-NMDA-receptor mediated eEPSC. In the catecholamine-depleted slices, MPH significantly enhanced NMDA-receptor mediated eEPSC, but produced no effect on non-NMDA-receptor mediated eEPSC; and 3) MPH significantly enhanced the NMDA-induced currents, and produced no effect on the glutamate-induced non-NMDA-receptor currents. The present study provides the first novel electrophysiological demonstration that treatment with MPH facilitates excitatory synaptic transmission in the mPFC through both pre- (DA and NE reuptake inhibitor) and post-synaptic mechanisms.

P5AM-5-24

CENTRAL NEURONAL PATHWAY ABOUT REGULATION OF APPETITE BY CART

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Couples of brain regions, including the lateral hypothalamic area (LH) and arcuate nucleus (ARH), are involved in the regulation of food consumption as well as anorexic and orexgenic factors exist in these regions. Our previous study showed that direct injection of cocaine- and amphetamine-regulated transcript (CART) peptide, an anorexic factor, into the shell of nucleus accumbus (AcbSh) significantly suppressed food intake in rats (Yang et al., 2005). The major goal in this study is to further verify the neuronal interactions between the AcbSh and ARH as well as LH in adult male Sprague-Dawley rats. The retrograde tracer, fluorogold (FG), was injected into the AcbSh, and we found that the CART neurons in ARH and LH projected into AcbSh. The FG-immunoreactive (ir) neurons in the paraventricular nucleus of thalamus (PVT) were also close to CART-ir terminals. Furthermore, we injected the FG into the PVT and found that the FG was retrograde back to CART-ir neurons in ARH. Our evidence indicates that leptin regulates food intake could be through the anorexic CART neurons in ARH and then connected to the AcbSh and PVT, and the possible orexgenic neurons in LH also connected to the AcbSh. Therefore, the AcbSh is a putative center involved in the regulation of food intake or appetite.

P5AM-6-1

REDUCED DOPAMINE RELEASE IN PARKIN KNOCKOUT MICE

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Parkin is the causative gene of familial autosomal recessive Juvenile Parkinsonism (AR-JP, PARK2). Although preceding studies have consistently shown that parkin knockout mice (PKO) have little sign of Parkinsonism, there are a few reports suggesting physiological changes in dopaminergic neurotransmission. To make it clear, we directly measured dopamine (DA) release in the striatum of PKO, using in vivo voltammetry. Three to twelve months old PKO were evaluated. The evoked DA overflow in the striatum was detected by carbon fiber microelectrode following electrical stimulation of medial forebrain bundle (MFB). The amplitude of evoked DA was significantly lower in young PKO, and this difference was the most significant in young 3 months old animals. The difference was still significant after administration of nomifensine, a DA transporter-blocker. There was no significant difference in half-life time of falling phase of evoked DA, but the response to nomifensine was high in young PKO. Our results are consistent with slice study of PINK1 deficient mice, and different from in vivo study of alpha-synuclein deficient mice. Marked change in young age may implicate in young onset and slow progress in parkin deficient type of AR-JP.

P5AM-6-2

DETECTING REWARD-ASSOCIATED DOPAMINE RELEASE IN THE STRIATUM OF BEHAVING MICE

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Reward presentation is known to induce transient burst of midbrain dopamine neurons. We aimed at detecting the reward-related transient release of dopamine in behaving mice by using fast-scan cyclic voltammetry of 10 samples/sec. Carbon-fiber microelectrodes were implanted into the striatum of male C57BL/6 mice from 3 to 4 months old. The voltammetric current increased transiently at the timing of food delivery in five of the six mice, and the current-voltage property was similar to that of dopamine. One animal showed strong preference to a novel appetitive food (almond dice) than an ordinal pellet, and almonds induced larger current in this animal. In animals trained with conditioned stimuli (CS) of preceding tone and light, the most significant response appeared after reversing the CS+ and CS-. These results suggest that the measured dopamine current was associated with reward. Our method has wide application to many other mutant mice than C57BL/6.

P5AM-6-3

THE NOVEL CYCLOOXYGENASE-2 INHIBITOR GW637185X PROTECTS AGAINST L-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE TOXICITY

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The possible neuroprotective role of a novel and highly selective cyclooxygenase-2 inhibitor GW637185X was studied in a model of acute 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced injury of nigrostriatal dopaminergic (DA) neurons in the mouse. Stereological and microdensitometrical analysis of nigral tyrosine hydroxylase-immunoreactive cell bodies and striatal tyrosine hydroxylase-immunoreactive terminals, respectively, showed that GW637185X exerted a full protection against MPTP-induced degeneration of the nigro-striatal pathway. In contrast to earlier studies, these findings demonstrate that acute inhibition of cyclooxygenase-2 can result in a full neuroprotective effect not only on nigral DA cell bodies, but also on striatal DA terminals in the mouse MPTP model

P5AM-6-4

GHRELIN INHIBITS OXIDATIVE STRESS RESPONSES BY SUPPRESSING NUCLEAR FACTOR-κB ACTIVATION IN MES23.5 CELLS

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The role of oxidative stress is strongly implicated in Parkinson's disease (PD). Ghrelin, a 28-amino acid peptide, is an endogenous ligand for the growth hormone secretagogue (GHS) receptor. In the previous study, we have observed the neuroprotective effects of ghrelin on dopaminergic neurons in MPTP-treated PD mice. However, the underlying mechanisms are largely unknown. Objective: Our objective was to evaluate the possible antioxidant effects of ghrelin on MES23.5 cells. Methods : The present study was carried out on three groups in MES23.5 cells: control, MPP^+-treated (200 μM) and MPP^++ghrelin(10-9 M,15 -20 min pretreated) groups.The levels of malondialdehyde (MDA) , the indicator of lipid peroxidation, and superoxide dismutase (SOD) ,catalase (CAT) were measured. The expression of NF-κB was also examined. Results: MDA was significantly increased, while antioxidant enzymes were notably decreased in MPP^+-treated group. Ghrelin alleviated the change. Moreover, ghrelin was able to inhibit the activation of NF-κB induced by MPP^+. Conclusion: Our data suggested that ghrelin protect MES23.5 cells by inhibiting oxidative stress responses. The inhibition of NF-κB activation in MES23.5 cells may contribute to the antioxidant effects of ghrelin.

P5AM-6-5

THE EFFECT OF TREADMILL EXERCISE ON MPP^+-INDUCED NIGROSTRIATAL DOPAMINERGIC NEURODEGENERATION

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Chronic and moderate exercise induced mild oxidative stress which may activate antioxidant defense system. In response to oxidative stress, transcription factor of nuclear factor-erythroid 2 related factor 2 (Nrf2) is activated to regulate the expression of antioxidant enzymes, such as gamma-glutamylcysteine synthetase (GCS) and heme oxygenase-1 (HO-1). 1-methyl-4-phenylpyridium (MPP^+) selectively induces oxidative insult in nigrostriatal dopaminergic (DA) neurons and generates an animal model of Parkinson's disease. However, it is unknown whether chronic exercise upregulates Nrf2-induced antioxidant enzymes to protect DA neurons against MPP^+ toxicity. MPP^+ was injected to striatum of rats after 4-week treadmill exercise and killed 1 week after MPP^+ injection. We found that exercise training prevented MPP^+-induced nigrostriatal DA neurodegeneration and the downregulation of Nrf2 and GCS protein expression. However, MPP^+ upregulated HO-1 expression in substantia nigra and striatum which was prevented by exercise training. Our results suggested that the HO-1 upregulation was a compensatory response to oxidative stress induced by MPP^+. Therefore, the prevention of Nrf2 and GCS downregulation by exercise may contribute to its neuroprotective effect and reduced HO-1 upregulation.

P5AM-6-6

LOW CONCENTRATION OF HYDROGEN GAS HAS PROTECTIVE EFFECTS ON DOPAMINERGIC NEURONS IN PARKINSON'S DISEASE MODEL MICE

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It has been reported that molecular hydrogen (H_2) selectively reduces the hydroxyl radical, the most cytotoxic of reactive oxygen species (ROS), and can thereby effectively protect cells. Thus, inhalation of H_2 gas strongly suppressed ischemic and reperfusion brain injury by buffering the effects of oxidative stress. Oxidative stress damage to the brain is also induced in Parkinson's disease (PD). 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated mice have been widely used as an animal model of PD. MPTP is converted into MPP+ within nondopaminergic cells, and taken up into dopaminergic neurons via the dopamine transporter (DAT). Inside the neuron, MPP+ inhibits mitochondrial complex1, inducing neuronal apoptosis. We show here that H_2 in drinking water significantly reduced loss of dopaminergic neurons in PD model mice using both acute and chronic administration of MPTP. MPTP-induced production of super oxide detected by intravascular injection of hydroethidine, and accumulation of 8-oxoG in cellular DNA in striatum, were significantly decreased in mice drinking H_2-containing water. Thus drinking H_2-containing water may be useful in daily life to prevent or minimize acute and chronic oxidative stress.

P5AM-6-7

DEXAMETHASONE CAUSES SIGNIFICANT CHANGES IN STRIATAL NEUROTRANSMISSIONS WITHOUT HAVING ANY IMPROVING EFFECT ON DAMAGED NEURONS

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In the present work, we study the effects of steroidal anti inflammatory agent dexamethasone on striatal dopaminergic, glutamatergic and Gamma Amino Butyric Acid (GABA)ergic neurotransmission in normal and parkinsonian rats. Dexamethasone (0.15, 0.30, 0.60 and 0.8 mg/kg) was administered to normal or parkinsonian rats (i.p.) followed by the analysis of the striatal glutamate, dopamine and GABA concentrations using the microdialysis technique. Additionally, the effect of dexamethasone on the damaged SNc neurons has been investigated. Dexamethasone resulted in decreased striatum glutamatergic-GABAergic and enhanced dopaminergic neurotransmission in normal and parkinsonian rats ($p<0.05$). In addition acute treatment with dexamethasone did not improve the lesion at all. These findings suggest the new mechanism of action for dexamethasone in animal Parkinson's disease (PD) model and also the effectiveness of dexamethasone as an alternative therapeutic agent in treatment of neurodegenerative brain diseases such as PD because of its therapeutic action on striatal neurotransmission.

P5AM-6-8

NEUROPROTECTION BY NICOTINIC RECEPTOR ACTIVATION IN HEMIPARKINSONIAN RAT MODEL

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Parkinson's disease (PD) is characterized by relatively selective nigrostriatal dopaminergic degeneration and movement dysfunctions. Recent studies have reported that the stimulation of nicotinic acetylcholine receptors (nAChRs) is considered to confer a neuroprotective effect. However, the underlying mechanisms have not been fully investigated. The present study was therefore designed to assess the protective effect of nicotine against 6-hydroxydopamine (6-OHDA)-induced dopaminergic cell death in rat substantia nigra pars compacta (SNpc). Intranigral injection of 6-OHDA alone induced a massive loss of tyrosine hydroxylase (TH)-immunopositive neurons in the SNpc and methamphetamine-induced rotational asymmetry. Injection of nicotine prevented both dopaminergic neurodegeneration and methamphetamine-induced rotational asymmetry in 6-OHDA-injected rats. In addition, immunohistochemical analysis revealed that α7 nAChR subunit was detected on neural cell body in the SNpc. Although injection of 6-OHDA induced massive loss of α7 nAChR-immnopositive neurons, co-injection of nicotine inhibited this reduction. The present results suggest that α7 nAChR stimulation may be neuroprotective against 6-OHDA-induced dopaminergic neurodegeneration in PD rat model.

P5AM-6-9

THE EFFECT OF DOCOSAHEXAENOIC ACID ON A RAT MODEL OF PARKINSON DISEASE

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The aim of the study is to observe the effects of DHA supplementation on experimental Parkinson in adult rats. Rats were randomly divided into 4 groups as: Control, DHA-enriched group, Parkinson-induced group and Parkinson-induced+DHA-enriched group. In the DHA group, DHA was supplied daily by gastric gavage for 4 weeks at a dose of 36 mg/kg/day. In the Parkinson group, MPTP (100 µg/1 µl saline) was microinjected bilaterally into median forebrain bundle. Motor activity of rats was observed by the "Vertical Pole" and "Vertical Wire" tests. Apoptosis in SN was detected by enzymatic labeling of DNA strand breaks using TUNEL method. Caspase-3, Bcl-2 and Akt, p-Akt were evaluated by immunohistochemistry. Animals with experimental Parkinson model exhibited decreased locomotor activity and motor coordination and loss in sense of balance. Diminished Parkinsonism symptoms were detected in DHA supplemented group. DHA supplementation also decreased dopaminergic neuron death Bcl-2 and caspase-3. Additionally, Bcl-2 expression density was similar in DHA and Parkinson+DHA groups, while Bcl-2 and p-Akt were higher in both groups in comparison with Pakinson animals.

P5AM-6-10

EFFECT OF DOCOSAHEXAENOIC ACID ON VISUAL EVOKED POTENTIALS IN A PARKINSON MODEL

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This study aimed to investigate the effect of DHA on VEPs, brain and retina lipid peroxidation and antioxidant enzyme activities of PD model. Ten month old male mice were randomly divided into 4 groups as follows; control (C), DHA-treated (D), Parkinson induced (P), Parkinson induced+DHA-treated (P+D). DHA (36 mg/kg/day) was given daily by gavage for 4 weeks. MPTP was given (4x20 mg/kg) ip. Motor activity of mice were tested. VEPs were recorded, brain and retina tissues were extracted for immunohistochemical and biochemical parameters. TH cell decreased in SN of P. Although DHA diminished the increment of the cell death in the P+D, it did not improve decreased motor activity observed in P+D. VEP latencies were prolonged in the P group compared to C. DHA decreased VEP latencies of P+D when compared to P. Brain and retina GPx activity was found to be alike in all groups. Brain SOD activity was decreased in P and P+D while in retina remained similar. Brain CAT activity remained similar among all groups, while retina CAT activity decreased in D, P and P+D. Brain TBARS levels were increased in P and P+D while retina TBARS levels showed no differences.

P5AM-6-11

ROSMARINIC ACID INHIBITS 6-OHDA-INDUCED NEUROTOXICITY BY ANTI-OXIDATION IN MES23.5 CELLS

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Rosmarinic acid (RA) is a naturally occurring polyphenolic compound. It is found in several herbs in the Lamiaceae family, such as Perilla frutescens. RA has been reported to exert anti-oxidative effects on rat erythrocyte, liver and kidney cells. However, little is known about the effects of RA on dopaminergic cells. In the present study, we investigated whether RA could protect MES23.5 dopaminergic cells from 6-hydroxydopamine (6-OHDA)-induced neurotoxicity. The results showed that RA pretreatment significantly prevented 6-OHDA-induced cell viability reduction. Further experiments demonstrated that 6-OHDA induced intracellular ROS generation, the mitochondria membrane potential ($\Delta\Psi m$) decrease. These effects could be partially reversed by RA pretreatment. However, RA had no direct chemical reaction with 6-OHDA extracellularly in cell-free system. Taken together, these results suggest that RA could exert its protective effects against 6-OHDA-induced neurotoxicity through its anti-oxidation properties. Thus, we propose that RA should be viewed as a potential chemotherapeutic in Parkinson's disease patients.

P5AM-6-12

CURCUMIN ATTENUATED 6-HYDROXYDOPAMINE-INDUCED CYTOTOXICITY BY ANTI-OXIDATION AND NF-κB MODULATION IN MES23.5 CELLS

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Oxidative stress has been implicated in the degeneration of dopaminergic neurons in the substantia nigra (SN) of Parkinson's disease (PD) patients, and several anti-oxidants have been shown to be effective approaches on PD therapy. Curcumin has been previously reported to possess radical scavenger, iron chelating, anti-inflammatory properties in different tissues. The purpose of present study is to explore the cytoprotection of curcumin against 6-hydroxydopamine (6-OHDA)-induced neuronal death, as well as the underlying mechanisms in MES23.5 cells. The results showed that 6-OHDA significantly reduced the cell viability of MES23.5 cells. Curcumin protected MES23.5 cells against 6-OHDA-induced neuronal death by restoring the mitochondrial membrane potential and suppressing the increase in intracellular reactive oxygen species. Furthermore, curcumin pretreatment significanltly inhibited 6-OHDA induced NF-κB nuclear translocation. These results showed that the neuroprotective effects of curcumin were attributed to its potent antioxidant propoties and prevention of NF-κB translocation. So we proposed the brain penetrating property of curcumin may make it an important class of drugs for treatment of neurodegenerative diseases which are associated with oxidative stress, such as PD.

P5AM-6-13

FERROPORTIN1 BUT NOT HEPHAESTIN WAS INVOLVED IN THE 6-HYDROXYDOPAMINE INDUCED IRON ACCUMULATION IN MES23.5 CELLS

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Elevated iron was thought to play an important role in the pathogenesis of Parkinson's disease (PD). Our in vivo experiments suggested that iron transporters ferroportin1 (FP1) and hephaestin (HP) might account for the nigral iron accumulation in 6-hydroxydopamine (6-OHDA) lesioned PD models. However, the underlying mechanisms are unknown. In the present study, we observed FP1 and HP expression in 6-OHDA-treated MES23.5 cells. Both FP1 and HP were down-regulated, followed by the decreased iron efflux in these cells. To further clarify that the down-regulation of FP1 and HP was not due to the increased intracellular iron, these cells were overloaded with ferric ammonium citrate. FP1 showed a dose-dependent up-regulation while HP showed no response. In 6-OHDA treated cells, both iron regulatory protein (IRP) 1 and IRP2 were up-regulated, and silencing of IRPs by small interfering RNA in MES23.5 cells dramatically abolished 6-OHDA-induced FP1 down-regulation and even reversed HP down-regulation. Silencing of FP1 but not HP would lead to the intracellular iron accumulation in MES23.5 cells. These results suggested that down-regulations of both FP1 and HP by 6-OHDA were IRPs-dependent and FP1 but not HP was involved in the 6-OHDA induced iron accumulation in MES23.5 cells.

P5AM-6-14

DIVALENT METAL TRANSPORTER 1 IS INVOLVED IN THE NIGRAL IRON ACCUMULATION OF MPTP INDUCED PARKINSON'S DISEASE MODELS

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Iron plays a key role in Parkinson's disease (PD) and increased iron content was found in the substantia nigra (SN) in PD patients. However, the mechanisms for the iron accumulation in this specific area are largely unknown. We hypothesized that divalent metal transporter 1 (DMT1) might be involved in this process. In the present study, we observed DMT1 both with the iron responsive element (IRE, DMT1+IRE) and without IRE (DMT1-IRE) were up-regulated in the SN of MPTP-induced PD mice. Then we observed DMT1 expression in MPP+-treated MES23.5 cells. DMT1-IRE expression was up-regulated, followed by the increased iron influx in these cells. This was further confirmed by the results that MES23.5 cells with DMT1-IRE over-expression showed a significantly enhanced iron influx. This resulted in a decreased mitochondrial membrane potential, an elevated level of ROS production and activation of caspase-3, as well as the subsequent cell apoptosis. There were no changes for iron regulatory protein 1 (IRP1), despite decreased expression of IRP2 in MPP+-treated MES23.5 cells. Our data suggest that MPTP (MPP+)-induced iron accumulation involves DMT1-dependent iron influx and the regulation of DMT1-IRE by MPTP (MPP+) is in an IRE/IRP-independent manner.

P5AM-6-15

COMPARISON OF THE EFFECTS OF BONE MARROW STROMAL CELLS TRANSPLANTATION BY TAIL VEIN INJECTION AND BY CORPUS STRIATUM INJECTION FOR PD RATS

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Objective: This study was undertaken to compare the effects of bone marrow stromal cells (BMSCs) transplantation for Parkinson's disease (PD) rats by tail vein injection and by corpus striatum injection.

Methods: PD rats were made by unilateral medial forebrain bundle (MFB) injection of 6-OHDA. The rats were transplanted with GFP-fluorescent rats' BMSCs either by tail vein injection (TVI group) or directly by corpus striatum injection (CSI group) four weeks after MFB lesion. Bromodeoxyuridine was administrated to label new born cells. Apomorphine-induced rotation was used to evaluate the behavior changes of PD rats.

Results: The number of BrdU-positive cell in corpus striatum of CSI group is higher than that of TVI group either 2 weeks or 3 weeks after transplantation. The Apomorphine-induced rotation number in CSI and TVI groups was decreased 38.37±2.81%, 10.3±8.25% two weeks after transplantation and 39.21±11.56%, 16.86±8.62% three weeks after transplantation, respectively.

Conclusion: The new born cells were increased after BMSCs transplantation either by direct corpus striatum injection or by tail vein injection in PD rats. The rotational behavior was also improved in both groups. The effects of direct corpus striatum injection are better than that of tail vein injection.

P5AM-6-16

NONLINEAR RELATIONSHIP OF SURGICALLY ALLEVIATED RIGIDITY WITH THALAMIC β-BAND ACTIVITIES IN PARKINSON DISEASE AND RELATED DISORDERS

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The microelectrode-guided thalamotomy for Parkinson disease (PD) has revealed that β-band activities are exaggerated in the thalamic ventrolateral nucleus (VL). We examined how these activities were related with surgically alleviated motor symptoms. Patients with PD (n = 138) and related disorders (n = 22) gave their informed consent to undergo the surgery. According to the preoperative UPDRS in each of rigidity (R), tremor (T) and bradykinesia (B) we divided the patients into eight groups scored 0, 0.5, 1, 1.5, 2, 2.5, 3, and 4, and estimated their postoperative reductions (dR, dT, dB). We rated the occurrence of β-band local field potentials by the mean time integral (mβ) of 13-27 Hz wavelets in percent of 3-sec sample records. The mβ was progressively increased with increases in R and B from 0 to 1.5-2, and decreased in 3-4. Among the dR, dT and dB, only the dR-to-mβ relationship was positive, but non-linear of histeresis nature. The histeresis may arise by the time lag of plasticity from the pathology represented by β-band activities to the expression of rigidity. In conclusion, the VL mβ represented rigidity but not for a part of severe rigidity, indicating that the late pathology was in progress, involving other sites beyond the VL.

P5AM-6-17

INVOLVEMENT OF SYNAPTIC PLASTICITY IN THE THERAPEUTIC ACTION OF DBS ON THE SYMPTOMS OF PARKINSON DISEASE MODEL RATS

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Deep brain stimulation (DBS) of the subthalamic nucleus (STN-DBS) has provided marked reduction of bradykinesia, rigidity and tremor in patients with Parkinson disease (PD). Yet, the mechanism of action of STN-DBS remains uncertain. We hypothesized that synaptic plasticity (LTP) in the surrounding inhibitory system including the globus pallidus may be involved in the mechanisms underlying the long-lasting therapeutic action of STN-DBS on the motor manifestations of PD. To test this hypothesis, using cylinder test, we examined whether a systemic administration of the blocking agent of LTP expression, a selective blocker of NMDA receptor, prevents the therapeutic action of STN-DBS seen in the 6-OHDA-induced PD model rats. As a result, the intra-peritoneal infusion of MK801 (0.1 mg/kg) completely prevented the improvement of the motor dysfunction of PD model rats by the STN-DBS.

P5AM-6-18

ELECTRO-ACUPUNCTURE STIMULATION IMPROVES MOTOR DISORDERS IN PARKINSONIAN RATS

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Electro-acupuncture (EA) proves to be effective in alleviating the motor symptoms in Parkinson's disease patients. In a rat hemiparkinsonian model induced by unilateral transection of the medial forebrain bundle (MFB). EA at a high frequency (100 Hz) significantly reduced the abnormal rotation of hemiparkinsonian rats. EA at 100 Hz also prevented the loss of TH-ir in the substantia nigra. Moreover, 100 Hz EA reversed the MFB lesion-induced decrease in substance P protein levels in the ventral midbrain the increase in the GAD-67 mRNA expression in the midbrain, while it did not alter the lesion-induced increase in enkephalin proteins in the globus pallidus. These results demonstrate the effectiveness of EA in normalizing neurochemical behavioral responses to the dopamine lesion in an animal model. The therapeutic effect of EA of parkinsonian rats may be derived from its ability to restore the homeostasis of dopaminergic transmission in the basal ganglia circuit.

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P5AM-6-19

PREVENTION AND TREATMENT OF ELECTRO-ACUPUNCTURE ON A RAT MODEL OF PARKINSON' DISEASE

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Parkinson's disease (PD) is a progressive neurodegenerative disorder of the extrapyramidal motor system, which characterized by degeneration of dopaminergic neurons in substantia nigra pars compacta (SNc). In this study, unilateral transection of the medial forebrain bundle (MFB) was performed using a retractable wire knife to set up the animal model of PD. Our group has certificated that four weeks of EA stimulation with 100 Hz greatly reduced the abnormal rotation significantly increased the rod time of MFB-transected rats. 100 Hz EA stimulation prevented the loss of dopaminergic neurons in the SNc visualized by the method of tyrosine hydroxylase (TH) immunohistochemical staining. However, 100 Hz EA stimulation didn't promote the contents of DA in the ipsilateral striatum, but it significantly increased the content of DA in the ipsilateral SN contrast to the MFB transected group.This study was Supported by National Basic Research Program of China-973 project (2006CB500700), National Natural Science Foundation of China (30472245), key project of Beijing Education Committee (kz200510025014), Talent Training plan of Beijing (20081d0501800206) the fund of Capital Medical University (107420).

P5AM-6-20

THE APPLICATION OF AN EXTRACT OF THE TRADITIONAL CHINESE HERB (T10) IN THE TREATMENT OF NEURODEGENERATIVE DISEASES

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Neuroinflammation has been demonstrated involving in the development of some neurodegenerative diseases, such as Parkinson's disease (PD) or Alzheimer's disease (AD). In present study, an extract of the traditional Chinese herb Tripterygium wilfordii Hook F (TWHF), T10, had been studied its anti-inflammation activity and therapeutic effect on a rat model of PD. The results showed that (1) T10 inhibited the microglial activation induced by lipopolysaccharide (LPS) or β-amyloid (Aβ) in primary cultures, which might be related to the inhibition of NF-κB and JNK signal pathway. (2) T10 inhibited the cytotoxic effect of neurotoxins on PC12 cells. (3) T10 promoted the axon growth and astrocyte release of nerve growth factor. (4) T10 alleviated the abnormal behaviors of PD rats, due to the inhibition of microglial activation and the expression of neurotrophic factors. In conclusion, our results suggest that T10 possess neuroprotective and neurotrophic effects through its anti-inflammation property and the capacity of facilitating the generation of some neurotrophic factors. Considering TWHF has been used in clinic for treatment of rheumatoid arthritis for a long time, it is highly expected that T10 may be prospective in the ancillary therapy of neurodegenerative diseases.

P5AM-7-1

ENHANCED CREB PHOSPHORYLATION BY FAD MUTANT PRESENILIN-ASSOCIATED EXAGGERATED Ca SIGNALING

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Mutations in presenilins 1 (PS1) and 2 (PS2) genes account for most early-onset Familial Alzheimer's Disease (FAD). Disruption of intracellular Ca signaling may play a proximal, and perhaps central role in AD pathogenesis, but links between disrupted Ca homeostasis and cell signaling are obscure. Here, we demonstrate that PS1-FAD mutant M146L constitutively enhances phosphorylation of the transcription factor cAMP-response element binding protein (CREB) in a Ca dependent way. CREB is constitutively phosphorylated in M146L expressing PC12 cells, that was abolished by depletion of endoplasmic reticulum Ca stores with thapsigargin. Both CAMKIV and Ca-dependent protein kinase C, but not MAPK, were involved in CREB phosphorylation. The activity of the InsP3 receptor Ca release channel is potentiated by PS1-FAD mutant M146L. Inhibition of PLC, responsible for the IP3 generation, treatment of the cells with Xestospongin B, a specific InsP3 receptor inhibitor, or RNAi against InsP3-1, the main neuronal isoform, each completely inhibited CREB phosphorylation. Our results demonstrate that exaggerated Ca signaling in PS mutant-expressing cells affects CREB phosphorylation through activation of the InsP3 receptor and may suggest a pathway involved in AD pathogenesis.

P5AM-7-2

FAMILIAL ALZHEIMER'S DISEASE-ASSOCIATED PRESENILIN REGULATES SPONTANEOUSLY ACTIVATED Ca^{2+} INFLUX VIA PIP_2

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Familial Alzheimer's disease (FAD) mutations in PS1 and PS2 affect the processing of amyloid precursor protein (APP) and linked to cellular Ca^{2+} homeostasis. However, the definitive mechanisms underlying the regulation of Ca^{2+} homeostasis by presenilins remain unknown. In a previous study, we showed that the activation of TRPM7-associated Mg^{2+}-inhibited cation (MIC) currents is suppressed in cells expressing FAD-associated PS1 mutants, including PS1-deltaE9, and that the down-regulation of PIP_2 contributes to the observed channel deficits. Since MIC channels could mediate spontaneously activated Ca^{2+} influx, we tested whether PS1 regulates this activity. We found that spontaneously activated Ca^{2+} influx was reduced in PS1-deltaE9 cells and the reduced Ca^{2+} influx was restored by supplying PIP_2. In contrast, deficiency of PS1 and PS2 induced the up-regulation of the spontaneously activated Ca^{2+} influx. Ca^{2+} nflux under the regulation of PS was blocked by the MIC channel blocker, 2APB. These results suggest that presenilins play an important role in regulating spontaneously activated Ca^{2+} influx. Thus, reduced Ca^{2+} influx through this pathway may underlie the altered Ca^{2+} homeostasis observed in FAD-associated PS mutants.

P5AM-7-3

ELECTROCONVULSIVE STIMULATION BLOCKS INTRACELLULAR AMYLOID-BETA-MEDIATED SUPPRESSION OF BK CHANNELS IN NEOCORTICAL PYRAMIDAL NEURONS

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Recent studies have shown that amyloid-β(Aβ), prior to accumulating extracellularly, is increased first intracellularly, thereby inducing cognitive deficit in the early stage of Alzheimer disease(AD). Here we investigated the pathophysiological significance of intracellular Aβ by injecting Aβ protein into rat and mouse neocortical pyramidal neurons through whole-cell patch pipettes. Intracellular $A\beta_{1-42}$, but not $A\beta_{1-40}$, broadened spike width and augmented Ca^{2+} influx via voltage-gated Ca^{2+} channel. However, this class of channel turned out to elude direct modulation by Aβ. On the other hand, charybdotoxin mimicked and occluded these effects of $A\beta_{1-42}$, suggesting that intracellular $A\beta_{1-42}$ caused the suppression of large-conductance Ca^{2+}-activated K^+(BK) channel. In agreement, electroconvulsive stimulation (ECS), which we previously showed to facilitate BK channel opening via homer-1a/vesl-1S expression, blocked the BK channel suppression by $A\beta_{1-42}$ in rats and wild-type mice, but not in homer-1a knockout mice. These findings suggest that the suppression of BK channel mediated by intracellular $A\beta_{1-42}$ may represent an early dysfunction in the AD brain, which is counteracted by activity-dependent expression of Homer-1a during ECS.

P5AM-7-4

AMYLOID-β PROTEIN-ACTIN COMPLEX IS MORE NEUROTOXIC THAN AMYLOID-β PROTEIN ALONE

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We previously reported that amyloid-β protein (Aβ) impaired in vitro axonal transport. The cytoskeleton actin should be released from damaged neurons to the extracellular space in ischemia and neurodegenerative disorders, and we hypothesized that Aβ may interact with extracellular actin. Here we examined the effects of Aβ-actin complex on axonal transport in cultured rat hippocampal neurons. The mixture of G-actin and Aβ fragments, $A\beta_{31-35}$, $A\beta_{25-35}$ or incubated $A\beta_{1-42}$ (including oligomers), immediately produced aggregates of fibrous Aβ-actin complex, whereas $A\beta_{15-20}$ or freshly dissolved $A\beta_{1-42}$ (not including oligomers) did not interact with G-actin. The mixture of $A\beta_{25-35}$ and actin or that of incubated $A\beta_{1-42}$ and actin impaired axonal transport more severely than corresponding Aβ alone. The impairment was not restored by wash, and aggregates of Aβ-actin complex remained attached to neurons. These results suggest that Aβ is more neurotoxic when it binds to actin.

P5AM-7-5

APOLIPOPROTEIN E4 SUPPRESSES I_A AND I_K IN HIPPOCAMPAL NEURONS

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Lots of studies show a clear association between AD and the APOE4. The present study investigates possible effects of apoE4 on K^+ current. The data was got by patch-clamp technique in the whole-cell configuration. Our data showed that: 1) application of apoE4 in extracellular solution suppressed I_K, it decreased by 19.1± 3.5 % and 17.7 ± 3.9 % at the membrane potential of +30 mV and +60 mV. 2) application of apoE4 in intracellular solution suppressed both I_A and I_K. In the case of I_K, the reduction rate were 54.4 ± 7.7 % and 58.5 ± 7.3 % at +30 mV and +60 mV, respectively; in the case of I_A, they were 57.7 ± 9.9 % and 64.2 ± 8.9 % at +30 mV and +60 mV; 3) in the contrast, application of a apoE3 did not exhibit any effects on I_K or I_A. These results indicate that apoE4 molecules could suppress the K^+ currents in hippocampal neurons when act on the either inner or outter side of the neuronal membrane. We propose that the overproduction of apoE4 in neurons may suppress K^+ currents and thus be responsible for the late-developed neuronal damages related to the pathogenesis of AD. Meantime, the observation that intracellular apoE4 exerted a more powerful and harmful effect on neurons is noteworthy, for it is generally known that apoE4 is expressed in cells, including neurons.

P5AM-7-6

DETERMINATION OF PLASMA AMINO ACID CONCENTRATIONS IN HEALTHY YOUNG AND ELDERLY PEOPLE, COMPARE WITH NEURODEGENERATIVE DISEASE SUBJECTS

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A large number of amino acids act as precursors of important neurotransmitters. The most important ones are glutamate and aspartate, as well as GABA and glycine; few studies have investigated how aging affects the plasma amino acids pattern. The aim of this research work was to investigate whether there was a difference in the plasma amino acid release profile among a healthy young (HY), a healthy elderly (HE) population and patients with a neurodegenerative disease. Amino acids were derivatized with o-phtalaldehyde and were determined using an HPLC system.

We found that HE population got higher concentrations of histidine, glycine, threonine and citrulline and lower concentrations of glutamatic acid, valine, triptophan and leucine than did the HY population. Moreover, when comparing the HE population and AD patients´ results, we found differences in the concentrations of histidine, threonine, and citrulline; whereas when comparing the HY population with AD patients´ results they did not show any difference in the concentrations between both. Plasma amino acid profiles of elderly patients with AD showed differences in regards to the HE population. Our study suggests that the aging may cause a change in the plasma amino acids release profile. SDEI-05.5, IN-216907, 24784-M

P5AM-7-7

DOCOSAHEXAENOIC ACID IMPROVES BEHAVIORAL IMPAIRMENT IN AMYLOID β (Aβ) INFUSED RATS, BY DECREASING Aβ FIBRILLATION

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Docosahexaenoic acid (DHA) plays crucial roles in the development, function and maintenance of the CNS throughout life. We previously reported that dietary DHA improves and/or protects against impairment of cognition ability in amyloid β (Aβ)-infused Alzheimer's disease-model rats, associating with a decrease of $A\beta_{1-40}$ levels in the detergent-insoluble membrane fractions of their cerebral cortex. Here we investigated the effects of DHA on the *in vitro* formation of fibrillation of $A\beta_{1-40}$ and $A\beta_{25-35}$. DHA significantly reduced the degree of fibrillation, as shown by the intensity of the thioflavin T. Transmission electron microscopy revealed that DHA-incubated samples were virtually devoid more of defined and structured than of amorphous fibrils, and granular-type aggregates were prominent. It also decreased the levels of oligomers in the course of their maturation to full fibrils. Gel electrophoresis revealed that the molecular size of the oligomers of Aβ was 10 kDa (equivalent to decamers of Aβ) and that DHA reduced these decamers. These results suggest that DHA decreases the *in vitro* fibrillation of Aβ by inhibiting the oligomeric amyloid species and, therefore, Aβ-related neurotoxicity or behavioral impairment is restrained by DHA.

P5AM-8-1

CIRCADIAN OSCILLATION OF EPILEPTIC DISCHARGES DETECTING BY THE SYSTEM USING CONTINUOUS WAVELET TRANSFORM AND ARTIFICIAL NEURAL NETWORK

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We have developed a new system based on continuous wavelet transform (CWT) and artificial neural network (ANN) in order to detect the specific waveform correlated with seizures in EEG. Animal model for epilepsy, Wakayama Epileptic Rat (WER) was used in this study. WER exhibits both absence-like seizures and tonic-clonic seizures. Absence-like seizures were extracted in long-term EEG monitoring. The accuracy of classifying absence-like seizure activities was improved to 92.4%. There were clear day/night patterns of absence-like seizures in the epileptic rats. The proportion of absence-like seizures occurring from the light period was significantly less than dark period. An isolated single-spike and wave form was one of deserved epileptic signals as pre-clinical wave. It was also analyzed and suggested that pre-clinical wave was occurred different mechanisms from absence-like seizures. The hybrid system comprising CWT and ANN might have good resolution and performance high enough for identifying mechanisms of epilepsy.

P5AM-8-2

FAST EEG RIPPLES AND BEHAVIOR SEIZURES INDUCED BY INTRAPERITONEAL INJECTION OF NIKETHAMIDE IN MICE

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We try to study the characteristics of neocortical fast EEG ripples and behavioral seizures induced by accumulatively intraperitoneal injection of nikethamide. Experiments were performed on KM mice. The stainless screws were fixed on cranial bone over the surface of bilateral neocortex for EEG recording. After injection of nikethamide EEG or behavioral seizures were recorded respectively in two group mice. Result showed: (1) Bilateral neocortical fast EEG ripples at the bandpass of 60~1700 Hz considered as EEG seizures. (2) Abnormal respiration: sighing, apnea or postsigh apnea, gasping, extremely Irregular patterns were observed. (3)theta-like EEG rhythm phase-locked with the same rhythmic respiratory movement. (4)Nikethamide-induced mice behavioral seizures included mainly some patterns switched from one to another following the usage of accumulatively increased doses: sighing, wet dog shakes, fast washing face-like clonic behavioral seizures, jumping and rolling. The results implied a possible relation between fast neocortical EEG ripples or theta rhythmic EEG activities and irregular respiratory movement or behavioral seizures.A new epilepsy model will be considered as an integrated epileptic network that central nervous system or peripheral system or organs were involved in.

P5AM-8-3

STAGE- AND REGION-SPECIFIC CYCLOOXYGENASE EXPRESSION AND EFFECTS OF A SELECTIVE COX-1 INHIBITOR IN THE MOUSE AMYGDALA KINDLING MODEL

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In an attempt to elucidate the involvement of cyclooxygenase (COX) enzymes, particularly COX-1, in epileptogenesis, the localization of COX-1 and COX-2 expression in the mouse kindling model was analyzed by immunohistochemistry. COX-2 was predominantly observed in brain neurons and its concentration in the hippocampus increased with progressing seizures, as reported previously. COX-1 was predominant in microglia and its concentration was also enhanced in the hippocampus and areas around the third ventricle during the progression of seizures. These regions are thought to play an important role in the propagation of limbic seizures. Moreover, the administration of SC-560 (a selective COX-1 inhibitor) or indomethacin (a nonselective COX inhibitor) retarded the progress of seizures. Although the precise function of COX-positive cells in microglia and elsewhere is not clear, our results suggest that COX-1 as well as COX-2 may be involved in epileptogenesis, and that certain COX inhibitors can potentially prevent the occurrence of seizures.

P5AM-8-4

ROLES OF THE BASAL GANGLIA IN RHYTHM GENERATION OF THE ABSENCE EPILEPSY

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Absence seizures consist of a brief and sudden impairment of consciousness. This symptom is accompanied by bilaterally synchronized spike and wave discharges (SWDs) in the electroencephalogram. Recently some studies suggest the involvement of the basal ganglia in the SWD generation in rats. However, (1) this involvement is not shown in mice model, and (2) roles of the basal ganglia in the SWD generation are not known in any animal models. To address these issues, we performed *in vivo* experiments as well as *in vitro* experiments of *tottering* (*tg*) mice, a well established model of absence epilepsy. (1) Extracellular recordings and blocker microinjections in *tg* mice showed that the cortico-subthalamo-nigral pathway was involved in the SWD generation. (2) Slice patch clamp recordings from the subthalamic nucleus (STN) neuron showed that the membrane excitability in STN neurons was enhanced in *tg* mice. And this enhancement seemed to result from the decrement of the Ih channel activities in STN neurons. Unilateral blockades of the Ih channel in the STN of *tg* mice extended the mean SWD duration. With these results, it was suggested that the basal ganglia have a positive role in the SWD generation through enhancing the membrane excitability in the STN neuron by decreasing the Ih channel.

P5AM-8-5

NF-κB REGULATE DOWNSTREAM TARGET GENE - COX-2 EXPRESSION IN PC12 CELLS

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Epilepsy is a serious disease of nervous system and Cox-2 over expression may be responsible for this. NF-κB could efficiently induce the transcription of downstream target genes and adjust the expression of immune and inflammatory response factors. However, the correlation between NF-κB activation and Cox-2 over expression remains unclear. In this experiment, PC12 cells were divided into 3 groups, experimental group: adding κB-decoy ODNs, and the control group: adding scrambled-decoy ODNs, and normal group. After transfection, PC12 cells were treated with LPS. Western blot was performed to detect the expression of NF-κB and Cox-2. Results: 1. In normal group, NF-κB expression increased obviously ($P<0.01$) and reaches the peak 2 hours after treatment with LPS while that of Cox-2 increased obviously ($P<0.01$) and reaches the peak 4 hours after treatment with LPS. 2. The expressions of both NF-κB and Cox-2 in experimental group were significantly reduced compared with control group after treatment with LPS for 4 hours. These results shows that κB-decoy could reduce the expression of NF-κB and Cox-2 in pathologic PC12 cells, which suggest that NF-κB could regulate the downstream target genes - Cox-2 gene expression in PC12 cells.

P5AM-8-6

$Ca_v2.1$ DYSFUNCTION MAY BE A GENETIC MODIFIER OF SEVERE MYOCLONIC EPILEPSY IN INFANCY

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Mutations of SCN1A, encoding the voltage-gated sodium channel α1 subunit, represent the most frequent genetic cause of severe myoclonic epilepsy in infancy (SMEI). The purpose of this study was to determine if mutations in other seizure susceptibility genes are also present and could modify the disease severity. All coding exons of SCN1B, GABRG2, and CACNB4 genes were screened for mutations in 38 SCN1A-mutationpositive SMEI probands. We identified one proband who was heterozygous for a de novo SCN1A nonsense mutation (R568X) and another missense mutation (R468Q) of the CACNB4 gene. The latter mutation was inherited from his father who had a history of febrile seizures. An electrophysiological analysis of heterologous expression system exhibited that R468Q-CACNB4 showed greater Ba^{2+} current density compared with the wild-type CACNB4. The greater $Ca_v2.1$ currents caused by the R468Q-CACNB4 mutation may increase the neurotransmitter release in the excitatory neurons under the condition of insufficient inhibitory neurons caused primarily by the SCN1A mutation.

P5AM-8-7

PRENATAL ACUTE STRESS ATTENUATED EPILEPTIFORM ACTIVITIES IN NEONATE MICE

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The development of the CNS is dependent on interactions between genetic and epigenetic factors, some of which could affect the susceptibility of the developing brain to subsequent damaging insults. Gestational stress has been shown to be a potential factor associated with a higher risk to develop some neurological and psychiatric disorders. The present study tested the hypothesis that exposure to prenatal acute stress would sensitize the offspring to neonatal epilepsy. Pregnant mice were exposed to restraint stress 2 times per day for 3 days in the beginning of last week of gestation. Ten days after birth animals decapitated and hippocampi extracted. The hippocampi were resected intact and bathed in low magnesium artificial cerebrospinal fluid to induce spontaneous seizure-like events recorded from CA1 neurons. Both number of recurrent seizures and seizure time decreased in stressed group. Stress induced a significant rise of circulating corticosterone levels both in pregnant mothers and in newborn pups. These findings suggest that prenatal acute stress which may mimic acute stress in human pregnancy probably could be a novel determinant for susceptibility to temporal lobe epilepsy in children. The underlying mechanism may be raise of neurostroids both in blood and brain.

P5AM-8-8

STUDY OF VALERIAN OFFICINALIS ROOT AQEOUS EXTRACT ON PTZ-INDUCED SEIZURE IN MICE

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Introduction: Phamacognostic studies shown that the root of valerian officinalis (VO) contains gamma-amino butyric acid and other materials. In traditional medicine anticonvulsant effect of VO is reminded. number of studies have been shown that this herb has anticonvulsant and hypnotic effects in a some of animal seizure models.
The aim of this study was to investigate the effect of aqueous extracts of VO on PTZ-induced clonic seizure threshold in mice.
Material & Methods: After determination of the intravenously PTZ-induced seizure threshold in control group, extract of valerian officinalis root (0.25, 0.5 and 1 g/kg) orally administration and investigate the effects of VO on clonic seizure threshold.
Results: Our results shown that the PTZ-seizure threshold was 34.25 ± 0.75
in control group in animals pretreated with VO the PTZ seizure threshold dose dependently increased, significantly ($P<0.05$).
Conclusion: Our results suggest that VO posse's anticonvulsant activity probably is via GABAergic effect.
Key words: valerian officinalis root, PTZ, Clonic seizure threshold, mice.

P5AM-8-9

MICE LACKING THE SEIZURE-RELATED GENE, *Sez12* , ENCODING A NOVEL C-TYPE LECTIN DISPLAY SEIZURE-LIKE ACTIVITY AND BEHAVIORAL ABNORMALITIES

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Sez12 is a seizure-related gene, whose expression is down-regulated in the mouse brain after seizure is induced with pentylenetetrazol (Kajiwara, K. et al., BBRC 219, 795-799, 1996; BBRC 222, 144-148, 1996). The *Sez12* protein is a transmembrane protein containing in its extracellular region a C-type lectin domain, which is probably involved in cell-cell interaction. In order to examine the physiological role of the *Sez12* protein, we generated mice with a deletion in the *Sez12* gene. About 85% of *Sez12* -deficient mice displayed slight but long-lasting seizure-like activity and abnormal gait. A behavioral study showed decreased motor activity in the open field and anomalous motor coordination in rotarod performance. Histological analyses of these *Sez12* -deficient mice showed vacuolated perikarya in the Purkinje cell layer, where the expression of the knock-in gene encoding the green fluorescence protein was prominent. However, there were no pathological changes in the skeletal muscles involved in some of the behavioral abnormalities. These findings suggest that the behavioral phenotypes of *Sez12* knockout mice are caused by a loss of cell-cell interaction in the Purkinje cells.

P5AM-9-1

RELATIONSHIP OF SERUM LEVELS OF BRAIN-DERIVED NEUROTROPHIC FACTOR AND CORTISOL AND PSYCHIATRIC ASPECTS AFTER SUDARSHAN KRIYA YOGA COURSES

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Sudarshan Kriya yoga (SKY) breathing contains a sequence of breathing technique Ujjayi, Bhastrika, and Sudarshan Kriya. It has been reported that regular practicing of SKY has antidepressant effect. The purpose of this study is to investigate whether the improved psychiatric conditions after the SKY courses and regular practicing is correlated with changed levels of serum brain-derived neurotrophic factor (BDNF) and cortisol. Four groups of human subjects: depressed controls without SKY (DC), with SKY (DS); healthy controls without SKY (HC), with SKY (HS). Blood samples were collected and psychiatric questionnaires [Beck Depression Inventory(BDI); Symptom Checklist-90(SCL-90); Maudsley Personality Inventory(MPI)] were self-evaluated before and after the SKY courses, and traced for 3 months. Results showed that (1) decreased BDI, SCL-90, and MPI-30 scores in DS and HS. (2) serum BDNF increased in DS and HS. (3) serum cortisol levels in DS and HS were lower than control groups. (4) a positive correlation between BDNF and extroversion in depressed group, and between cortisol and phobic anxiety in healthy group. In conclusion, some improved psychiatric symptoms were correlated with increased serum BDNF levels or decreased cortisol levels after the SKY course and regular practicing.

P5AM-9-2

THE PEOPLE WITH TENDENCY OF DEPRESSION HAVE REDUCED AUTONOMIC NERVOUS SYSTEM ACTIVITY IN JAPANESE APPARENTLY HEALTHY PEOPLE

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Depressed psychiatric patients have been found to possess altered autonomic nervous system (ANS) function compared with healthy people. However the extent to which the ANS contributes to apparently healthy people remains inconclusive. The present study was to evaluate whether sympathetic (SNS) and/or the parasympathetic nervous (PNS) activities were altered or not, in the tendency of depression (TD) on apparently healthy people. Ninety four subjects with no personal history of physical and mental diseases participated in this study. ANS activity was assessed by means of heart rate variability power spectral analysis during resting condition. The subjects were also assessed with the "Center for Epidemiologic Studies Depression (CES-D)" rating scale, and divided into two groups on their depressive mood by the CES-D scores; high (TD, 16≤) and low (non-depression or control: CO, <16). The TD demonstrated a significantly lower ANS (351.2 ± 95.9 *vs*. 631.8 ± 69.1 ms ^{2}, $p < 0.05$), and SNS (190.7 ± 50.1 *vs*. 358.3 ± 46.1 ms ^{2}, $p < 0.05$) activities compared with the CO. Although causes and consequences are unknown, the present data suggest that the ANS activity could be a physiological factor associated with the state of depression.

P5AM-9-3

BEHAVIORAL INVESTIGATIONS OF FG7142-INDUCED ANXIETY-RELATED BEHAVIORS IN MICE

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Animals under stress take adaptive actions that may lead to various types of behavioral disinhibition. Such behavioral disinhibition, when expressed excessively and impulsively, can result in harm in individuals and cause a problem in our society. In the present study, we firstly confirmed the effect of diazepam or fulmazenil on benzodiazepine receptor inverse agonist N-methyl-β-carboline-3-carboxamide (FG7142)-induced anxiety-related behaviors using the light / dark box test to clarify the suitable dosage for evaluation of FG7142-induced anxiety-related behaviors in mice. FG7142 shortened light area spent time in a dose-dependent manner in the light / dark box test. In addition, FG7142 also increased the locomotor activity in a dose-dependent manner in the open field test. Nextly, we evaluated the possibility of cliff avoidance test to determine the impulsivity in mice. In the cliff avoidance test, FG7142 shortened open area spent time, but diazepam chanceled the FG7142-induced effect. In addition, to confirm the possibility of cliff avoidance test as the suitable methods for evaluation of impulsivity, we investigated the hyperemotional behaviors in FG7142-treated mice.

P5AM-9-4

VESICLE ASSOCIATED PROTEIN CAPS2 KO MICE SHOW IMPAIRED SYNAPSE FUNCTION OF HIPPOCAMPUS AND EXHIBIT DEPRESSION-LIKE BEHAVIOR

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CAPS2 is a secretory vesicle-associated protein that is involved in dense-core vesicle exocytosis. We previously showed that CAPS2 KO mice have a significant reduction in release of BDNF in the cerebellum and cerebrum, and display some abnormal behavioral phenotypes including autistic-like decreased social and exploratory behaviors. Although these previous data suggest an importance of CAPS2 in brain function and behaviors, a functional role of CAPS2 in hippocampus, however, remains unclear. In this study, we examined hippocampus-associated phenotypes of CAPS2 KO mice. In CAPS2 KO mice, a significant decrease in BDNF expression level at P21, but not P8, was observed. Synapse structures and synaptic vesicle distribution were slightly affected in the CA1 but significantly changed in the CA3 region. Short and long-term synaptic plasticity in CA3-CA1 synapses were altered in CAPS2 KO mice. In spite of their morphological and physiological changing, CAPS2 KO mice showed no obvious deficit in various hippocampus-associated behavioral tests. On the other hand, it is notable that CAPS2 KO mice tended to exhibit anxiety- and depression-like phenotypes. These data suggest that CAPS2 regulates BDNF secretion in hippocampus and is involved in anxiety- and/or depression-like behaviors.

P5AM-9-5

ESTROGEN (E_2)-DEPENDENT EFFECT OF THE SELECTIVE SEROTONIN REUPTAKE INHIBITOR (SSRI) FLUOXETINE ON ANXIETY-LIKE BEHAVIORS IN FEMALE RATS

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Little is known regarding the E_2-dependent effect of anxiolytic drugs in female animals. We investigated here the interaction between E_2 and fluoxetine (Flx) on anxiety-like behaviors in ovariectomized (Ovx) rats treated for 4 wks with 10 μg/kg E_2 s.c. (Ovx+E_2), 10 mg/kg Flx p.o. (Ovx+Flx) or a combination of both (Ovx+E_2+Flx). The results from elevated plus-maze (EPM) and T-maze (ETM) tests showed that Ovx+E_2 and Ovx+E_2+Flx rats manifested a reduction in the anxiety-like behavior when compared with Ovx rats, whereas Flx alone did not have such effects. In contrast to female rats, the anxiolytic action of Flx alone was seen in male rats. Changes in the anxiety parameters were not due to the impairment of locomotion as demonstrated by the open field test. Furthermore, quantitative real-time PCR revealed that E_2 and E_2+Flx upregulated tryptophan hydroxylase 2, the key enzyme for serotonin synthesis, in the dorsal raphe. However, both E_2 and Flx had no effect on the expression of serotonin reuptake transporter in the frontal cortex, hippocampus, septum, amygdala and periaqueductal gray. In conclusion, E_2 and E_2+Flx exerted anxiolytic actions, in part, by altering serotonin metabolism in the dorsal raphe, and E_2 was required for the action of Flx in female, but not in male rats.

P5AM-9-6

THE ASSOCIATION BETWEEN CYTOKINES AND COGNITIVE PERFORMANCE IN BIPOLAR DISORDER

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Objective: To explore the association between inflammatory cytokines and cognitive function in euthymic individuals with bipolar disorder (BD). Method: Euthymic individuals (N=20; target N=50, age 18-55) with DSM-IV-TR-defined BD-I/II were enrolled. Cognitive performance was measured with the California Verbal Learning Test (CVLT) and Process Dissociation Task. All subjects provided a morning blood sample after an overnight fast. Cognitive deficits were operationalized as a one standard deviation below the norm. Pro-and anti-inflammatory cytokines (IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-13, TNFα, INFλ) were measured with the Mutliplex Bead Immunoassay. Preliminary evidence indicates that individuals with BD manifest cognitive deficits on short-delay and long-delay free recall (30.6% and 25.7%, respectively). A significant correlation (r=0.456, p=0.043) was found between TNFα and total number of intrusions as well as between IL-8 and total number of repetitions (r=0.458, p=0.042) on the CVLT. Recollection deficits on the Process Dissociation Task were negatively associated with INFλ (r=-0.466, p=0.044). These preliminary results indicate that cognitive deficits in BD persist into euthymia and may be subserved by inflammatory response systems.

P5AM-9-7

LOW DOSE BISPHENOL A; A POTENTIAL FACTOR OF PSYCHOLOGICAL ILLNESS

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We have reported the low dose effects of bisphenol A (BPA) on the sexually dimorphic brain and behaviors. BPA impaired the sexual differentiation of locus ceruleus and exploratory behaviors even if the dosage was below the reference dose (Kubo et al. 2003, Fujimoto et al. 2006). In addition, we demonstrated that prenatal BPA exposure altered the emotional behaviors in which depressive response was enhanced in the forced swimming test (FST). To acquire the further evidences, we examined the effects of BPA on general motor activity and avoidance response to predator odor. The smell of fox predominantly suppressed locomotor activity and enhanced avoidance response in the BPA-treated rats. This result suggested that BPA-rats might be vulnerable to an environmental stress such as a predator odor. Not only prenatal exposure, we also examined the FST in the postnatal BPA exposure rats. Although, postnatal exposure route (via milk) was different from prenatal one (via placenta), postnatal treatment more effectively enhanced the depressive behavior. Recently, many studies have focused on the effects of BPA on rodents central nervous system and nonreproductive behaviors. We have to discuss the potentiality of BPA as a risk factor of psychological illness such as a depression.

P5AM-9-8

DETERIORATED BACKWARD MASKING IN SCHIZOPHRENIC PATIENTS AND THEIR RELATIVES

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Over the past years, studies of unaffected first-degree relatives of schizophrenic patients have reported cognitive deficits in the domains of executive functions, memory, and attention. However, these deficits may rely on lower level information processing deficits. We investigated visual information processing with a visual backward masking task. A vernier target was followed by a grating mask. Observers had to indicate the offset direction of the vernier. We determined the SOA between the vernier and the grating onset for schizophrenic patients, their healthy first order relatives, and a healthy control group. Schizophrenic patients needed SOAs about three times longer than healthy controls to obtain comparable performance; backward masking performance of unaffected relatives was significantly better than the one of patients but significantly worse than performance of controls. It seems that deteriorated performance in visual backward masking reveals an innate vulnerability to suffer from schizophrenia. Therefore, studies on healthy relatives of schizophrenic patients allow one to study prolonged processing without the effects of the illness itself or a corresponding drug treatment.

P5AM-9-9

BEHAVIORAL CHARACTERISTICS AND THE EXPRESSION OF NMDA RECEPTOR SUBUNIT IN THE SERINE RACEMASE KNOCKOUT MICE

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Serine racemase (SR) is an enzyme that synthesizes D-serine, an endogeneous coagonist of the N-methyl-D-aspartate (NMDA) receptor. NMDA receptor hypofunction is considered central to schizophrenia pathophysiology. Therefore, a genetic variant of the SR gene might be associated with the disease. Knockout (KO) mice lacking the SR gene were recently generated. In the present study, we analyzed the behaviors related to pain, anxiety, or fear and investigated the brain expression of NMDA receptor subunit in $SR^{-/-}$ KO and $SR^{+/+}$ wild-type (WT) mice to characterize the behavioral consequence of a lack of serine racemase and to evaluate the $SR^{-/-}$ KO mice as model animals for schizophrenia. We found that the $SR^{-/-}$ KO mice generated significantly less ultrasonic vocalization during noxious stimulation than the $SR^{+/+}$ WT mice did. We also found that the $SR^{-/-}$ KO mice displayed a trend toward increased anxiety-like behavior compared to the $SR^{+/+}$ WT mice in the open field. Furthermore, we found that the expression of the NR2B subunit of NMDA receptor was enhanced in the $SR^{-/-}$ KO mice using real-time RT-PCR. We investigated additional behavioral tests, including the prepulse inhibition responses, to ensure our speculation that the $SR^{-/-}$ KO mice could be used as model animals for schizophrenia.

P5AM-10-1

PREVALENCE OF ADULT OF ADHD COMPAIRMENT BETWEEN HIGHLY EDUCATED SUBJECTS AND ORDINERY ONES

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Introduction : If ADHD is not diagnosed and treated in childhood it can turn to "ADULT ADHS ". Here we tried to find out is prevalence in Iran and find out some differences

Material and method : The examination was single blind with 262 samples.

They were between 18-24 years old. Half of them were medical students and another half not. 6 questions questionery of WHO. The answers were analyzed by special softwere program. The scores were between 0-4. If the score was 4 or higher it was a high suspection to adult ADHD. Scores from 3 (including 3) were not per se. study was a single blind.

RESULTS : Our results showed ahthough ADHD may interrupt normal life may some times, but its not a rule. 1)Percent of ADHD at all was 15.27% 2)Percent of ADHD in ordinary subjects was 12.98% 3)Percent of ADHD in highly educated was 17.56% 4)Comparison of percents between two groups was non-significant.

Conclusion : Our results showed altought ADHD may interrupt normal life, but its not a rule. We suggest first, factors of. this condition must be considered more, another suggestion is that the high percent of adult ADHD must be experienced again in large groups .

Poster Session

P5AM-10-2

THE EFFECT OF METHYLPHENIDATE ON THE AUTONOMIC FUNCTION IN THE RAT

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Methylphenidate (MPH) is widely used for the treatment of attention-deficit hyperactivity disorder (ADHD). Abundant animal studies show that MPH increases the concentration of dopamine (DA) and noradrenaline (NA) in a synapse throughout the brain. The increments of DA and NA contents in the central nervous system possibly affect the autonomic nervous system. However, the effect of MPH on the autonomic nervous system is still unknown. We first examined the effect of MPH on heart rate using Wistar rats under urethane anesthesia. Intra- peritoneal application of a high dose of MPH increased the heart rate. To explore the possibility that the effect of MPH on the rat heart rate could involve the alternation of autonomic nervous system, we evaluated the power spectral analysis of heart rate variability (HRV). The results showed that only high dose MPH (4.0 mg/kg) significantly increased LF/HF as an index of the sympathetic nerve tone, while lower concentrations of MPH (0.5 and 1.0 mg/kg) did not change LF/HF. HF of the indicator the parasympathetic nerve tone is significantly decreased by MPH (1.0 and 4.0 mg/kg). This study demonstrates that at a relatively high dose, MPH increases the sympathetic nerve activity and mid dose MPH reduces parasympathetic nerve activity.

P5AM-10-3

LOCALIZATION OF BRAIN REGION RESPONSIVE TO NEONATAL SOCIAL ISOLATION

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Neonatal social isolation induces prolonged stress and can lead to variety of psychiatric disorders. However, brain regions responsive to social isolation are poorly known. Here we investigated brain areas responsive to neonatal social isolation. We isolated a male pup from its mother and other siblings for 1 or 6 hours at P7-P11. Then, pups were sacrificed and brains were carefully removed. Brain was sectioned, and expression of c-fos, an immediate early gene, was analyzed with immunohistochemistry. We found that neurons in amygdala of isolated pups showed increased c-fos expression, indicating social isolation-induced increase of neuronal activity in this region. This suggests that amygdala responds to social isolation and can be a sensor of "loneliness". We will also report other brain areas activated with social isolation.

P5AM-10-4

QUALITATIVE AND QUANTITATIVE EEG ABNORMALITIES IN VIOLENT OFFENDERS WITH ANTISOCIAL PERSONALITY DISORDER

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Resting eyes closed electroencephalogram was studied in a group of violent offenders evaluated at Psychiatric Department of the Legal Medicine Institute in Cuba (18 with antisocial personality disorder, ASPD, and 10 without psychiatric diagnosis). Characteristics of the EEG visual inspection and the use of frequency domain quantitative analysis techniques (narrow band spectral parameters) are described. Both groups were compared to Cuban normative database. High incidences of electroencephalographic abnormalities were found in both groups of violent offenders. The most frequent were: electrogenesis alterations, attenuated alpha rhythm and theta and delta activities increase in the frontal lobe. In the quantitative analysis theta and delta frequencies were increased and alpha activity was decreased in both groups. Differences appear for the topographical patterns present in subjects of both groups. EEG abnormalities were more severe in ASPD than in control group. Results suggest that EEG abnormalities in violent offenders should reflect aspects of brain dysfunction related to antisocial behaviour.

P5AM-10-5

EFFECT OF MUCIMOL AND PICROTOXIN INJECTION IN AC NUCLEUS OF AMYGDALE ON AGGRESSIVE BEHAVIOR IN RATS

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One neurochemical system most consistently linked with aggression is the GABAergic system. So, the aim of present investigation is study of the effect of muscimol (GABAA agonist), picrotoxin (GABAA antagonist) injection in amygdaloideus centralis nucleus (ac) of amygdala on aggression behavior.
Thirty adult male rats weighing 200-240 were kept under controlled temperature (21-24°C). Water and food were ad libitum. Cannula was implanted into ac nucleus of amygdala using stereotaxic method. Aggression was induced by 2 mA current every 3 seconds for 5 minutes, e.i., and each animal were received 100 electrical shocks every session. After electrical shock, another rat was placed in electroshock chamber. Data were analyzed by one and two ways of ANOVA and Tucky as Post-hoc test. Significant level was $P<0.05$. Our data showed that after injection of muscimol in ac nucleus of amygdala induced a significant increase in aggression ($P<0.05$). Injection of picrotoxin in ac nuclei of amygdala significantly ($P<0.05$) increased aggressive behaviors.
Our result indicated ac nucleus of amygdala modulate aggressive behavior due to GABAA receptors.

P5AM-10-6

EFFECT OF MUSCIMOL AND PICROTOXIN INJECTION IN AM NUCLEUS OF AMYGDALE ON AGGRESSIVE BEHAVIOR IN RATS

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The aim of present investigation is study of the effect of muscimol ($GABA_A$ agonist), picrotoxin ($GABA_A$ antagonist) injection in amygdaloideus medialis nucleus (am) of amygdala on aggression behavior.
Thirty adult male rats weighing 200-240 were kept under controlled temperature (21-24°C). Cannula was implanted into ac and am nuclei of amygdala using stereotaxic method. Aggression was induced by 2 mA current every 3 seconds for 5 minutes, e.i., and each animal were received 100 electrical shocks every session. After electrical shock, another rat was placed in electroshock chamber. Data were analyzed by one and two ways of ANOVA and Tucky as Post-hoc test. Significant level was $P<0.05$. Our data showed that after injection of muscimol in am nucleus of amygdala induced a significant increase in aggression ($P<0.05$). Injection of picrotoxin in am nucleus of amygdala significantly ($P<0.05$) increased aggressive behaviors.
Our result indicated am nucleus of amygdala modulate aggressive behavior due to $GABA_A$ receptors.

P5AM-10-7

CHARACTERIZING AND AMELIORATING LEARNING DEFICITS OF DOWN SYNDROME MICE USING A COMPUTERIZED OLFACTOMETER

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We tested the learning performance of trisomic Ts65Dn mice and disomic littermates in a computerized go-no go task where mice learned to discriminate between the odors of citral (10% in mineral oil) and mineral oil. We concluded that at least some of the trisomics are able to perform the task, but the disomics require about half the number of trials to reach a learning criterion (disomic=343±249 trials, trisomic=716±342 trials, n=6, P=0.035, paired student t-test). In addition, when we presented new odor pairs the disomic mice performed significantly better than trisomics. We also performed an additional odor search test were the animal has to dig to find a petri dish containing peanut butter, and we observe that the disomics find the peanut butter about two times faster than the trisomics. Our hypothesis is that learning deficits of trisomic Ts65Dn mice are due to decreased cholinergic innervation of key brain areas. After finishing characterization of behavioral deficits of the trisomic mice we plan to alleviate the deficits by performing deep brain stimulation in the cholinergic basal forebrain, and i.p. injections of galantamine to improve performance of synaptic transmission by basal forebrain cholinergic neurons.

P5AM-11-1

DELETION OF TAU AMELIORATES HEAT SHOCK-INDUCED INJURY IN CULTURED CORTICAL NEURONS

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Early studies implicated that microtubule associated protein tau is involved in amyloid-beta-induced neurotoxicity and glutamate-induced excitoxicity. However, role of tau in neuronal responses to other insults remained unclear. Here, we examined whether deletion of tau would change cell injury induced by heat shock in primary cultured cortical neurons. After 45°C heat shock insult for 30 min, the maximum of lactate dehydrogenase release was reduced 1.5-fold by tau deletion. The processes of WT neurons showed more dramatic abnormalities than those of TKO neurons after the same insult treatment. Both WT and TKO neurons exhibited a similar pattern in the elevation of HSP70 level, but different in the time course of Akt phosphorylation. In contrast to an early and brief response in WT neurons, TKO neurons displayed a late but long-lasting increase in phosphorylation of Akt as well as its downstream GSK3β. Additionally, inhibition of Akt activity suppressed cell viability in both WT and TKO neurons exposed to heat shock. In conclusion, our results demonstrated that deletion of tau ameliorated heat shock-induced neuronal injury and suggest that enhanced Akt response in absence of endogenous tau may represent a compensatory mechanism in regulation of cell survival to stress stimuli.

P5AM-11-2

PATHOPHYSIOLOGY OF HEMIFACIAL FLUSHING AND CONTRALATERAL ANHIDROSIS WITH CROSSED HYPOHIDROSIS BELOW THE CHEST

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Hemifaicial flushing and contralateral anhidrosis in adulthood is sometimes observed in sympathetic trunk lesion, e.g. mass lesion, thoracic outlet syndrome, or malignant tumor. However, we observed the symptom in three young boys (1 yr, 2 yr, and 9 yr), possibly due to damage at delivery. Case 1 and 2 had right himifacial flushing with higher skin temperature of right arms and contralateral anhidrosis. Case 3 had the similar symptoms in the opposite side with the right Horner syndrome. Minor method presented anhidrosis in the face contralateral to flushing to C4 level in case 1, to C5 level in case 2, and to C6 level in case 3 with crossed hypohidrosis of lower parts than the levels respectively in all cases. Laser doppler blood flowmetry showed low skin blood flow volume at anhidrosis area without the laterality of lower part in case 2 and 3. The lesions were speculated between the left superior and middle for case 1, near middle for case 2, and between the right middle cervical and stellate ganglion. We suspected these symptoms were caused by minor cervical damages when the patients went though the birth canal, associated with direction and rotation of the fetal head. Pathophysiological examination may be necessary when diagnosing facial dysautonomia.

P5AM-11-3

ROLE OF $[Ca^{2+}]_i$ AND $[Cl^-]_i$ ALTERATIONS IN DISTINCT PATTERN OF GLIOBLASTOMA CELL MIGRATION

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Malignant glioma invasively grows to widespread regions of brain. During their extensive migration, glioma cells change in the motility, polarity and cell shape. However, it is not well known about cellular mechanism how these changes were orchestrated in a migrating cell. To address this, we have examined the role of alterations in intracellular ions such as Ca^{2+} and Cl^- during migration. By using time-lapse imaging, we observed that malignant glioma cells migrate with changing its direction and the rate of the movement on matrigel-substrate. To examine the distinct pattern of changes in $[Ca^{2+}]_i$ and $[Cl^-]_i$ during migration, we monitored the temporal and spatial changes in $[Ca^{2+}]_i$ and $[Cl^-]_i$ of migrating glioma cells. Time-lapse Ca^{2+} imaging experiments revealed that Ca^{2+} oscillation was often observed in the monopolar or bipolar shaped cells. We also monitored the spatio-temporal dynamics of $[Cl^-]_i$ by FRET imaging using a genetically-encoded indicator, Clomeleon. Interestingly, treatment with bumetanide, a Na-K-Cl co-transporter inhibitor, accelerated motility, whereas chlorotoxin, a Cl^- channel inhibitor, suppressed. How bumetanide affects the glioma cell migration by modulating $[Ca^{2+}]_i$ and $[Cl^-]_i$ has been studied.

P5AM-11-4

AMYOTROPHIC LATERAL SCLEROSIS AND SOD1 MUTATION

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Amyotrophic lateral sclerosis (ALS) is an age-dependent neurodegenerative disease that causes motor neuron degeneration. Mutations in Cu, Zn superoxide dismutase (SOD1) are one cause for the familial form of this disease. Transgenic mice expressing mutant SOD1 develop age-dependent motor neuron degeneration, muscle weakness, paralysis and death. The mechanism whereby mutant SOD1 induces motor neuron degeneration is not understood but widespread mitochondrial vacuolation has been observed during early phases of motor neuron degeneration. To determine which of these possibilities are true, we examined the vacuolar patterns in detail in transgenic mice expressing mutant SOD1G93A. Vacuolar patterns revealed by electron microscopy (EM) suggest that vacuoles originate from the expansion of the mitochondrial intermembrane space and extension of the outer mitochondrial membrane. Immunofluorescence microscopy and immuno-gold electron microscopy reveal that vacuoles are bounded by SOD1 and mitochondrial outer membrane markers. Vacuoles lack lysosomal signal but contain abundant peroxisomes and SOD1 aggregates. Mutant SOD1, possibly by toxicity associated with its aggregation, causes mitochondrial degeneration by inducing extension and leakage of the outer mitochondrial membrane.

P5AM-11-5

DELETION OF SHN2 CAUSES ABNORMAL BEHAVIORS RELATED TO PSYCHIATRIC DISORDERS AND FAILURE IN MATURATION OF THE DENTATE GRANULE CELLS IN MICE

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Schnurri-2 (Shn2) is a mouse homologue of human HIVEP2. Shn2 knockout (KO) mice showed hyperactivity, impaired prepulse inhibition, impaired working memory and an exaggerated infradian rhythm. Hippocampal transcriptome pattern of mutant mice was strikingly similar to that of alpha-CaMKII heterozygous KO mice that also show abnormal behaviors related to psychiatric disorders, with more than 70 differentially-expressed probes shared with these mutants. Histological analysis revealed that calbindin-D 28k, a marker for mature granule cell, was almost abolished in the dentate gyrus (DG) of mutant mice. Electrophysiological properties of the mutant DG granule cells were similar to those of immature neuron in normal mice. These results suggest that abnormality in DG may underlie abnormal behaviors related to psychiatric disorders.

P5AM-11-6

IS MYOFIBRILLOGENESIS REGULATOR 1 A TRULY CAUSATIVE GENE OF PAROXYSMAL DYSTONIC CHOREOATHETOSIS? - ANALYSIS OF HUMAN CASES AND HAMSTER MODEL

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Familial paroxysmal dystonic choreoathetosis (PDC) is a neurological disorder, which is characterized by episodes of involuntary movements. Recently, myofibrillogenesis regulator 1 (*MR-1*) gene has been reported to be a causative gene for PDC. PDC model hamsters (dt^{sz}), whose clinical symptoms are similar to those of human PDC, are also reported. However, a causative gene of dt^{sz} remains unclear. To clarify the pathogenesis of PDC, we performed genetic analysis of human and hamster *MR-1* genes. The linkage of PDC to chromosome 2q32-36 was confirmed in a Japanese PDC family. A7V mutation in *MR-1* gene was found in PDC-affected patients, which was consistent with the mutations reported in American PDC families (A7V and A9V). We also performed mutational analysis in dt^{sz} hamster model after investigating genome structures of hamster *MR-1* gene. Interestingly, mutations identical to those found in human cases were not observed. These results imply that truly causative genes of dt^{sz} may be channel/transporter proteins that are related to MR-1. We are now performing genetical analysis of candidate genes of dt^{sz}. Further analysis of *MR-1* and related genes in the patients with PDC may help facilitate the development of effective therapies for paroxysmal neurological disorders.

P5AM-11-7

ABNORMAL NEURAL FIRING AND CORTICALLY EVOKED LONG-LASTING INHIBITION OF PALLIDAL NEURONS IN A DYT1 TRANSGENIC MOUSE MODEL OF DYSTONIA

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Dystonia is a neurological disorder characterized by sustained or repetitive involuntary muscle contractions and abnormal postures. A major group of early-onset generalized dystonia arises from a mutation in the DYT1 gene, which encodes torsinA protein. To understand the pathophysiology of dystonia, neurophysiological analyses were performed on a mouse model of early-onset generalized dystonia, DYT1 transgenic mice overexpressing mutant torsinA. The DYT1 transgenic mice showed abnormal muscle activity, such as co-activation of agonist and antagonist muscles and sustained muscle activation. Recording neuronal activity of both pallidal segments in awake state revealed markedly decreased spontaneous activity and abnormal neural firing patterns with irregularly grouped discharges and intermittent long pauses. Motor cortical stimulation evoked abnormal responses with a long-lasting inhibition, which were never observed in the normal mice. In addition, somatotopic arrangements in both pallidal segments were disorganized. These results suggest that the abnormal neural firing patterns, long-lasting inhibition to the cortical stimulation and disorganization of somatotopic arrangements in globus pallidus participate in abnormal motor control in dystonia.

P5AM-11-9

ALTERATIONS IN MONOAMINERGIC NEURONS IN OREXIN NEURON-ABLATED MICE

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The neuropeptides orexin A and B are crucial regulators of sleep and wakefulness. Loss of orexin-producing neurons results in narcolepsy. Orexins are exclusively produced in hypothalamic neurons (orexin neurons), which abundantly project to monoaminergic neurons that are important in sleep-wake regulation. Orexin neuron-ablated mice (*orexin/ataxin-3* mice) showed cataplexy and sleep/wake fragmentation. We examined electrophysiological property of monoaminergic neurons in *orexin/ataxin-3* mice.

A neuronal activity of serotonergic dorsal raphe (DR) and noradorenergic locus ceruleus (LC) neurons was recorded extracellulary under monitoring electroencephalographic (EEG) and electromyographic (EMG) activity. Sleep-wakefulness stages were judged with EEG/EMG signals.

There was no significant difference in activity of DR neurons between *orexin/ataxin-3* mice and wild type mice during all stages. Meanwhile, firing frequency of LC neurons in *orexin/ataxin-3* mice was significantly higher than that in wild type mice during wakefulness and transitional non REM sleep. Firing rate of the LC neurons did not decrease immediately after transition from wakefulness to non REM sleep in *orexin/ataxin-3* mice. These results indicate that loss of orexin neurons resulted in chronic alteration of LC neurons.

P5AM-11-10

OLFACTORY STIMULATION WITH GRAPEFRUIT OIL AND LAVENDER OIL ON AUTONOMIC OUTFLOWS

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This report deals with effects of olfactory stimulation with scent of grapefruit oil (SGFO)and lavender oil (SLVO) on the activity of sympathetic branch innervating white adipose tissue, brown adipose tissue, adrenal medulla and gastric branch of vagus nerve in anesthetized rats. Stimulation with SGFO facilitated sympathetic nerve activity and suppressed vagal nerve activity to stomach, and elevated plasma glycerol level. On the contrary, stimultion with SLVO suppressed sympathetic and facilitated vagal nerve, and decreased plasma glycerol level. A 15-min exposure to SGFO three times a week in non-anesthetized rats reduced food intake and body weight, however, same procedure with SLVO resulted in opposite responses. It can be mentioned that SGF0 activates body function and increase in energy expenditure, and SLVO shows sedative and assimilative effects on body function through modulation of auonomic nerve activity. These results may give physiological bases on aromatherapy.

P5AM-11-11

ESTRADIOL INTERACTS WITH OPIOID PEPTIDES TO MODULATE BEHAVIORAL SENSITIZATION TO COCAINE IN FEMALE RATS

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The present study investigated the role of opioid receptors in estrogenic modulation of behavioral sensitization to cocaine in females. Rats were ovariectomized (OVX), half received a Silastic implant filled with estradiol benzoate (EB), the other half received an empty implant. A week later, they were tested for their locomotor response to cocaine (15 mg/kg, i.p.) in the presence or absence of a mu (naloxonazine (NLZ); 15 mg/kg, i.p.) or kappa (nor-binaltorphimine (nor-BNI), 10 mg/kg, sc) opioid receptor antagonist. Nor-BNI exacerbated the acute response to cocaine in OVX-EB rats whereas NLZ had no effect. In contrast, NLZ abolished the development of behavioral sensitization to cocaine in OVX-EB rats, whereas nor-BNI had no further effect. fMRI studies revealed that the increased neural activity observed in OVX-EB rats sensitized to cocaine was also decreased by NLZ pretreatment. Mu and kappa opioid peptides exert opposing effects on cocaine-induced behavioral sensitization in female rats and this effect is dependent on plasma estradiol. Supported by NINDS-U54NS39405 and NIGMS-S06GM08224.

P5AM-11-12

METHAMPHETAMINE EXPOSURE RESULTS IN DIFFERENTIALLY EXPRESSED PROTEINS IN THE RAT FRONTAL CORTEX: A PROTEOMIC APPROACH

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The effects of methamphetaine are primarily mediated via the mesocorticolimbic dopamine system which projects from the ventral tegmental area to the forebrain. One of these areas, the frontal cortex, has been suggested to be a major role player in drug addiction and reward, and in the initiation drug seeking behaviour, craving and relapse. We used proteomic techniques to investigate the effects of methamphetamine on intracellular, membrane and membrane associated proteins in the frontal cortex. Two-dimensional gel electrophoresis detected 62 and 44 differentially expressed stained spots in the cytosolic and membrane fractions respectively. The differentially expressed proteins were excised and subjected to mass spectrometry analysis for identification. These proteins represented several functional categories, including signal transduction, energy metabolism, cellular transport, binding proteins, protein synthesis, cell division, cell structure and rescue, and protein degradation. This study therefore provides further insight into the extensive molecular effects of methamphetamine on neuron structure and function that may be relevant to addictive behaviour.

P5AM-12-1

REGULATION OF SLEEP BY THE HABENULAR NUCLEUS: INVOLVEMENT OF ADENOSINE AND SEROTONIN

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The habenular nucleus, as an important link between the limbic forebrain and the midbrain nuclei, has been implicated in the regulation of sleep. The present study investigated the role of the habenular nucleus in this process and the possible underlying molecular mechanism, using both a rat model with habenular lesion and a hypnotic-treated rat model. The results showed that habenular lesions induced waking and reduced both non rapid-eye movement sleep and rapid-eye movement sleep in electroencephalographic recordings. A microarray analysis also demonstrated up-regulation of waking-related genes in the hypothalamus after habenular lesions. The expression of adenosine and serotonin related genes was examined by RT-PCR and shown to be up-regulated in the hypothalamus of the rats with habenular lesions. In contrast, the expression of these genes was down-regulated in the hypothalamus and the habenular nucleus of rats treated with a well-known hypnotic, zolpidem. The present results provide the first functional and molecular evidence indicating that the habenular nucleus has a sleep promoting role in sleep regulation. They also strongly support the involvement of both adenosine and serotonin in mediating the sleep regulation process in habenular nucleus.

P5AM-12-2

OPTICAL CONTROL OF OREXIN/HYPOCRETIN NEURONAL ACTIVITY

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Orexin/hypocretin is a neuropeptide which is specifically expressed in neurons in the hypothalamus. Orexin has an important role in the regulation of sleep/wakefulness. The animals lacking prepro-orexin or orexin neurons shows a fragmentation of sleep/wakefulness and sudden muscle atonia. Those symptoms are observed in narcoleptic patients. To further study physiological significance of orexin neuronal activity on maintenance of waking state, we generated transgenic mice in which orexin neurons specifically express halorodopsin. The activity of halorodopsin expressing neurons are inhibited by emitting yellow light since halorodopsin is a yellow light activated chrolide pump. Immunohistochemical study revealed that more than 90% of orexin-immunoreactive neurons express halorodopsin in this transgenic mice brain. Slice patch clamp analyses showed that yellow light irradiation hyperpolarized membrane potential and terminated spontaneous action potentials in the orexin neurons. These transgenic mice might allow us to control the activity of orexin neurons in in vivo.

P5AM-12-3

EFFECTS OF PERIPHERAL AND CENTRAL H1 HISTAMINE BLOCKERS ON DAILY SLEEP AMOUNTS AND ARCHITECTURES IN RATS

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Classic H1 histamine blockers produce sleepiness in humans, whereas modern more specific H1 blockers, which reduced permeability through the blood-brain barrier, represent less sedative effects. Although these suggest cerebral histamine as an arousal regulator, specific H1 blocker functions on sleep-wake cycles have not been analyzed in details. Here, we approached this in rats. Rats received 0.04-4 mg/kg i.p. injections of chlorpheniramine (CPA), the first generation H1 blocker, at the dark onset time dose-dependently increased non-rapid-eye-movement (non-REM) sleep and reduced REM sleep for subsequent 3 hours. The i.p. injection of cetirizine, the third generation H1 blocker, failed to modulate sleep, consistent with reported pharmacokinetics of these H1 blockers. Continuous intracerebroventricular (i.c.v.) infusion of CPA or cetirizine further demonstrated that 10 μmol CPA infusion increased drowsiness but not non-REM sleep with a partial inhibition of REM sleep whereas 10 μmol cetirizine continuously increased non-REM sleep and decreased REM sleep and wakefulness for more than 10 hours. Therefore, these results demonstrate that specific H1 blockers in the brain work as non-REM sleep-increasing and REM sleep-suppressing reagents with inhibition of arousal levels.

P5AM-12-4

ROLES OF OREXIN RECEPTOR SUBTYPES AND HISTAMINERGIC SYSTEM IN SLEEP/WAKE STATES

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Orexin A and orexin B were initially identified as endogenous ligands for two orphan G protein coupled receptors, named orexin-1 (OX1R) and orexin-2 receptor (OX2R). The finding that orexin deficiency causes narcolepsy in human and animals suggests that these hypothalamic neuropeptides play a critical role in regulating sleep/wake states. In this study, we examined roles of each orexin receptor in regulation of sleep/wake states by EEG/EMG recordings. While OX1R-/- mice showed almost normal sleep/wake behavior, OX2R-/- mice were affected with cataplexy attacks and fragmentation of sleep/wake states. OX1R-/-/OX2R-/- mice showed more severe narcoleptic phenotype, comparable to orexin-/- mice. These observations suggest that, although both OX1R- and OX2R-mediated pathways play roles in maintenance at arousal, OX2R plays a highly important role in maintenance of wakefulness.

OX2R is abundantly expressed in the histaminergic TMN. To further dissecting downstream effectors of the OX2R-mediated pathway, we generated OX1R-/-; H1R-/- mice, and examined their sleep/wake behavior. Unexpectedly, OX1R-/-; H1R-/- mice showed normal sleep/wake phenotype. These results suggest that basal maintenance of sleep/wake system is completely achieved by OX2R without the H1R-mediated pathway.

P5AM-12-5

ADMINISTRATION OF Am80, AN AGONIST OF RETINOIC ACID RECEPTOR, IMPROVES THE AGING-RELATED DECREASE OF REM SLEEP IN SAMP8 MICE

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SAMP8 mice exhibit an accelerated-aging in learning and memory compared with normal aging (SAMR1) mice. It is also reported the SAMP8 shows the deterioration in the sleep-wake states. Vitamin A (VA) is a lipophilic vitamin, which function is induced by the binding of retinoic acid (a metabolite of VA) to retinoic acid receptor (RAR) or retinoid X receptor (RXR). Many studies reported that VA improves the aging of learning and memory. We hypothesized the aging of sleep-wake architecture would be also improved by the activation of RAR. To test this hypothesis, we carried out the 24-hour sleep recording in SAMP8, SAMR1 and SAMP8 administrated RAR agonist (Am80).

SAMP8 showed decreases in rapid eye movement (REM) sleep and theta power compared with SAMR1. Am80 administration induced the recovery of REM sleep and theta power. Quantitative RT-PCR analysis indicated decreases in RARalpha, RXRbeta, transthyretin (TTR) and choline acetyltransferase expression in SAMP8 hippocampus compared with SAMR1. Am80 administration induced increases of TTR in hippocampus and vesicular acetylcholine transporter in brainstem.

Our result suggests the possibility that Am80 administration improves the aging-related decreases in REM sleep and theta power via cholinergic neuromodulation.

P5AM-12-6

NEUROVASCULAR COUPLING IN THE HIPPOCAMPUS IS ALTERED DURING REM SLEEP IN RATS

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The present study aimed to examine relationship between hippocampal CA1 neuron activity and hippocampal regional cerebral blood flow during sleep-wake cycle in rats. Wistar male rats were instrumented chronically with multiple electrodes for the measurement of the hippocampal CA1 neuron activity and bipolar electrodes for measurement of electroencephalogram and electromyogram. In a separate group, rats were instrumented chronically with a laser-Doppler flow probe made with a pair of glass fiber in the CA1 region of the hippocampus, and EEG and EMG electrodes. The mean value of the CA1 neuron activity was lowest during REM sleep compared with the other behavioral states including non-REM sleep, quiet awake, moving, grooming states while the CA1 neuron activity was highest during moving states. However, the mean value of the local cerebral blood flow in the CA1 region of the hippocampus was the highest during REM sleep compared with other behavioral states. During REM sleep, the relationship between CA1 neuron activity and regional cerebral blood flow was dissociated from those obtained during the other behavioral states. Thus, it is concluded that neurovascular coupling in the hippocampus seems to be altered during REM sleep in rats.

P5AM-12-7

EFFECT OF SLEEP ON AUDITORY EVOKED POTENTIAL

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Early potentials recorded within the first 10 ms after a brief auditory stimulus, which are called brainstem auditory evoked potentials (ABRs), reflect the activation of auditory structures in the brain. Previous papers reported that no significant effect of sleep on ABR was observed in humans. In order to confirm it, we recorded ABRs across sleep-wake states in rats and investigated the effect of sleep on ABR latency and amplitude. We found that, i) ABR amplitude during NREM sleep was bigger than that during wakefulness and REM sleep, and ii) ABR latency during NREM sleep was smaller than that during wakefulness. In addition, we examined the effect of injection of atropine, a muscarinic receptor antagonist, on ABRs and found that iii) injection of atropine tended to increase ABR amplitude, and iv) ABR latency was almost no changed after the injection. Because atropine blocks cholinergic transmission and cholinergic neurons in the brainstem are active during wakefulness and REM sleep and inactive during NREM sleep, our results suggest that ABR amplitude that changes across sleep-wake stages reflects the activity of cholinergic system.

P5AM-12-8

EEG DELTA POWER IN NREM SLEEP IS ENHANCED BY REFEEDING AFTER 24-HR FASTING IN TIME-DEPENDENT MANNER

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There are many evidences that energy balance influences sleep/wake patterns. It is reported that fasting and high-fat feeding can change sleep/wake time. It has been demonstrated that during fasting, wake time is increased, and rapid eye movement (REM) sleep time is decreased. It remains, however, unclear how delta power in non-REM (NREM) sleep which is accepted to be a parameter of sleep depth would change by fasting. We therefore observed the delta power during fasting and refeeding period. We monitored body temperature, electroencephalogram and electromyogram for 24 hours under light-dark condition throughout the experiment (3 days).On the first day, mice were fed with diet ad libitum. On the second day, the mice were fasted from zeightgeber time 1 (ZT1). On the third day, the mice were refed at ZT1. The delta power in NREM sleep was significantly increased after refeeding on the third day, while it was not affected during fasting period (day2). Interestingly, when we changed the refeeding time from ZT1 to ZT7, no increase of delta power by refeeding was observed. Thus the increase in delta power by refeeding is suggested to be dependent on time.

P5AM-12-9

INFLUENCES OF SLEEP SPLINT ON ELECTROENCEPHALOGRAM

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A sleep splint is used as a dental countermeasure against sleep apnea syndrome and snoring. It brings the lower jaw four millimeter forward aiming at maintenance of one's airway opening. However, the result of the Bi-Digital O-Ring Test (BDORT) *was not necessarily desirable. We manufactured a personal sleep splint for the individual using the BDORT to make one relax and promote good sleep.

In this study, we compared the effects of an ordinary splint and the BDORT adjusted splint on electroencephalogram (EEG) in five healthy men. Delta, theta, alpha and beta waves were analyzed in each case.

Results showed beta waves were dominant under ordinary splint conditions. While, alpha, theta and delta waves were increased in BDORT adjusted splint.

From these results, we found BDORT adjusted sleep splint decreased stress and sedated the brain activity. This splint may be able to induce one into a deep and qualitative sleep. Biting conditions must be counted as one of the important factors for the treatment of the disease and health promotion.

*:Omura Y., Acupunct Electrother Res. 1981;6(4):239-54

P5AM-12-10

THE EFFECTS OF SLEEP DEPRIVATION ON CORTICAL FUNCTIONS AND PLATELETS RECTIVITY IN NORMAL SUBJECTS

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Introduction: in healthy humans, sleep deprivation (SD) has consistently been demonstrated to impair different cortical parameters. The objective was to assess the changes in cortical functions and platelets reactivity after sleep deprivation in normal subjects. Materials and methods: the variables analyzed were: quantitative EEG, the event-related potential (P 300) and neurobehavioral parameters (Attention Test and Memory Test performance). We recorded EEG and P300 and performed Memory and Attention Tests after a normal night sleep (baseline data) and also after 30 hours of sleep deprivation. The platelets reactivity was assessed by evaluation of platelets circulating aggregates. Results: electrophysiological recording show a delayed P300 and a change in EEG topography as compared to the baseline. Attention-Tests and Working Memory-Tests performance also decreased The platelets circulating aggregates were significantly increased in sleep deprivation subjects (were assumed to cathecolamine incresases). Conclusions: sleep deprivation compromises the cortical functions, especially a drastic decrease of alertness. The compromised cortical functions were associated with platelets hypereactivity and may lead consequently to the development of cerebrovascular morbidities.

P5AM-12-11

CHANGE IN THE THRESHOLD OF REFLEXIVELY-EVOKED SWALLOWING DURING SLEEP IN MAN

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Although it is known that swallowing is episodic, with long swallow-free periods during sleep, little is known about the influence of sleep in initiation of swallowing in response to peripheral stimulation. The aim of the present study was to test if the threshold of swallowing reflex is increased during sleep in man. In three healthy volunteers, a custom-made monopolar silver electrode connected with flexible teflon-coated multi strained stainless steel wire was introduced into the pharynx via the nasal cavity under the endoscopical observation. The stimulating electrode was fixed on the posterior wall of the oropharynx or hypopharynx and the indifferent electrode was placed on the forehead. Then, 30 trains of electrical pulses (1 ms duration at 30 Hz, maximum intensity < 0.8 mA) were delivered. Swallows were identified by visual observation of movement of larynx and electromyographic (EMG) burst of suprahyoid muscles. Electroencephalogram was recorded to determine wakefulness and sleep. Swallowing was successfully elicited by electrical stimulation of oropharynx or hypopharynx during wakefulness. However, the incidence of such reflexively-evoked swallowing was decreased during sleep. The finding suggests that the threshold of swallow reflex is increased during sleep.

P5AM-13-1

INHIBITORY EFFECT OF MOXIBUSTION FOR THE SERUM IL-6 LEVELS IN COLLAGEN-INDUCED ARTHRITIS MICE

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We have reported that moxibustion to MEIMON (GV4) acupoint suppressed severity of collagen-induced arthritis (CIA) in DBA/1J mice. However, the mechanism in the effect of moxibustion is still not clear. We studied whether moxibustion (MOX) inhibited inflammatory cytokine (IL -6) related to the onset of autoimmune disease.

Male DBA/1J mice were separated into four groups. Group 1: control (no treatment), Group 2: control+MOX (MEIMON, 1.0mg), Group 3: CIA (immunized with bovine type II collagen), Group 4: CIA+MOX (1.0mg). Moxibustion (MOX): applied moxa cone on the acupoint 5 times a day, 3 days a week, for 2 weeks. We evaluated the severity of arthritis by using arthritis score method in all the groups and determined the serum IL-6 levels on day35. Moxibustion to GV4 (1.0mg) significantly suppressed the arthritis score and incidence compared to CIA group. The CIA+MOX group inhibited the serum IL-6 levels in comparison with the CIA group significantly.

The results suggested that the moxibustion suppressed arthritis symptom in CIA mice, at least in part, through the inhibition of the inflammatory cytokine IL-6.

P5AM-13-2

EFFECT OF MOXIBUSTION ON THE SERUM IFN-γ LEVELS IN COLLAGEN-INDUCED ARTHRITIS MICE

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Recent studies provide evidences that Th17 cell has a critical role in the pathogenesis of several autoimmune disorders. It is reported that IFN-γ interferes with the differentiation of Th17 cell in the rheumatoid arthritis in vitro. We have reported that the moxibustion(Mox) inhibited the symptom and progress of collagen-induced arthritis(CIA), but its mechanism has not been clear yet. We measured serum IFN-γ levels in CIA mice to examine the association with inhibitory effect of moxibustion on CIA. Male DBA/1J mice were separated into four groups. CIA group: immunized with bovine type II collagen twice, CIA+Mox group: immunized and applied with moxibustion, by 1mg moxa cone 5 times a day to the MEIMON(GV4) 3 times per week for 2 weeks, Control group: not immunized and no treatment, and Control+Mox group: applied with moxbustion only. We evaluated the severity of arthritis using arthritis score method and also determined the serum IFN-γ levels by ELISA. Moxibustion to GV4 significantly suppressed the arthritis score and incidence compared to CIA group. The serum IFN-γ levels in CIA+Mox group was significantly higher than that in CIA group on day28. The results suggested that moxibustion increase the serum IFN-γ levels in CIA mice, and then inhibit CIA.

P5AM-13-3

ANTI-INFLAMMATORY ACTIVITIES OF TAMARINDUS INDICA L. AQUEOUS FRUIT EXTRACT

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The aim of study: to evaluate the anti-inflammatory activity and mechanism of action of Tamarindus indica L. The anti-inflammatory activity of the Tamarind fruit aqueous extract, administrated orally at doses 60, 100, 300 and 600 mg/kg, was assessed using carrageenan induced rats paw edema model. It was found that Tamarind possessed inhibitory activity on acute phase of inflammation caused by carrageenan. The effects of highest dosage of the extract were then examined on the rat paw edema caused by inflammatory mediators, histamine and serotonin. At 600mg/kg, Tamarind caused maximum inhibitions of inflammation induced by carrageenan (83.33%), by histamine (83.67%) and serotonin only (46.08%). Loratadine and mianserin were used as antagonist for histamine and loratadine respectively. In sub-chronic inflammation model, Tamarind provoked a significant reduction of both proliferative and transudative phase when tested on cotton pellet-induced granuloma model. At 600mg/kg, Tamarind (600mg/kg) caused maximum inhibition of granuloma with 22.00%. These results show that *Tamarindus indica* L. aqueous fruit extract has anti-inflammatory activities consistent with the use of the extract in management of pain in folkloric medicine.

P5AM-13-4

ROLE OF SEX STEROIDS IN MODULATING INFLAMMATORY CYTOKINES CHANGES AFTER TRAUMATIC BRAIN INJURY

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Background: Following a traumatic brain injury (TBI), the excessive release of inflammatory cytokines is a major cause of cerebral edema. This study examined the changes in production of proinflammatoy cytokines IL-1β, IL-6, TNF-α, TGF-β after sex steroids treatment in brain- injury rats at 24h post injury.

Material And Methods: ovariectomized (OVX) rats received either diffuse brain injury or sham surgery (control). The hormones were given intraperitoneally at 1h by combined from [using estrogen (E) plus progesterone (P) in four groups with different doses of these steroids]. The protein concentrations of IL-1β, IL-6, TNF-α, and

TGF-β in brain were measured using ELISA.

Results: we found that, E1+P1 group, significantly suppressed the injury induced up regulation of IL-1β and TGF-β while having no effect on TNF-α. E2+P2 group also resulted in a significant decrease in IL-6 levels. The elevation of IL-6, TGF- β, are significantly suppressed by E1+P2.

Conclusion: our results suggest that combined sex hormones exposure may attenuate the production of proinflammatory cytokines early after TBI, and this may be one mechanism by which these sex steroid hormones reduce cerebral edema.

Key words: Estrogen, Progesterone, Brain Edema, Cytokines, Traumatic Brain Injury

P5AM-13-5

STUDY TO EVALUATE CORRELATION BETWEEN EXPERIMENTALLY INDUCED VARIOUS INFLAMMATORY MODELS SEIZURE AND BIOCHEMICAL PARAMETERS IN RATS

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Study was to produce various inflammatory models and seizure and to understand the effect of different drugs on seizure and to find out the correlation with antioxidant parameters.

Total of 54 male rats were divided into 3 groups of acetic acid colitis, adjuvant arthritis, and cotton wool granuloma. Thalidomide was used in first groups while etoricoxib was used in cotton wool granuloma group. After development of various inflammatory models, a sub-convulsive dose of pentylenetetrazole(40mg/kg i.p.) was injected ip to note seizure onset and seizure score. Parameters were assessed morphology and histology including plasma and brain biochemical parameters like MDA, SOD, GPx.

The models of colitis and arthritis were effectively produced as evidenced by morphology scores ($p<0.001$). Seizure onset was reduced and grade was increased ($p<0.001$). Thalidomide reduced the morphological ($p<0.002$) and seizure grade ($p<0.001$) while increased seizure onset ($p<0.001$) in the arthritis group. There was an increase in MDA levels in the brain of thalidomide treated arthritis group ($p<0.05$) while there was a no significant raise in SOD and GPx levels.

Study showed decreased threshold to PTZ induced seizure with increased lipid peroxidation and reduced SOD and GPx following oxidative stress.

P5AM-13-6

EFFECT OF MOXIBUSTION ON THE LYMPHOCYTE SUBPOPULATIONS IN COLLAGEN-INDUCED ARTHRITIS IN MICE

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It is known that Moxibustion (Mox) affects immune system activity. We have reported that Mox applied to the Meimon acupoint (GV4) suppressed severity of collagen-induced arthritis (CIA). To examine whether immune cells are involved in suppressive effect of Mox, we investigated the change of lymphocyte subpopulations in inguinal lymph nodes, which is regional lymph nodes of GV4, by flowcytometric analysis. Male DBA 1J mice were separated into 4 groups. Group 1, Control: no treatment. Group 2, Control+Mox: treated with Mox by 1mg moxa cone 5 times a day, 3 days a week, for 2 weeks. Group 3, CIA: immunized with bovine type II collagen. Group 4, CIA+Mox: immunized and treated with Mox. Clinical symptom was observed until day 35 in CIA, while the symptom was significantly suppressed in CIA+Mox at day 35. At day 28 and 35, the ratio of B cell / T cell was increased and the ratio of CD4 / CD8 was decreased significantly in CIA and CIA+Mox. The percentage of $CD25^+CD4^+$ T cells in $CD4^+$ T cells was significantly increased in CIA+Mox compared with CIA. It is known that $CD25^+CD4^+$ T cells suppress autoimmune diseases such as rheumatoid arthritis. Present study suggested that the increase of $CD25^+CD4^+$ regulatory T cells is involved in the mechanism of the suppressive effect of moxibustion.

P5AM-13-7

PERIPHERAL AND CENTRAL ANTAGONISM OF CYTOKINES ENHANCES THE RESOLUTION OF ANOREXIA, LETHARGY AND FEVER INDUCED BY LIPOPOLYSACCHARIDE

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Although fever, anorexia and lethargy may be beneficial sickness responses initially they may become detrimental to the host if they continue for prolonged periods. We therefore investigated whether antagonizing the biological action of putative mediators of these responses, interleukin (IL)-6 and IL-1β, could reduce the duration of these sickness responses, measured as changes in food intake, voluntary activity and body temperature, induced by subcutaneous administration of lipopolysaccharide (LPS). Male Sprague-Dawley rats received a caspase-1 inhibitor to reduce the synthesis of IL-1β or vehicle intrac erebroventricularly and antiserum to IL-6 (IL-6AS) or pre-immune serum intraperitoneally before receiving LPS or saline. LPS administration induced a fever (~1.3±0.2°C) which resolved within 2 days, while lethargy and anorexia continued for 3 days. Rats pre-treated with IL-6AS had no fever and attenuated sickness behaviours which resolved within 2 days, whereas rats pre-treated with a caspase-1 inhibitor exhibited attenuated fever and sickness behaviours which resolved within 2 days. Thus antagonizing the biological action of IL-6 in the circulation or IL-1β in the brain reduces the duration of anorexia, lethargy and fever induced by LPS administration.

P5AM-13-8

ITCH-RELATED RESPONSES OF DORSAL HORN NEURONS TO CUTANEOUS ALLERGIC STIMULATION

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Itch is a sensation that provokes a desire to scratch. It has been shown that distinct spinal dorsal horn (DH) neurons respond to histamine and allergic itch stimuli. The present study was done to identify the spinal DH neurons responsive to allergic itch stimuli. ICR mice were immunized and challenged in the caudal back and hind paw, respectively, with extract of the mosquito salivary gland. Biting behavior was observed as an itch-related response. The recording of the activity of single DH neurons was done with a tungsten recording electrode. We examined 98 units responsive to cutaneous allergy; 90 showed only immediate responses which subsided before the onset of itch-related behavior and eight showed immediate and sustained responses, the latter of which was similar in duration to itch-related behavior, suggesting the involvement of sustained units in itch signaling. Sustained units were localized in the superficial, but not deep, layers of the DH. They were wide-dynamic range or nociceptive-specific, but not low-threshold and 4 of 8 were noxious heat-sensitive. The results suggest that a small minority of neurons in the superficial dorsal horn are involved in allergic itch signals.

P5AM-13-9

CENTRAL CHANGES IN EXPERIMENTAL GLAUCOMA

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Relatively high incidence of glaucoma has become a serious medical and social issue in rapidly aging societies, including Japan. We wanted to investigate how visual brain responds to the degeneration of optic nerve in experimental glaucoma. In monkeys with ocular-hypertension glaucoma experimentally induced in one eye, imaging of positron emission tomography (PET) was performed. We found in 2-[^{18}F]fluoro-2-deoxy-glucose PET studies that monocular stimulation of the affected eye yielded significantly reduced neural responses in the visual cortex ipsilateral to the affected eye. The expression of an immediately early gene, c-fos upon visual activation was also reduced in V1 when the affected eye was stimulated. We also noted that selective accumulation of activated microglia was bilaterally induced at the level of lateral geniculate nuclei. Anatomical tracing of the retinal axons with WGA-conjugated HRP revealed abnormal pattern of their central projection. The present findings support the importance of noninvasive molecular imaging for diagnosis of early phase of glaucoma. It has also been suggested that central visual pathway is reorganized by slowly developed degeneration of the retinal outputs.

P5AM-14-1

MODULATION OF EXPRESSED HOMOMERIC KCNQ2 CURRENT BY ENVIRONMENT HIGH TEMPERATURE

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Mutations in the voltage-gated potassium channel genes KCNQ2 and KCNQ3 have been found to cause BFNC. Channels from these genes have been suggested to underlie the neuronal M current, which regulates the subthreshold electrical excitability in the CNS. Febrile convulsions represent the majority of childhood seizures. In this study, we asked whether KCNQ2 current may be altered following increased environment temperature. Whole-cell voltage-clamp recordings were obtained from homomeric expressed KCNQ2 on tsA-201 cells. Bath solution was preset to certain temperature before recording and keep on perfusion as 1ml/min during the recording. Analysis of KCNQ2 tail-currents indicated that the voltage-dependence activation has the trend to be decreased by elevated temperature, V1/2 was right-shifted to _38.2 ± 1.3 mV ($P > 0.05$). While the late current of KCNQ2 is decreased from 176±36pA/pF to 102±15pA/pF ($p<0.05$).These data indicate that KCNQ2 currents decreased by exposing to high temperature. Given that the limited cell number and relatively physiological range temperature, the tendency of down-regulation on KCNQ2 currents by stimulation of hyperthermia may represent a mechanism that febrile convulsion was triggered by altered M channel function through pathological hyperthermia.

P5AM-14-2

THE EFFECT OF AMYGDALA LESIONING ON ANTERIOR CLAUSTRUM-KINDLED SEIZURES AND ULTRA-STRUCTURAL CHANGES IN THE RAT ANTERIOR CLAUSTRUM

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The claustrum connects with the allocortical and neocortical regions and also projects to the hippocampus and amygdala. It is currently unclear what the role of this nucleus is in complex partial seizures. Thus, this study investigated the effect of amygdala lesion on anterior claustrum-kindled seizures in rat and the ultra structural changes of claustrum neurons after seizures . Animals were implanted with both a bipolar electrode in the basolateral amygdala and a tripolar electrode in the anterior claustrum. After a recovery , animals were kindled by the claustrum (60Hz, 2s, 1 ms p/ d), and kindling parameters were measured. In the lesion groups, animals received unilateral lesion of basolateral amygdala before and after claustrum kindling. We found that lesioning the amygdala before claustrum-kindling retarded claustrum-kindling by increasing the number of stimulations required to reach seizure stages. Lesioning reduced the severity of anterior claustrum seizures, duration of stage 5 seizures, after-discharge duration, and seizure duration. Claustrum neurons involved in partial seizures did not show definite ultra-structural changes. Although the lesion of basolateral amygdala had significant effects on anterior claustrum epileptic seizures.

P5AM-14-3

THE EFFECTS OF CARBENOXOLONE ON POWER SPECTRA IN PENICILLIN-INDUCED EPILEPTIFORM ACTIVITY

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Epilepsy is one of the important neurological disorders. Recent studies claimed that gap junctions have a critical role in epileptic activity. The aim of present study is to investigate the effects of carbenoxolone, a gap junction blocker, on power spectra in penicillin-induced experimental epilepsy. Permanent screw electrodes and permanent cannula were placed into the cranium of rats under general anesthesia. Epileptiform activity was generated by injecting 300 IU crystallized penicillin through the ventricular cannula. Epileptiform activity was monitored from a digital recording system (PowerLab/4SP). Carbenoxolone (100, 200, 500 nmol) was applied after 1 h penicillin injection in the same way with penicillin. Effects of carbenoxolone on epileptiform activity were assessed by power spectral analysis. This analysis was performed with a fast Fourier transform by using Chart program. Power spectrum of delta, theta, alpha, beta and gamma bands was increased to 1484%, 725%, 1024%, 1736% and 1251% by the penicillin, respectively. According to our findings, carbenoxolone was reversed penicillin-induced power spectral changes in dose dependent manner. The results of this study suggest that the blockade of electrical synapses may contribute to the amelioration of epileptic activity.

P5AM-14-4

EFFECT OF AOPCP INJECTION ON SYNAPTIC ACTIVITY OF DENTATE GYRUS IN PERFORANT PATH KINDLED RATS

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Introduction: Low frequency stimulation (LFS) has inhibitory effect on kindling acquisition. In the present study, the anticonvulsant effect of LFS and intraventricular injection of AOPCP (inhibitor of the ecto-5-nucleotidase) was investigated in kindled seizures.

Methods: Animals were kindled by perforant path stimulation in a rapid kindling manner (12 stimulations per day) and afterdischarges were recorded from the dentate gyrus. LFS was applied after termination of each kindling stimulation. Field potentials and paired pulse indices were recorded just before kindling stimulations.

Results: Application of LFS retarded the kindling acquisition and decreased the afterdischarge durations and behavioral seizure stages, significantly. LFS application also prevented an enhancement of the field EPSP slope and population spike amplitude during kindling acquisition. In addition, LFS significantly reduced the kindling induced increase in early and late paired pulse depression. intraventricular injection of AOPCP increase the field EPSP slope and population spike amplitude during kindling acquisition.

P5AM-14-5

THE EFFECT OF LIPOPOLYSACCHARIDE ON BLOOD-BRAIN BARRIER PERMEABILITY IN CONVULSIONS CREATED IN NORMOGLYCEMIC AND DIABETIC RATS

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The purpose of this study is to examine the effect of lipopolysaccharide (LPS) on Blood Brain Barrier (BBB) permeability and the changes observed in coloring of Zonula Occludens-1(ZO-1), Glial Fibriller Acidic Protein(GFAP) and in the release of serum TNF-α, and IL-10, IL-12(p40) cytokines during epileptic attacks occurring in diabetes. The attacks occurring in diabetes caused a remarkable the increase of BBB permeability ($p<0.01$). While LPS administration provided protective effects during epileptic attacks on BBB permeability ($p<0.01$), it did not cause a significant change in BBB permeability during epileptic attacks occurring in diabetes ($p>0.05$). ZO-1 in diabetic rats and coloring power of GFAP in only diabetic rats were observed to reduce. It was determined that TNF-α levels in LPS, LPS+ PTZ, Diabetes +LPS groups ($p<0.01$), IL-10 levels in LPS, LPS+PTZ,Diabetes+LPS, Diabetes+LPS+PTZ groups ($p<0.01$), IL-12(p40) in groups treatment with PTZ ($p<0.05$), increased significantly. Although LPS caused an increase in immune reactivity of ZO-1 and GFAP and in TNF-α and IL-10 levels, it was determined that LPS did not display a protective effect on BBB permeability during epileptic attacks occurring in diabetes.

Poster Session

P5AM-14-6

ENHANCED EXCITABILITY ON HIPPOCAMPAL CA3 IN EL MICE

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<Purpose> The aim of this study is to test the hypothesis that EL mice would show hyper-excitability which might cause the seizure susceptibility. <Methods> Experiment (Exp.) 1: We observed the changes in intracellular Ca^{2+} on adult EL and control DDY mice hippocampal slice after oxygen-glucose deprivation (OGD) using Ca^{2+} imaging with Rhod2-AM. Exp.2: We also performed the same experiments in developing animals (2-8w). Exp.3: We examined the changes in Ca^{2+} signal under the conditions of calcium free or applying of NMDA antagonist AP5 or AMPA antagonist CNQX. <Results> Exp.1: In EL mice, cytosolic Ca^{2+} on CA1 and CA3 was significantly increased after OGD. Control DDY showed increase just on CA1 region. Exp.2: In developing EL mice, Ca^{2+} increase was less than the adults evenly in CA1 and CA3. The fluorescent intensity was successively increased in both CA1 and CA3 as it grows. Exp.3: Extracellular Ca^{2+} free condition, application of AP5 and CNQX partially decreased the intracellular calcium increase after OGD. <Conclusions> These results suggest that enhanced excitability on CA3 in EL mice might cause epileptogenesis. Hyper-excitability occurs gradually as they get older, not after growth and might be related with both glutamate NMDA and AMPA receptors.

P5AM-14-7

SURVIVAL PROLONGATION AND NEUROPROTECTION IN EXPERIMENTAL HEATSTROKE BY COMBINATION DRUG TREATMENT

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Treatment with a combined therapeutic approach has been repeatedly advocated in the various cerebral ischemia experiments. The aim of this study was to investigate whether the combined agent (dexamethasone and hydroxyethyl starch) had more beneficial efficacy to improve heatstroke-induced neuronal damage in experimental heatstroke by attenuating the concentration of monoamines, and hydroxyl radical productions in rat brain and plasma levels of cytokines and lipid peroxidation associated with heatstroke. It was significantly decreased in values of cerebral ischemic and cellular injury markers after immediate treatment with the combined agent in rats during heatstroke. The combined agent also diminished the heatstroke-induced high plasma levels of cytokines and malondialdehyde, and high cerebral striatal levels of dopamine, serotonin and hydroxyl radicals in rats, and led to ameliorate the condition of heatstroke-induced central neuronal damage. Our findings suggest that immediate treatment with this combined agent confers significant protection against heatstroke-induced arterial hypotension, systemic inflammation, cerebral ischemia, cerebral monoamines, free radicals productions overload, and neurnal damage, and improve the survival time in experimental heatstroke.

P5AM-14-8

AUTOLOGOUS BONE MARROW MONONUCLEAR CELLS TRANSPLANTATION COMBINED WITH CHINESE MEDICINE TO TREAT LOWER LIMB ISCHEMIA

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Forty two patients with limb ischemia were treated and Granulocyte Colony-Stimulating Factor was used to stimulate the bone marrow. The mononuclear cells were separated from the aspirated bone marrow fluid in the stem cell studio. The transplantation was performed by intramuscular multi-injection. Chinese medicine was prescribed from the first day after operation. The pain evaluation, poikilothermia evaluation, the ulcer or necrosis and ankle/brachial index (ABI) of the ischemic limb were contrasted before and after the treatment. The pain score and poikilothermia score decreased one week after the transplantation, and increased a little two weeks after the treatment, and decreased steadily one month after the treatment. The ABI increased gradually after the treatment. The ABI one month after treatment was 0.23 higher than before-treatment averagely. The ulcer areas in these patients got smaller one month after the treatment. The patients with limb necrosis undertook amputation one month after the treatment, and the incision healed well. Autologous bone marrow nomonuclear cells transplantation combined with Chinese medicine improve the symptom and sign of severe lower limb ischemia efficaciously.

P5AM-14-9

THE PROTECTIVE EFFECT OF POST-CONDITIONING THERAPY AFTER CEREBRAL ISCHEMIA BY SUPPRESSING THE ACTIVATION AND EXPRESSION OF NADPH OXIDASE

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The purpose of this study was to determine whether the interrupting reperfusion post-conditioning therapy suppress the increasing activity and expression of NADPH oxidase induced by global ischemia/reperfusion. Male C57BL/6 mice were randomly divided into a sham operated group, control (global ischemia/reperfusion) group, postconditioning (global ischemia, 3 circles of 15 sec reperfusion/15 sec occlusion before permanent reperfusion) group (PC group). At second(+2D) and seventh(+7D) day after reperfusion, NADPH oxidase enzymatic activity and superoxide levels, $gp91^{phox}$ and $p47^{phox}$ mRNA levels, $gp91^{phox}$, cytosolic $p47^{phox}$ and membrane-translocated $p47^{phox}$ were analyzed. At +2D and +7D, NADPH oxidase enzymatic activity, superoxide levels increased sharply, but post-conditioning therapy prevented these increases significantly. mRNA expressions and protein subunits expressions had the similar increase tendency in both control group and PC group, while at +7D day after reperfusion both mRNA and protein expressions had smaller increase rate in PC group ($p<0.01$). The membrane-translocation of $p47^{phox}$ was also largely diminished by post-conditioning therapy. Post-conditioning therapy suppresses the injury of ischemia/reperfusion caused by NADPH oxidase.

P5AM-14-10

FUNDAMENTAL CONSIDERATION OF ^{31}P-NMR STUDY OF BRAIN ENERGY METABOLISM

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We have studied brain energy metabolism by measuring phosphocreatine (PCr) and ATP using ^{31}P-NMR. Rat brain slices are superfused with artificial cerebrospinal fluid (ACSF) with pO_2 of 550 Torr and 10mM glucose at 25°C. In order to conduct experiments under different conditions, we investigated effects of temperature, pO_2, extracellular $[K^+]$ and [glucose]. When temperature was raised without an increase in the flow rate, PCr and ATP signals disappeared, indicating that the flow rate was insufficient to deliver O_2 and glucose. In an attempt to load hypoxia (< 50 Torr), we noticed that pO_2 was increased to 100-120 Torr while ACSF passing through the tube due to dissolution and diffusion of O_2 into the wall of the tube. Intracellular pH during ischemia was decreased when [glucose] was increased while PCr and ATP were lowered when [glucose] was decreased. When extracellular $[K^+]$ was raised from 5 mM to 60 mM, energy expenditure was linearly increased, which was blocked by tetrodotoxin. Based on those findings, we are capable of conducting ^{31}P-NMR study in combination of appropriate temperature, [glucose], and flow rate. By lowering pO_2, brain slices are subject to ischemic/hypoxic stress. Using depolarizing high-$[K^+]$ ACSF, energy consumption of brain slices can be controlled.

P5AM-14-11

ALTERED EXPRESSION OF ERYTHROPOIETIN MRNA IN THE CENTRAL NERVOUS SYSTEM OF HYPOXIC RATS

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Erythropoietin (EPO) is a crucial hematopoietic cytokine, which is produced by renal tubulointerstitial cells in oxygen tension-dependent manner. In addition to renal EPO production, recent evidence from cell culture and developing animal experiments suggests that endogenous EPO produced in brain has a neuroprotective and neurotrophic function from hypoxic damage. However, the distribution and the time-dependent change in EPO mRNA remain unclear in adult central nervous system (CNS) under hypoxia. In this study, we detected the hypoxia-induced expression of EPO mRNA in the adult rat CNS. Hypoxic stimulation to male Wister rats (8 week-old) was sustained by using an airtight cabinet with a flow of gas containing 10% O_2 and 90% N_2 for 0, 4 and 24 hr. The samples for RT-PCR were quickly obtained from kidney and CNS. The expression of EPO mRNA in rat CNS was divided into two patterns; (1) Hypoxia induces the potential expression of EPO mRNA with the faint expression observed in normoxia (kidney, cerebral cortex, mesencephalon, hippocampus, olfactory bulb). (2) Hypoxia sustains or induces the continuous expression of EPO mRNA as observed in normoxia (hypothalamus, neurohypophysis). The present results indicate that hypoxia modulates EPO mRNA in region-specific manner.

P5AM-14-12

THE EFFECT OF SEX STEROID HORMONES ON BRAIN EDEMA, INTRACRANIAL PRESSURE AND NEUROLOGIC OUTCOME AFTER TRAUMATIC BRAIN INJURY

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The aim of present study was to investigate the role of sex steroid hormones on changes in brain edema, intracranial pressure (ICP), and cerebral perfusion pressure (CPP) after TBI in ovarectomized rats.

Method: rats were divided into five groups. Control, sham groups and other TBI groups include: vehicle, estrogen (1mg/kg) and progesterone (8mg/kg) groups , TBI was induced by Marmarou method , 30 minutes after TBI, hormones injected i.p. ICP was measured in spinal cord and CPP calculated by subtract the mean arterial pressure from ICP.

Results: after TBI brain water content was significantly lower in estrogen and progesterone groups compared to vehicle group, ICP was significantly higher in TBI rats. The ICP in estrogen and progesterone groups was significantly decreased at 4 and 24 hours after TBI as compared to vehicle group. The CPP in estrogen and progesterone groups at 24 hours significantly increased compared to vehicle. Also after TBI Nevrologic scores was significantly higher in estrogen and progesterone groups compared to vehicle at 1 hour and 24 hours for estrogen, but only at 1 hour for progesterone.

We conclude that improvement of ICP, CPP and neurologic scores produced by pharmalogic doses of estrogen and progesterone after TBI .

P5AM-14-13

DELAYED SERINE PROTEASE INHIBITOR TREATMENT REDUCES BRAIN DAMAGE AFTER INTRACEREBRAL HEMORRHAGE IN RAT

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We examined whether nafamostat mesilate (FUT), a serine protease inhibitor, can reduce ICH-induced brain damage. SD rats were received an infusion of autologous blood (100-µl), thrombin (5 U) or type VII collagenase (0.4 U) into the right basal ganglia. FUT (10 mg/kg) or vehicle was administered i.p. 6 hours after ICH and then at 12-hour intervals (6 treatments in total). There were four sets of experiments in this study. In the first set, the effects of FUT on ICH-induced brain injury were examined. In the second set, apurnic/apyrimidinic abasic sites (AP sites), hallmark of DNA damage, were examined. In the third set, the effect of FUT on thrombin-induced brain edema was investigated. The fourth examined whether FUT promotes rebleeding in a model in which ICH was induced by intracerebral injection of collagenase. Systemic administration of FUT starting 6 hours after ICH reduced brain water content 72 hours after ICH ($P < 0.05$). FUT treatment also ameliorated neurological deficits ($P < 0.05$). FUT attenuated ICH-induced changes in AP-sites ($P < 0.05$) and thrombin-reduced brain edema ($P < 0.05$). FUT did not increase collagenase-induced hematoma volume. FUT attenuates ICH-induced brain damage suggesting that serine protease inhibitor may be potential therapeutic agent for ICH.

P5AM-14-14

HYPERAMMONEMIA AND PSYCHOLOGICAL ILLNESS

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Introduction

There are a lot of uncertain points of the biochemical abnormality of the psychological illness.

I think about the possibility that the abnormality of a partial urea cycle takes part in a part of the psychological illness. There are some patterns of an abnormal amino acid of the patient who presents the hyperammonemia. So, I show two cases and discuss about hyperammonemia.

Case 1

A bipolar disorder patient, 28 year old man. He suffered from mood change and decreased judgment power and the impulse control. He was treated with the valproic acid. Serum ammonia showed 120, slight high. Therefore, I stopped valproic acid, then it decreased to 80, and the decrease in the judgment power was recovered.

Case 2

A bipolar disorder and eating disorder patient, 24 year old women. She suffered from difficulty of thinking and control of her emotion. Her serum ammonia was 120. She had the treatment of the energy metabolic and improved her difficulty of thinking and control of her emotion.

Consideration

The possibility for such slight abnormal ammonia to influence the thinking ability was thought, and I felt the necessity for the further examination.

P5AM-14-15

EFFECT OF MOOD STABILIZERS ON TREK-1 AND TREK-2 CHANNELS

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Earlier studies have reported that channel activity of TREK-1 and TREK-2, members of two-pore domain K^+ (K2P) channel family, are inhibited by antidepressants and antipsychotics such as fluoxetine, norfluoxetine, paroxetine, and chlorpromazine. This study was carried out to investigate the effect of mood stabilizers on TREK-1 and TREK-2 channels. Mood stabilizers are commonly used for treatment of bipolar disorder. In HEK-293A cells transfected with human TREK-1 and TREK-2, LiCl, gabapentin, valproic acid, and carbamazepin increased TREK-1 currents by 30%, but not TREK-2. These mood stabilizers had no effect on the TREK-2 channel activity. The application of lamotrigine failed to change channel activity of both TREK-1 and TREK-2. These results suggest that LiCl, gabapentin, valproic acid, and carbamazepin could have therapeutic potential for bipolar depression through modulation of TREK-1.

P5AM-14-16

GLIOMA CELLS EXPRESS NG2 PROTEOGLYCAN AND PLATELET-DERIVED GROWTH FACTOR α RECEPTOR, MARKERS OF OLIGODENDROCYTE PROGENITOR CELLS

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Glioma intrinsic to the central nervous system is one of the most difficult neopalsms to treat. One of the causes for the difficulties in creating the way to treat may be the fact that the cellular origin of glioma is not elucidated yet. In this study, to gain some insights into the origin of glioma cells, antigenic repertoire expressed by rat C6 glioma and 5 human glioma cell lines was compared with that expressed by primary cultured microglia, astrocytes and NG2 glia. Rat C6 glioma and some human glioma cell lines expressed NG2 chondroitin sulfate proteoglycan (NG2) and platelet-derived growth factor α receptor (PDGFαR), both of which are typical markers of oligodendrcyte progenitor cells (OPCs) or NG2 glia as revealed by immunocytochemistry, RT-PCR and immunoblotting. NG2 appeared to be processed in different ways in glioma cells from normal NG2 glia. Expression of glial fibrillary acidic protein (GFAP) was not remarkable in C6 glioma cells and some human glioma cells. Nestin, a marker of neural stem cells, was expressed only in C6 glioma and one human cell line. These results support that notion that glioma are derived from NG2 glia, which are the most actively cycling in the normal mature brain.

P5AM-14-17

RESEARCH OF HYPOGLYCEMIA INDUCED BY ELECTRICAL ACUPUNCTURE ON AURICULAR ACUPOINTS IN ZUCKER DIABETIC FATTY RATS

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The purpose of this study was to investigate the effects of electrical acupuncture (EA) on the blood glucose and insulin concentration. Lean Control (LC) rats and Zucker Diabetic Fatty (ZDF) rats were divided into three groups: Group A was administered with EA at auricular concha acupoints; Group B was applied in EA at the Zusanli (ST36) acupoint; Group C was administered with the same EA at Yishu (T8) acupoints. Electrical stimuli were applied (0.2 mA, 0.2 ms, 30 Hz, 30 min). The results indicated that the changes in ZDF rats: 1) In group A, the glucose concentration induced from 336.83±17.83 to 199.13±29.79 mg/dl ($P<0.05$) and the plasma insulin was raised from 50.66±2.15 to 58.49±2.44µmol/L ($P<0.001$) after EA. 2) In group B, the glucose concentration raised from 328.50±13.20 to 340.20±15.97 mg/dl and the plasma insulin concentration raised from 46.32±1.86 to 51.79±2.23µmol/L ($P<0.05$) after EA. 3) In group C, no significant difference in glucose concentration was induced by EA but difference in plasma insulin was found after EA (45.64±1.17 vs, 50.36±1.03µmol/L $P<0.05$). This study showed that the plasma glucose concentration could be reduced by EA auricular concha, also the plasma insulin could be raised by EA at auricular concha, ST36 and Yishu acupoints in diabetic rats.

P5AM-14-18

EFFECT OF HIGH FREQUENCY STIMULATION OF THE SUBTHALAMIC NUCLEUS ON THE NEURONAL ACTIVITIES OF SNR AND PF IN RATS

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Although high-frequency stimulation(HFS) of the sbuthalamic nucleus (STN) has been successfully used in the treatment of Parkinson's disease (PD), the underlying mechanism is not fully understood. We investigated the effect of stimulation from low to high frequencies of rat STN on the neuronal activities of the Substantia Nigra Pars Reticulata (SNr) and Parafascicular Nucleus (PF) using extracellular recording.

The low frequency STN stimulation had no obvious effect on the spontaneous firing activities of both nuclei, but STN-HFS(frequency 130Hz, intensity 0.4mA, width 0.06ms. duration 5s)significantly inhibited SNr neuronal firing activities, which could be blocked by bicuculline. Glutamate applied microelectrophoretically excited the PF neuronal activity, while MK801 inhibited the firing rates of the same PF neurons. STN-HFS increased the discharge rates of the PF neurons, which could be blocked by MK801. Our results suggest that the modification of the neuronal activities of STN-SNr-PF pathway is participated in the mechanism of STN-HFS to treat PD.

P5AM-14-19

TAURINE ATTENUATES THE WITHDRAWAL SYMPTOMS IN HEROIN DEPENDENT RAT

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The aim of this study was to examine whether taurine exerts beneficial effects on withdrawal symptoms of heroin dependent rats and if so whether changes in Ca^{2+} flux in the ventromedial hypothalamus (VMH), a situs rich in opioid receptors, are involved. Male Wistar rats (200-240 g) were used. Heroin was subcutaneously injected twice a day with a dose of 3 mg/kg at the day 1 then followed by increasing 1 mg per day until 30 mg/kg that was maintained for 3 days. Taurine (1 g/kg, i.p.) was administrated twice a day for 3 days before withdrawal provocation. Naloxone (5 mg/kg, i.p.) was applied to provoke precipitated withdrawal. Body weights were measured and behavioral responded signs were recorded and scored to evaluate withdrawal intensity. Taurine administration attenuated the withdrawal behavioral responses by 34% and reduced the body weight loss by 37% at 8 h, 45% at 24 h and 54% at 48 h after naloxone injection. In vivo microdialysis tests revealed a 39% increase of extracellular Ca^{2+} and 310% increase of extracellular taurine in the VMH after naloxone injection (5 mg/kg, i.p.) in the heroin dependent rats treated with taurine, indicating that the effects of taurine on withdrawal symptoms of the heroin dependent rats might be mediated by increase of Ca^{2+} efflux in the VMH.

P5AM-14-20

EFFECTS OF SIMULATED RECURRENT MYCOPLASMA INFECTION ON FEVER, GROWTH AND LEARNING AND MEMORY

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Repeated infections are major causes of morbidity and mortality and may put infected individuals at risk of growth failure. We used the synthetic lipopeptide FSL-1 (fibroblast-stimulating lipopeptide-1), derived from *Mycoplasma salivarium*, to investigate the effects of simulated recurrent *Mycoplasma* infection on body temperature, cage activity, body mass, food intake, and learning and memory in growing rats. Male Sprague-Dawley rats were assigned randomly to receive three intraperitoneal injections of either FSL-1 ($500\mu g.kg^{-1}$) or phosphate-buffered saline ($PBS;1ml.kg^{-1}$), spaced 10d apart. Radiotransmitters measured core body temperature and cage activity. Body mass and food intake were measured daily. Spatial learning and memory were tested in the Morris water maze. FSL-1 treated rats had a significant increase in body temperature and decrease in cage activity, food intake and body mass, compared with PBS treated rats. The magnitudes of fever, lethargy and anorexia, induced by FSL-1, were not significantly different following the three injections. Repeated administration of FSL-1, at 10d intervals, induced fever and sickness responses without the development of pyrogenic tolerance. Moreover, repeated administration of FSL-1 did not impair learning and memory in growing rats.

P5AM-14-21

ADMINISTRATION OF OUABAIN AGGRAVATES NEUROMUSCULAR PARALYSIS ASSOCIATED BY BOTULINUM NEUROTOXIN IN MICE

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The selective blockade of acetylcholine exocytosis by botulinum neurotoxin serotype A (BoNT/A) at neuromuscular junction causes prolonged neuromuscular paralysis. It was reported that ouabain attenuates BoNT/A-induced inhibition of acetylcholine release at the neuromuscular junction in isolated frog muscle. In this study, we examined the effect of ouabain on BoNT/A-induced neuromuscular paralysis. Ouabain (1 μmol/kg) was administered intraperitoneally to the mice immediately after a single injection of BoNT/A (0.1 ng) into gastrocnemius muscle, and BoNT/A-induced paralysis was assessed using digit abduction scoring (DAS) assay. The number of DAS, which increases with severer paralysis, was reached to the peak level at 2 days and then restored to the normal level at 8 days after BoNT/A injection. A single administration of ouabain significantly prolonged BoNT/A-induced neuromuscular paralysis. Moreover, a consecutive daily injection of ouabain increased the maximal DAS score and retained the peak level during the period of ouabain administration. We also found that administration of ouabain decreases myofibrillar cross-sectional area of the muscle 14 days after BoNT/A injection. These results suggest that ouabain aggravates BoNT/A-induced neuromuscular paralysis.

P5AM-15-1

MODELING AND TESTING THE RECEPTIVE FIELD PROFILE OF MACAQUE MT NEURONS

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Neurons in extrastriate visual areas have large receptive fields (RFs) compared with area V1, suggesting an extensive spatial integration. To examine the spatial integration of neurons in area MT, we modeled RFs of MT neurons based on a symmetrical (Gaussian) integration of V1 outputs and tested the model by single-unit recording from two fixating macaque monkeys. Because visual representation in V1 is logarithmically compressed along eccentricity, the resulting RF model is log-Gaussian along the radial axis in polar coordinates. To test the log-Gaussian model, RF of each neuron was mapped in a 5 x 5 grid manner using a small patch of random-dots drifting at the preferred velocity of the neuron. The majority of MT neurons had RFs with a steeper slope near the fovea and a shallower slope away from the fovea. Among various 2-dimensional Gaussian models fitted to the RFs, the log-Gaussian model provided the best description (N = 132, median R^2 = 0.94). The fitted parameters revealed that the range of sampling by MT neurons is constant across eccentricities and the sampling ranges along 2 orthogonal directions on V1 cortex are different. Our results suggest that MT neurons integrate inputs from V1 cortex with an elliptical 2-dimensional Gaussian weight.

P5AM-15-2

A SPATIO-TEMPORAL COMPUTATIONAL MODEL OF THE RETINAL CIRCUIT REALIZING MULTIPLE FUNCTIONS

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Previous studies have shown that output of the vertebrate retina, i.e. retinal ganglion cell (GC) output, is not merely an outcome of a light transduction but is a signal encoded in a highly nonlinear fashion by retinal circuitry to realize various visual functions such as object motion segregation and rapid neural coding. Those functions were found to be originated not at the GC but rather commence at inter-retinal layers. Specifically, the temporal asymmetry between ON and OFF bipolar cell (BC) pathways and inhibition driven by wide-field amacrine cells (wfACs) were shown to be critical for rapid neural coding and object motion segregation, respectively. Although previous models could realize either of these functions, multiple functions were not implemented in a single model as each class of retinal neurons was largely omitted. Here, we present a spatio-temporal computational model of the retinal circuit by including photoreceptors, horizontal cells, ON and OFF BCs, ON and OFF ACs, ON-OFF wfACs, and ON-OFF GCs and their anatomical connections. The simulations showed that the present model can realize not only spatio-temporal dynamics of the retinal cells but also implements two different functions (object motion segregation and rapid neural coding) on a single type of GC.

P5AM-15-3

CHAOS, MULTIFRACTALS AND EMD-DECOMPOSITION IN ANALYSIS OF THE EEG SIGNAL STRUCTURE

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The aim of the present talk is quantitative verification of the following hypothesis about the EEG signal dynamical components structure. Namely, we conjecture that the EEG signal consists of the noised chaos, periodic oscillations corresponding to the main rhythms of brain activity and low-frequency residue. In present work we studied the structure of the different components of the EEG signal in order to verify this hypothesis. EEG signals were decomposed via the EMD (Empirical Mode Decomposition) method. Obtained empirical modes were studied implementing spectral analysis as well as methods of multifractal analysis, dynamical chaos and artificial neural networks. It was shown that the first and second modes of the EEG signal recorded from C_z position can be considered as noised chaos. The modes with numbers from 3 to 8 are periodic oscillation corresponding to the main rhythms of brain activity. The residue consisting from modes with numbers grater than nine is low-frequency oscillation of unknown nature.

Summing the present study one can state that the hypothesis*" the EEG signal consists of the noised chaos, periodic oscillations corresponding to the main rhythms of brain activity and low-frequency residue"* is confirmed in the framework of used methods.

P5AM-15-4

METAL DEPOSIT MARK OF ELGILOY ELECTRODE IS DETECTABLE USING HIGH-FIELD MRI: AN *IN VIVO* METHOD TO LOCALIZE THE RECORDING SITES

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Elgiloy microelectrode has an ability to mark the recording sites with electrolytic metal deposits (Suzuki & Azuma, 1976). Here, we report that these metal deposits are detectable using high-field MRI. Using anesthetized monkeys, we inserted glass-coated elgiloy electrodes into brains and marked the recording sites by passing a direct anodic current of 2-10 μA for 3-30 min. Subsequent MRI with a fast spin-echo (FSE) and gradient-echo (GE) sequences (in-plane resolution, 150-200 μm) showed hypointense spots of 150-1600 μm at the marked sites. The size of the markers was correlated with the total charge used for the deposition ($p<0.05$) and was larger in GE than FSE sequences ($p<0.05$, paired *t*-test). The size of the markers was also affected by some MRI scan parameters: bandwidth and frequency encoding direction in the FSE sequences and TE in the GE sequences ($p<0.05$, *post-hoc* Tukey's test). Follow-up MRI scans showed that the markers remained detectable at least 18 months after the deposition. Prussian blue reaction in histological analyses confirmed the deposition of iron at locations that corresponded to the hypointense spots. This *in vivo* localization method would help to relate recorded neuronal activities to the fine anatomies of the brain.

P5AM-15-5

STAMPORATION: A NEW MICROINJECTION METHOD INTO VERIOUS CULTURED CELLS

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The transfer of DNA molecules into cells is performed by several different techniques, including lipid- or viral-mediated transfection, electroporation, and microinjection.

One of the advantages of this microinjection technique over the others is capability of pinpointing cells to be transfected.

Currently, microinjection through glass pipettes requires much experience and effort, and often causes cell damage.

Here we have developed a user-friendly and efficient microinjection technique, AC160BN (Olympus) etched by focused ion beam. When a needle penetrates a cell, a small puncture is created in the plasma djacent cells with cDNAs for different fluorescent proteins.

P5AM-16-1

MEMBRANE POTENTIAL MEASUREMENTS WITH ENGINEERED FRET SENSOR

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Ciona voltage sensitive phosphatase (Ci-VSP) is a unique enzyme discovered first in asicdian genome, in which phosphatase activity is regulated by transmembrane potential. Ci-VSP consists of a phosphatase domain and a preceding voltage sensing domain (VSD) which is homologous to the S1-S4 transmembrane domain found in conventional voltage-gated ionic channels. Possible mechanism of Ci-VSP should be that changes in transmembrane potential elicit conformational changes in the VSD, which then induce conformational changes in the phosphatase domain, regulating enzymatic activity. Analogously, by replacing the phosphatase domain with two fluorescent proteins that act as fluorescence resonance energy transfer (FRET) donor and acceptor, it is expected that transmembrane potential can be optically probed as FRET readout. Using two new coral fluorescent proteins, we developed such a membrane potential reporter, named Mermaid, that displays 40% changes in emission ratio per 100 mV change, allowing for visualization of spatiotemporal dynamics in electrical activities of excitable cells. We then like to present some results of imaging experiments using mermaid.

P5AM-16-2

AN IMPROVED GENETICALLY-ENCODED FLUORESCENT Ca^{2+} PROBE THAT LESS AFFECTS CALCINEURIN SIGNALING

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G-CaMP2 is a genetically-encoded fluorescent probe suitable for mammalian *in vivo* Ca^{2+} imaging. We previously generated the cardiac G-CaMP2 transgenic mice, which enabled visualizing Ca^{2+} transients in the beating heart of adult and embryonic mice *in vivo* and *in vitro* (*PNAS 2006, 103:4753*). However, the G-CaMP2 mice developed cardiac hypertrophy. In the course of clarifying the mechanisms underlying the hypertrophy, we found that G-CaMP2 significantly enhanced NFAT-, AP1-, and NFκB-signaling. The enhancement of these signaling was shown to be associated with the activation of calcineurin (CaN). By introducing several known mutations into the calmodulin part of G-CaMP2 to lower the probe affinity for CaN, we generated an improved probe, named "cyto-friendly (cf) G-CaMP2", which less interfered with signaling described above. Hypertrophy was not induced by this probe in cardiomyocytes. cfG-CaMP2 was also verified to function as a Ca^{2+} probe and have a similar affinity for Ca^{2+} to that of the original G-CaMP2. Our new probe may provide a better tool for *in vivo* Ca^{2+} imaging by less affecting CaN signaling not only in cardiomyocytes but also in other cells.

P5AM-16-3

VISUALIZATION AND QUANTITATIVE ANALYSIS OF THE NANO-SCALE OLFACTORY SENSORY CILIA

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Olfaction starts at the sensory cilia that display a nano-scale tubing structure (100~200 nm diameter). It is worth to visualize the cilia for olfactory system, because second messengers (e.g., Ca^{2+}or cAMP) are involved within such tiny biological structure. However, even a visualization of cilia has been accompanied with difficulties. Previously, a number of studies have been conducted to look at living cilia by using Lucifer Yellow (LY). It has been shown that LY has toxicity for the cell survival. It is therefore necessary to examine the LY effect whether it provides useful tool for visualization under the electrophysiological studies of the cell. In this work, we focused on 3 points. 1) Visualization of the nano-scale cilia with using LY. 2) Time-dependence of LY loading and UV bleaching. 3) Quantitative analysis of diffusion of cytoplasmic molecules into the cilia. The present study shows that LY incorporation into the soma stains the whole cilia beyond the diffusion barrier that has been though to be present at the basement. LY bleaching was time-dependent with half decay time of 19.5±4.5 min (n=8). Furthermore, the evidence that LY can diffuse from dendro-somatic membrane to the cilia indicates that molecules of comparable sizes can be transported as well.

P5AM-16-4

DEVELOPMENT OF FLUORESCENT cAMP SENSOR USING CYCLIC NUCLEOTIDE BINDING DOMAIN OF SEA URCHIN HCN CHANNEL

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The cyclic AMP (cAMP) is a crucial second messenger in many types of cells. In order to study the dynamics of this molecule in live cells, a fluorescent sensor for cAMP is indispensable. The first cAMP sensor was developed by the group of Roger Tsien in 1991 based on fluorescence resonance energy transfer (FRET) using chemically modified PKA. In 2000, genetically encoded sensors were developed based on the same principles, but using fluorescent proteins. Recently, several groups reported cAMP sensor based on intramolecular FERT using cyclic nucleotide binding domain (CNBD) of EPAC and fluorescent proteins. These new sensors have some advantages against intermolecular FRET sensor using PKA, but their dynamic ranges are still poor (below 50 % of fluorescent signal changes).

We are trying to develop more useful sensors for cAMP using several distinct strategies, which include: 1) intramolecular FRET sensor using circular permutated fluorescent proteins, 2) allosteric sensor using fluorescent protein (Non-FRET FP sensor), and 3) chemically modified sensor. We use a CNBD of sea urchin HCN channel for all types of sensors because of its high specificity on cAMP.

P5AM-16-5

ZERNIKE PHASE CONTRAST ELECTRON MICROSCOPY VISUALIZES DETAILED ULTRASTRUCTURES OF VITRIFIED BIOLOGICAL SPECIMENS

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Conventional transmission electron microscopy (CTEM) enables us to visualize ultrastructures of biological specimens. However, morphological and structural artifacts occur during the steps of specimen preparation such as chemical fixation and dehydration. By means of cryo-fixation method such as quick freezing and high pressure freezing method, vitrified biological specimens are able to keep original close-to-physiological state. Without heavy metal staining however, it requires a large amount of defocus to gain an adequate contrast for the observation. While large defocus values are effective for visualizing low frequency component of objects, they can cause a severe attenuation of the contrast of high frequency components. For the clear observation of close-to-physiological ultrastructures without a large amount defocus, Zernike Phase Contrast TEM (ZPC-TEM) was applied to vitrified specimens. We successfully observed close-to-physiological ultrastructures of vitrified specimens. The application of ZPC-TEM to vitreous biological specimens will be a powerful method to open a new field of imaging close-to-physiological ultrastructures.

P5AM-16-6

STUDIES ON POTENTIAL TUMOR VACCINE HSP110-HER2/neu ICD AND ITS ANTI-TUMOR EFFECTS

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HER2/neu is over-expressed in 20-30% of breast cancer cells and other tumor tissues. Since humoral and cellular immune reaponses against HER2/neu can be detected in many tumor patients, HER2/neu has the potential to be an efficient target for oncotherapy.

Recent studies have shown that another HSP, known as HSP110, also exhibits this immune-stimulating activity when purified from a tumor tissue and applied in a preventative therapy.

In our study, we binded recombinant HER2/neu ICD with recombinant HSP110 noncovalently forming a novel tumor vaccine.Since the vaccine aimed at HER2/neu ICD, it can be applied in any patient expressing this antigen.

The contents of this work are focused on the following parts: 1.Noncovalent binding between recombinant HER2/neu ICD and recombinant HSP110 in vitro 2.Studies on HSP110-HER2/neu ICD complex to induce specific immune reaction.

We confirmed the HSP110-HER2/neu ICD complex could induce specific immune responses for the first time. Our study made the foundation of using HSP110-HER2/neu ICD complex as a potential vaccine to cure tumors overexpressing HER2/neu and will help the further exploration on the immunifaction mechanism of HER2/neu ICD.

P5AM-16-7

NEW PARAMETER FOR EVALUATING FUNCTION OF THE PAROTID GLAND

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Purpose: Equivalent cross-relaxation rate (ECR) imaging (ECRI) with MRI can detect minute changes in organization and molecular structure. The aim is to determine that equivalent cross-relaxation rate (ECR) imaging (ECRI) with MRI is a feasible method for evaluating function of the parotid gland.

Materials and Methods: A total of 15 patients with head-neck cancer underwent ECRI and salivary gland scintigraphy. The single saturation transfer pulse frequency was employed at the frequency 7 ppm downfield from the water resonance. ECR (ECR7) value was defined as the percentage of signal loss between unsaturated and saturated images. ECR7 value shows high values by the increase of cell density or by the decrease of extracellular component. ECR7 values were compared between non- and post-radiated parotid glands. ECR7 value was compared with maximum uptake rate (MUR), a functional parameter obtained by salivary gland scintigraphy.

Results: A correlation was detected between ECR7 and MUR ($r=-0.55$, $p<0.01$). Moreover, ECR7 values were elevated in the post-radiated parotid gland with significant difference ($p<0.01$). This result showed the decrease of extracellular component in post-radiated parotid glands.

Conclusions: ECRI is new parameter for evaluating function of the parotid gland.

P5AM-17-1

GENE EXPRESSION PROFILE OF THE SMALL INTESTINE IN REELER MICE

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Reelin is an extracellular matrix protein that plays a pivotal role in neuronal migration during brain development in mammalian. Reelin was first considered as brain-specific but it has also been detected in non-nervous systems, where its function remains unknown. We have previously shown that rat small intestine expresses reelin and the reelin signalling system. Using microarray analysis, the current work evaluates whether the lack of reelin (reeler mice) modifies the small intestinal gene expression. RNA was isolated from either enterocytes or the whole small intestine of reeler and normal mice. All procedures were accord with current national/local ethical guidelines.

The gene expression profiles revealed that the expression of 148 genes (55 up-regulated and 93 down-regulated) were significantly altered in the small intestine of reeler mice ($p < 0.01$). These genes are involved in processes, such as cell proliferation, development, differentiation and apoptosis (48 genes), transport and metabolism (36 genes), immune response (23 genes), transcription and signaling (16 genes) and cell adhesion and communication (13 genes). The results suggest that reelin might be involved in development of mice small intestine.

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P5AM-17-2

GENE EXPRESSION OF PROTEINS INVOLVED IN THE REGULATION OF CALCIUM SENSITIZATION PATHWAY IS MEDIATED BY NF-κB IN HYPERTROPHIED SMOOTH MUSCLE

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The expression of proteins (RhoA, ROK and CPI-17) involved in the Ca2+-sensitization of smooth muscle tone is altered in the urinary bladder smooth muscle (UBSM) in response to obstruction-induced hypertrophy. Partial bladder outlet obstruction (PBOO) was created in male mice and kept for 2-weeks. Normal mice served as a control. The expression of RhoA, ROK β and CPI-17 were upregulated in the UBSM following PBOOas shown by immunoblot and RT-PCR analyses. Analysis of the transcription factor profiling in the nuclear extract isolated from UBSM from PBOO using protein/DNA arrays revealed enhanced binding of NF-κB to their cognate DNA sequences. The transient transfection of NF-κB cDNA in murine UBSM cell cultures increased the RhoA, ROKβ and CPI-17 mRNA and protein expression. The RT-PCR and immunoblot anlalyses of RhoA, ROKβ and CPI-17 from NFkB/p65 knockout mice showed reduced amount of mRNA transcripts and proteins compared to control, indicating that NF-κB is required for the transcriptional regulation of these proteins. The upregulation of these proteins could alter the signaling pathways that regulate DSM contractility. An understanding of the gene regulation of these regulatory proteins would help to develop therapies for PBOO-induced bladder dysfunction.

P5AM-17-3

ASSOCIATION OF GENETIC VARIANTS IN MTHFR AND PON-1 GENES WITH HOMOCYSTEINE, FOLATE AND VITAMIN B12 IN CORONARY ARTERY DISEASE

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Background: The aim of the present study was to investigate whether genetic variants in metylenetetrahydrofolate reductase (MTHFR) and Paraoxonase 1 (PON1) 55/192 are associated with total homocysteine (tHcy), folate, B12, as well as paraoxonase levels in patients with coronary artery disease (CAD).
Methods: The study included 235 patients with CAD and 268 controls.
Results: LL and LM genotypes and L allele of PON1 55 were over-represented in patients. In contrast, MM genotype and M allele were more frequent in controls. QQ genotype and Q allele of PON1 192 and CT genotype of MTHFR were significantly diminished and QR genotype and R allele were significantly elevated in patients compared to controls. The plasma tHcy were elevated but B12 diminished in patients. PON1 55 and 192 were significantly associated with PON1, triglyceride, cholesterol, tHcy and, HDL-C and LDL-C in patients, respectively.
Conclusion: Genetic variats of PON1 55/192 and MTHFR were associated with CAD.

P5AM-17-4

PREVENTION OF MUSCLE ATROPHY BY ELECTROACUPUNCTURE IN A MURINE HINDLIMB SUSPENSION MODEL

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Electroacupuncture (EA), one of the acupuncture treatment, that is stimulated by using acupuncture needles with low-frequency microcurrent. Our previous study provided molecular evidence that EA suppresses myostatin gene expression and causes a satellite cell-related proliferative reaction in skeletal muscle. In this study, we investigated the EA effect for the treatment of muscle atrophy prevention in the Hindlimb Suspended (HS) mouse model. We then analyzed myostatin gene expression in skeletal muscle from HS mice which received alternate-day EA treatments repeated after the onset of suspension for up to 14 days. RT-PCR showed that long-term EA suppressed myostatin gene expression in these mice. EA treatment significantly prevented decreased soleus weight per body weight in HS mice (EA/HS mice) compared with no EA treatment of HS mice, at 7 days and 14 days. The myofiber diameter for EA/HS mice was also significantly larger than that of HS mice. Histochemical ATP staining showed no significant differences in proportions of muscle fiber types for HS, EA/HS, and wild-type mice. These findings indicate that EA can effectively prevent and treat muscle atrophy.

P5AM-17-5

REGULATION OF KALLIKREIN 1b26 (klk1b26) EXPRESSION IN MOUSE SUBMANDIBULAR GLAND BY POST-TRANSCRIPTIONAL MODIFICATION OF ITS mRNA

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We studied hormonal regulation of klk1b26 expression in submandibular gland (SMG) of ICR mice. The klk1b26 protein in male SMG was abundant by 9-fold comparing to female and was decreased by castration. 5α-dihydrotestosterone (DHT) administration into castrated mice or females increased the protein level. Therefore, these events are thought to be due to the increased mRNA synthesis by androgen. We found, however, that when klk1b26 mRNA was examined by RT-PCR with primers targeted to the middle regions of the mRNA, there was no sex difference in the PCR product levels. Either the castration or the DHT administration showed no effect on the PCR product levels. While the RT-PCR was done with primers targeted to the 5'-terminal region of the mRNA, the PCR signals revealed sex difference, and was influenced by castration and DHT administration to females or castrated mice as observed in case of klk1b26 protein levels. Moreover, incubation of total RNAs from male SMGs with SMG extract resulted in the decrease in PCR signals with primers for 5'-terminal regions of klk1b26 mRNA but not in that with primers for middle regions. These results suggest that klk1b26 protein expression in female SMG is regulated by posttranscriptional decomposition of the mRNA near the 5'-terminal region.

P5AM-17-6

THE EXPRESSION OF COFILIN IN ADAPTIVE RESPONSE INDUCED BY GAMMA-IRRADIATION

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Cells exposed to low dose of radiation play a role on radioresistance to subsequently high dose of radiation, which is called 'adaptive response'. However, the precise mechanism by which cells adaptively respond to radiation remains unclear. In this study, we identified differentially expressed proteins related with radio-adaptive response in human fibroblasts by proteomic approach. Human normal fibroblast cells were irradiated with 4Gy (H) alone or 1cGy priming dose prior to 4 Gy (LH). We found that cells in LH group underwent adaptive response by micronucleus assay. After MS/MS analysis, we identified several consistently up- or down-regulated proteins in LH group compared to H group. In particular, the level of cofilin protein was decreased in H group compared to LH group. Interestingly at 4Gy irradiation, cofilin was highly phosphorylated, whereas in cells undergoing adaptive response the phosphorylation of cofilin was not occurred. Further analysis revealed that the cofilin was phosphorylated by PI3K-dependent pathway. When the level of cofilin mRNA was knockdowned by RNA interference, cellular protein oxidation was increased by impairing redox regulation. Taken together these findings suggest that cofilin could be influenced in radiation induced adaptive response.

P5AM-17-7

DECREASE OF MUSCLE CELLS PROLIFERATION AND DIFFERENTIATION IN HYPOXIA IS NOT RESTORED BY EPO

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Hypoxia (1% O_2) has been shown to alter both mouse myoblast proliferation and differentiation. Conversely, Erythropoietin (Epo) has been shown to improve the accumulation of myogenic precursor cells in C2C12 cultures. We first analyzed the Erythropoietin receptor (EpoR) expression in L6 myoblasts and secondly evaluated the effects of Epo when L6 cells are cultured in normoxia (21% O_2) and hypoxia with and without human recombinant Epo (RhEpo). The presence of the EpoR was assayed by RT PCR and western blotting. Proliferation was evaluated by the determination of the doubling time and the kinetics of culture. Differentiation was evaluated by the percentage of Myosin heavy chain expressing cells and Myogenic Fusion Index by immunostaining. Myogenin and myosin heavy chain expression was determined using western blotting technique. We found that L6 myoblasts express the Epo receptor mRNA and protein. After a-96h culture, L6 myoblast doubling time was increased by about two hours in hypoxia; the rate of cells expressing fast MHC and the myogenic index fusion were reduced. The expression of myogenin and MHC were also reduced. Epo did not counteract the effects of hypoxia. Hypoxia considerably alters myoblasts proliferation and differentiation. Epo does not prevent this effect.

P5AM-17-8

LDH AND TNF-ALPHA IN ODONTOGENIC KERATOCYSTS

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Based on consideration that odontogenic cysts have a neoplastic potential it is very interesting to analyzed values of Lactate dehydrogenase (LDH) and TNF-α in our type of oral disease. We compared values of LDH by the spectrometric assay and cytokines activity by ELISA assay in 32 patients with our cysts type. Cysts fluid were obtained from patients undergoing surgery, under local anaesthesia, and after aspiration from non-ruptured cysts. We confirmed diagnosis by clasical histology, immunohistology and by routine radiological and clinical analyses. The significantly ($p < 0.05$, Mann-Whitney U-test) higher concentration of LDH in cystic fluids were associated with smaller radicular cysts, higher protein concentration, higher presence of inflammatory cells in peri cystic tissues, (analyzed by immunohistochemistry), cysts wall thickness and higher degree of vascularisation (determined by enumeration blood vassels) (Mann-Whitney U-test, $p<0.05$). No correlation was found, based on these parameters in respect to TNF-α, but all cysts have detectable concentrations of TNF-α. We here for the first time present that LDH as marker of anaerobic metabolic disturbance is more elevated in comparison to TNF-α as inflammatory cytokine.

P5AM-17-9

THE EFFECTION OF ALCOHOL METABOLISM ON CAVEOLIN-1 TRANSPORTING

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Alcohol metabolism in hepar may produce a large amount of free radical. Ethanol-related metabolic enzymes present in the smooth endoplasmic reticulum. When intake excessive ethanol, the free radical produced will cause the smooth endoplasmic reticulum structure damage. Caveolin-1 is a key protein of Caveolae which is the structure of animal cell membrane. Caveolin-1 is synthesized in the smooth endoplasmic reticulum, then transported into the cytoplasm. Reported that when liver damaged seriously, the expression of Caveolin-1 is significantly reduced. Objective: To study if it would affect Caveolin-1 transporting into the cytoplasm when liver injured by ethanol. Methods: Established the liver injury models by ethanol; Use Western Blot method to detect the expression of Caveolin-1 in different locations. Results: Compared the control group, the total Caveolin-1 expression of the alcohol group is significantly reduced, and it is the same tendency in the cytoplasm. Discussion: It shows that when excessive alcohol intaked, a large number of free radical produced in liver caused the endoplasmic reticulum damaged, inhibiting the Caveolin-1 form a coating into the cytoplasm, so that Caveolin-1in the cytoplasm is significantly reduced.

P5AM-17-10

THE ROLE OF PPARγ IN THE REGULATION OF COAGULATION AND FIBRINOLYTIC SYSTEM IN VASCULAR ENDOTHELIAL CELLS

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To investigate the role of PPARγ on the regulation of coagulation and fibrinolytic system in vascular endothelial cells (VECs), cultured VECs were established from thoracic aorta of LETO rat by the explant method. The mRNA expressions were measured using the comparative RT-PCR method. Binding activity of PPARγ to coactivator (CBP) was determined by ELISA. RT-PCR revealed that PPARγ mRNA was constitutively expressed in cultured VECs. The mRNA expressions of PAI-1 and thrombomodulin (TM) were significantly decreased by treatment with PPARγ antagonist, GW9662, but u-PA and t-PA mRNA expressions were not. Synthetic PPARγ agonists, troglitazone (TRO) and telmisartan (TMS) were also tested for their effect on those mRNA expressions. TRO and TMS activated PPARγ with the EC_{50} values of 3.5 μM and 52 μM under the cell-free condition, respectively. The mRNA expressions of PAI-1, u-PA and TM were significantly decreased by treatment with TMS (50 μM), but not by TRO (10 μM). Thus, transcriptions of PAI-1 and TM might be constitutively upregulated by intrinsic PPARγ agonists and TMS possibly downregulates PAI-1, u-PA, and TM mRNA expressions through an atypical PPARγ pathway.

P5AM-17-11

UPREGURATION OF Ki-67 IN ORAL SQUAMOUS CELL CARCINOMAS ENHANCED THE CERVICAL LYMPH NODE METASTASIS THROUGH THE INCREASED LYMPHANGIOPLASTY

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It is hypothesized that the higher population of Ki-67 positive cells at the invasive front of tumor occur the more frequent metastasis in human oral squamous cell carcinomas (OSCCs). To test the hypothesis, we investigated the relationship between the number of tumor cells expressing Ki-67 and the frequency of lymph node metastasis in 44 surgically treated patients with OSCCs. All participants provided informed consent to participate in this study. None of the patients showed evidence of lymph node metastasis at initial clinical evaluation. Immunohistochemical study with anti-Ki-67 monoclonal antibodies was performed to quantitate the number of tumour cells expression Ki-67 in the formalin-fixed, paraffin-embedded biopsy samples and the acquired images were analyzed using NIH image. In 16 of 44 patients, secondary lymph node metastasis occurred. As compared with other Grade types of OSCCs, the frequency secondary lymph node metastasis was increased in Grade 4C and 4D type, in which the number of tumor cells expressing of Ki-67 was increased. These results suggest that the expression Ki-67 in tumor cells may promote secondary lymph node metastasis by lympangioplasty. Subsequently, the release of VEGF from cells expessing Ki-67 in OSCCs may increase the lymphangioplasty.

P5AM-17-12

MOLECULAR MECHANISMS OF TERMINATING AND RESTARTING CIRCADIAN RHYTHMS IN THE MOUSE SUPRACHIASMATIC NUCLEUS

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Circadian rhythms are widely observed in physiological functions and a transcriptional-translational negative feedback loop has been considered as molecular clockworks for the intracellular rhythm generation in the mammalian central clock which resides at the suprachiasmatic nucleus of hypothalamus (SCN). However, the dynamics of molecular loop is still poorly understood. Since prolonged protein synthesis inhibition by cycloheximide (CHX) is known to terminate the circadian oscillation, we applied CHX into the SCN explants for different duration, and studied the mechanisms for the molecular loop to turn. We monitored *Bmal1*, a core clock gene, expression in real time using the transgenic mice carrying a luciferase reporter gene. A robust circadian rhythm was observed in *Bmal1* expression, and the amount of bioluminescence was decreased to the background level upon the CHX administration. The level of bioluminescence was recovered and the overt rhythm resumed after the washout of CHX, the phase of which was depended on the duration of treatment. We also directly measured mRNA and protein products of various clock genes during and after the application of CHX. From these results, the molecular mechanism for starting the oscillation will be discussed.

P5AM-17-13

INFRARED LASER MEDIATED GENE INDUCTION IN A TARGETED SINGLE NEURON OF C. ELEGANS

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A technique for inducing expression of a given gene in a completely directed fashion with respect to time and space would be useful for studying neuron circuits. Heat shock promoters provide a means of the temporal control of gene expression by external heat stimuli. If we can manage to heat a targeted cell, a gene under the control of a heat shock promoter would be expressed only in the target. One way to heat a single cell is irradiation with a laser beam. Previous attempts by using a 440 nm dye laser, however, failed to induce gene expression efficiently and caused detrimental effects on target cells. Recently, a novel microscope system, InfraRed Laser-Evoked Gene Operator(IR-LEGO), has been developed. The wavelength of IR laser (1,480 nm) can heat water with higher efficiency than the 440 nm laser. By applying IR-LEGO to *C. elegans*, we have succeeded in inducing gene expression in epidermal cells efficiently. Here we report on our attempts to regulate gene expression in targeted single neurons.

P5AM-17-14

EXPRESSION AND FUNCTIONAL ANALYSIS OF ITGB4 ON BRONCHIAL EPITHELIAL CELLS

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Integrin beta 4 (ITGB4) expressed on the airway epithelial cells play a vital role in maintaining the airway architecture and functional homeostasis. Our previous study indicated that ITGB4 is relevant closely to asthma susceptibility, but its biological function or significance is still unclear. Here, real-time PCR, immunocytochemistry and flow cytometry were used to detect the expression of ITGB4. It is demonstrated that increased expression of ITGB4 are detected either on the edge cells of mechanic wounded area or ozone stress cells in culture. High expression of ITGB4 obliviously promotes the wound repair and anti-oxidative ability which can be blocked by ITGB4 siRNA. Then, correlation analysis suggested that the expressions of the ITGB4 is highly correlated to the lung resistance on an OVA-challenged asthma model. It also indicated that FAK (focal adhesion kinase) phosphorylation can promote would repair and anti-oxidative ability which were inhibited by ITGB4 siRNA. In conclusion, the expression of ITGB4 mRNA is highly correlated to airway resistance; ITGB4 may contribute the wound repair and anti-oxidative process of 16HBE14O-. Further, it may serve to laminin mediated cell-extracellular adhesion and its signal transduction.

P5AM-18-1

GLUCOSE-6- PHOSPHATE DEHYDROGENASE (G6PD) STATUS IN NEONATAL JAUNDICE AND ITS RELATIONSHIP WITH SEVERITY OF HYPERBILIRUBINEMIA

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Objectives: To observe G6PD status in male, term, neonates with jaundice and its relation with total serum bilirubin level.

Method: This study was carried out on 90 male term neonates with jaundice(group B) and 30 apparently healthy neonates(groupA) aged 3 to 12 days. Group B was further divided into B-I (TSB <15mg/ dl), B-II (TSB 15 to 20 mg/ dl) and B-III (TSB> 20mg/dl). Erythrocyte G6PD level, serum Bilirubin, ALT , Hb%,hematocrit, TC of RBC, and PBF was examined. Data were analyzed by ANOVA,unpaired t test and pearson correlation coefficient test.

Results: 7.7% of study group had G6PD deficiency which was higher in severe hyperbilirubinemic neonates. Significant difference in mean G6PD level was observed between hyperbilirubinemic neonates and control. Though , difference between groups A and B-II was significant but the values are similar for control and B-I. Serum ALT was significantly higher in B-III than A,B-I,and B-II. Hb%,PCV,RBC were significantly lower in B-III and B-II than B-I and A. Conclusions: Results suggest that G6PD deficiency in neonates is related with hyperbilirubinemia. Therefore, early detection of this enzymopathy in newborns may be important in reducing the complications of severe hyperbilirubinemia.

P5AM-18-2

AGE-RELATED CHANGES IN BODY WEIGHT AND LENS PROTEIN STRUCTURE FROM DIET-RESTRICTED RAT

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It is known that restricting the food intake of animals such as mouse and rat increases longevity, and the diet-restricted experimental animals are often used for the study of aging processes.

Male Donryu rats were divided into two groups (group AL, rats were fed *ad libitum*; group DR, rats were fed at about 60% of the mean caloric intake of group AL rats), and have been studied age-related changes in their body weight, and lens protein structure by using Raman spectroscopy. The longevity of group DR has extended obviously from that of group AL. During life span, difference in body weight between group DR and group AL rats was well correlated with that in their caloric restriction. Peptide backbone in lens protein structure was no significant difference between both groups during their life span examined. Both microenvironment for protein side-chains (cysteine, tyrosin and tryptophan) and water content, that are estimated by their specific Raman bands, were also no significant difference between two groups.

These findings suggest that, although dietary restriction in Donryu rats might be influenced on systemic parameters such as body weight, structural components in closed organ system such as proteins in lens might not be affected by dietary restriction.

P5AM-18-3

RESPONSE OF FENUGREEK SEEDLINGS TO VARYING LEVELS OF CHROMIUM

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Seed germination and seedling growth are important events in the life cycle of plants.Chromium interfere with such events.Fenugreek is an important minor spice crop of Gujarat.Seeds of fenugreek were germinated in DW and varying levels of chromium 200,400,600,800 and1000µg/ml of sodium dichromate in petri plates under lab conditions,experimental period was 96h.Response of fenugreek seedling was studied by noting elongation,fresh weight and dry weight of seedlings at regular interval.Root elongation,shoot elongation,fresh weight and dry weight of seedlings were gradually lowered by gradual increase in sodium dichromate concentration,root was target organ.Moderate concentration of Cr increased soluble protein during first 8h of germination,but later on it was lowered, while severe concentration significantly lowered it.Peroxidase activity was lowered by Cr during 8h then it was stimulated.Cr enhanced polyphenol oxidase activity,severe concentration was more effectiveThe lowering in soluble protein and stimulation in peroxidase and polyphenol oxidase activities in sprouting seeds may be considered as biochemical symptoms of Cr toxicity,significant changes were found in peroxidase activity.Response of fenugreek seedlings to Cr may be evaluated with the help of peroxidase activity.

P5AM-18-4

EXPRESSION OF CALCIUM BINDING PROTEINS IN SKELETAL AND HEART MUSCLES OF RATS WITH ALTERED THYROID STATUS

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Thyroid hormones modify MyHC isoform content and this can result in transformation of slow to fast muscle fibers or vice versa. However, for the correct performance, other physiologically important components of ECC machinery should occur. We have therefore investigated whether the alterations of thyroid hormone levels will alter expression of selected calcium binding proteins in the slow soleus (SOL) and the fast extensor digitorum longus (EDL) hind limb muscles and in the heart of adult female inbred Lewis strain rats. HY rats were treated with 0.05 % solution of methimazole (2-mercapto-1- methylimidazole, Sigma) in drinking water, the TH status was induced by intraperitoneal injections of 3, 3',5-triiodo-L-thyronine (Sigma, sodium salt, T3, 150 µg/kg body weight) 3 times a week. Protein levels were determined by SDS-PAGE followed by western blot analysis and gene expression was assessed using reverse transcription and subsequent real time polymerase chain reaction (RT-PCR). In the poster, we will describe the protein and mRNA transcript levels for calsequestrin 1 and 2, parvalbumin and phospholamban in euthyroid, hypothyroid and hyperthyroid rats. Approved by the Expert Committee.

P5AM-18-5

THE ENERGY METABOLISM IN AGGREGATING SLIME MOLD

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Living things appeared on the earth as unicellular organism and evolved into multicellular one. A reason for this evolution attracts our interests. In order to investigate the advantages in the energy metabolism of multicellular organism, we employed Dictyostelium cells and measured cellular NADH, which could be an indicator of ATP synthesis. A fluorescence microscope was used to quantify NADH fluorescence by UV-irradiation. A serious damage induced by long irradiation required optimizing the protocol of irradiation. The protocol was established to monitor the NADH level in the single cell without any critical damage. Our monitoring system could detect the difference of NADH level between single cell amebas. We further observed that the NADH level increased by addition of cyanide in time-lapse analysis. These results strongly suggest that our method is a powerful tool for detecting the energy metabolism in single cell ameba. It was found that the NADH level in the cells decreases as forming multicellular structure and that high density of cells declines the NADH level much quickly. In this article, the effect of cell-cell contact and/or adhesion on the NADH level will be discussed.

P5AM-18-6

MICE OVEREXPRESSING DOMINANT NEGATIVE Cdk5 IN THE PANCREATIC BETA CELLS SHOW THE DIABETES MELLITUS

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Cyclin dependent kinase 5 (Cdk5) is a serine/threonine kinase. Cdk5 and its activators, p35 and p39 are enriched in neuron and regulate neurotransmission, synaptic plasticity and neural development. Recently, however, studies have shown that high Cdk5 activity is also detected in pancreatic beta cells and Cdk5 regulates glucose-stimulated insulin secretion. Recent four studies identified single-nucleotide polymorphisms in Cdk5 regulatory subunit-associated protein 1-like 1 (CDKAL1) gene in type 2 diabetes mellitus. CDKAL1 shares considerable protein domain and amino acid homology with Cdk5 regulatory subunit associated protein 1 (Cdk5RAP1), a known inhibitor of cdk5 activation.

In the present study, we produced transgenic mice overexpressing dominat-negative Cdk5 in the pancreatic beta cells. The mice showed higher HbA1c than wild-type mice in the age of 6-weeks and 12-weeks. Intraperitoneal glucose tolerance test (IPGTT) revealed the glucose-stimulated insulin secretion was impaired in the transgenic mice. Moreover, the volume of pancreatic beta cell was small in the transgenic mice. These results suggest that Cdk5 may be involved in the development of pancreatic islets, and the transgenic mouse may be a new model of the patients with diabetes mellitus.

P5AM-19-1

MODIFICATIONS ON THE EQUILIBRIUM TEST IN SIMULATION OF THE SPACE MOTION SICKNESS CAUSED BY REVERSING PRISMS

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To clarify the sensory conflict theory hypothesizing for the space motion sickness, we examined the functional equilibrium tests while human subjects wore right-left reversing prisms for 6 hours. Only 7 of all 21 subjects completely tolerated and chief complaints were nausea, vertigo and a headache. In vertical writing test, deviation angle were shifted into transient zone during reversing prisms were worn. Both total of each length and start-end distance in square drawing test elongated in an early stage of experiment. Under the reversed visual conditions, transitional positions of stepping test were localized similarly to those under blindfold one. It is observed in statokinesigram that trajectory length and enveloped area slightly increased, while Romberg ratio decreased, and that power spectrum ratio of low frequency below 0.20 Hz increased along X (right-left) axis. Functional equilibrium test revealed that reversing prisms are appropriate for the simulating method of space motion sickness. Reversed visual information brings two possible deteriorations; one is the vestibular nucleus and another is the cerebellum. We discuss effects of reversing prisms upon the autonomic systems, and also partly refer to mechanisms of space motion sickness.

P5AM-19-2

VESTIBULO-OCULAR RESPONSES IN FLAT FISH FOR THE CHANGES OF ACCELERATIONS

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On Earth, signals from otolith organs can be interpreted either as linear motion or as tilt with respect to gravity. In microgravity, static tilt will no longer give rise to changes in otolith activity. However, linear acceleration as well as angular acceleration stimulates the otolith organ. Flatfish provide a natural model for the study of adaptive changes in the vestibulo-ocular reflex. During metamorphosis, vestibular and oculomotor coordinate of flatfish displaced 90 degrees about the longitudinal body axis. In this study, we analyzed the eye movements for body tilting in normal flatfish and in unilaterally otolith removed flatfish. The eye movements for body tilting along the different body axis were video-recorded. The vertical and torsional eye rotations were calculated from the images digitized by computer.

In normal flatfish, the properties of vertical and torsional eye movements for body tilt were almost the same as that in goldfish. After removal of left utricular otolith, the amplitude of vertical eye movements decreased. Especially for 180 degrees tilting, the vertical eye movements almost disappeared. These results suggested that utricular otolith play some roll in flatfish.

P5AM-19-3

EFFECT OF GRADED LOAD OF ARTIFICIAL GRAVITY ON VESTIBULE-CEREBELLUM IN HUMANS

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We developed the short-arm centrifuge device which consisted of a rotating rod with a diameter of 4 m weighing 400 kg, a rotation motor, and a base weighing 2 tons. In addition, the centrifuge device was equipped with a detachable electromechanically braked bicycle ergometer (model V70, Senoh, Tokyo, Japan). It has been examined whether centrifuge-induced artificial gravity with ergometric exercise could reduce developing cardiovascular deconditioning, the myatrophy and osteoporosis in humans during ground-based simulated spaceflight. Anti-G tests with graded load of artificial gravity were employed before and after exposure to pseudo microgravity because there were large individual differences in tolerance to the artificial gravity although the subjects wore a head set and a head-mount display (Glasstron, Sony, Tokyo, Japan), through which audiovisual instructions were provided. However, effect of the graded load on vestibule-cerebellum in humans does not have been reported. In this study, acceleration of the centrifuge device and electrooculogram were recorded during the graded load of artificial gravity. Furthermore, we compare stabilograms before with after the load.

P5AM-19-4

THE EFFECT OF PHASIC ELECTRICAL VESTIBULAR STIMULATION ON THE 3 G ENVIRONMENT-INDUCED ATTENUATION OF THE VESTIBULO-CARDIOVASCULAR REFLEX

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We have demonstrated that the vestibular system has a significant role in arterial pressure (AP) response to gravitational change. The vestibular system is known to be highly plastic, and recent study from our laboratory demonstrated that if rats were reared under 3 G environment, the attenuation of the gravitational change-induced AP response was observed. This attenuation might be due to the decrease of phasic inputs to the vestibular system since the movement activity in rats under hypergravity environment was significantly suppressed. If this is true, we hypothesized that the electrical vestibular stimulation might prevent the decrease of phasic inputs to the vestibular system. In order to examine this, rats were reared under 1 G or 3 G environment with or without electrical vestibular stimulation for 6 days, and then the linear acceleration-induced pressor response was measured. In 1G rats, the AP was increased by 23 ± 1 mmHg in the headward acceleration, and it was significantly suppressed in 3G rats (12 ± 1 mmHg). However, the pressor response was recovered in 3G rats with electrical vestibular stimulation (20 ± 1 mmHg). In conclusion, electrical vestibular stimulation during 3 G load could prevent the attenuation of vestibulo-cardiovascular reflex.

P5AM-19-5

FEASIBILITY OF EMPLOYING GVS TO BLOCK THE VESTIBULO-CARDIOVASCULAR REFLEX UPON GRAVITATIONAL CHANGE IN HUMAN STUDY

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The role of the vestibular system in controlling arterial pressure (AP) has been the focus of many studies on both human and animals, and in these studies on animals, the AP response to gravitational change was compared between intact and vestibular lesioned (VL) animals. In human studies, however, such invasive and irreversible method like VL could not be employed. Thus, in human studies, an alternative method for acutely interrupting the vestibular-mediated AP response is required in order to examine the role of the vestibular system in controlling AP during gravitational change. Galvanic vestibular stimulation (GVS) is known to create an imbalance in the vestibular inputs, thus it is possible that the simultaneously applied GVS obscures adequate gravity-based inputs to the vestibular organs or modifies an input-output relationship of the vestibular system, and then impairs the vestibular-mediated response. To examine this, the AP responses to gravitational change were compared among rats with or without GVS and rats with VL, and found that the effects of GVS on the AP response were qualitatively and quantitatively similar to that caused by the VL. Using this method, the role of the vestibular system in AP control during head-up tilt was examined in young and aged subjects.

P5AM-19-6

EFFECTIVENESS OF ARTIFICIAL GRAVITY AND ERGOMETRIC EXERCISE AS A COUNTERMEASURE - COMPARISON BETWEEN EVERYDAY AND EVERY OTHER DAY PROTOCOLS

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Effectiveness of centrifuge-induced artificial gravity and ergometric exercise as a countermeasure to spaceflight deconditioning induced by 20 days of head-down bedrest was examined in 12 healthy men in 2006, and 8 healthy men in 2007. Bedrest was performed with 2300 kcal of diet. Water intake was recommended more than the urine volume in a previous day. A new protocol for artificial gravity with ergometric exercise was adopted, with 1.6 G of artificial gravity at heart level and 60 W of exercise every day in 2006, and every other day in 2007. The load was suspended when subjects complained all-out, and was continued until 30 min cumulative total load time. Gravity was stepped up by 0.2 G or exercise load was stepped up by 15 W alternately when the subject endured the load for 5 min. Gravity tolerance was examined by using centrifuge, and anti-G score was determined before and after the bedrest. Not all result has been analyzed, however, effectiveness of artificial gravity with ergometric exercise was evidenced in orthostatic tolerance, physical fitness, cardiac function, myatrophy, and bone metabolism in everyday protocol, but not in every other day protocol. We concluded this everyday protocol was effective in cardiovascular deconditioning myatrophy, and bone metabolism.

P5AM-19-7

EFFECTIVENESS OF ARTIFICIAL GRAVITY AND ERGOMETRIC EXERCISE AS A COUNTERMEASURE AGAINST CARDIOVASCULAR DECONDITIONING INDUCED BY HDBR

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Centrifuge-induced artificial gravity with ergometric exercise has been employed for countermeasures to spaceflight deconditioning. We examined that effectiveness of combined artificial gravity and ergometric exercise on orthostatic tolerance induced by -6◦ head-down bed rest (HDBR). We subjected 12 healthy male volunteers in 2006 & 8 healthy male volunteers in 2007 to 20 days of the HDBR. Both 2006 & 2007 control groups were not allowed to sit up during the HDBR while countermeasure group were performed ergometric exercise with centrifuge-induced artificial gravity for cumulative 30 min per day. The countermeasure was carried out during every day in 2006 & every other day in 2007. Muscle sympathetic nerve activity, blood pressure & heart rate were measured during head-up tilt test before & after the HDBR. In the control group, the blood pressure has decreased though MNSA enhance as the slope increases after the HDBR. On the other hand, the blood pressure of the countermeasure group did not difference before & after the HDBR. We conclude that the countermeasure of every day is necessary to prevent the decrease in the orthostatic tolerance after the HDBR.

P5AM-19-8

REVIEW OF STRUCTURAL AND FUNCTIONAL DEVELOPMENT OF THE RAT AORTIC BAROREFLEX IN MICROGRAVITY

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Baroreceptors sense pressure change and send this information as afferent nerves activities in the baroreflex to the brain. Changing position within a gravity field activates the baroreflexes, therefore, the amount of these stimuli is reduced in microgravity. Nine-day-old rats with their dams were flown on the Space Shuttle for 16 days spaceflight (FLT) and age-matched rats remained on the ground (two ground controls) in 1998. On landing day, we carried out the analysis of the baroreflex function and the histological preparation for electron microscopic analysis of the aortic afferent in the anesthetized rats. The number of aortic unmyelinated fibers (UMF) was significantly less in the FLT group than in each ground control, which correlated with the lower index of baroreflex sensitivity (IBRS) at the peak increase in arterial pressure (Yamasaki et al., 2004). Some of the animals were remained in the breeding facility on the ground until 30 days after landing and were examined. The number of UMF remained reduced in the FLT but no statistical differences in the IBRS were observed among the three experimental groups (Waki et al, 2005). In this congress, we summarize the development of rat aortic baroreflex under microgravity including postnatal growth of these afferent fibers.

P5AM-20-1

FASTING ELEVATED COLONIC CIRCADIAN-CLOCK GENES AND THEIR RELATED GENES

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Circadian-clock genes comprise positive and negative regulation loops to generate circadian rhythm. This molecular mechanism allows most organisms on earth to anticipate predictable daily changes in their external environment and involve in multiple metabolic processes. The gastrointestinal tract also exhibits circadian rhythms in many physiological functions such as gut motility and proliferation rates. The main focus of the present study is to investigate whether the daily patterns of the circadian-clock genes expression in colon were influenced by food restriction and examined by RT-qPCR in adult male Spraque-Dawley rats. We found that most of expressions of circadian-clock genes and their related genes, including *Per1*, *Per2*, *Per3*, *Cry1*, *Cry 2*, *Bmal1*, *Clock*, *CK1ε*, *Dbp*, *Rev-erα*, *Rev-erβ*, *Id2*, *PGC1α* and *PGC1β*, exhibited daily rhythm in the colon. Fasting did not alter the rhythmic pattern of circadian-clock genes and their related genes expression, but increased their expression levels. Prolong fasting schedule enhanced this elevation in colonic circadian-clock genes and their related genes expression. These results indicate that the expression levels of circadian-clock genes and their related genes might be responded to the metabolic status of colon.

P5AM-20-2

CIRCADIAN-CLOCK GENES AND THEIR RELATED GENES EXPRESSION IN THE RAT COLON MODULATED BY DAY-TIME FEEDING

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From transcription to physiology and behavior most organisms on earth express daily rhythm which is affected by the external time cues, including light and food schedule. The gastrointestinal tract also exhibits daily rhythms in many physiological functions such as gut motility and proliferation rates. Some of these rhythms are conceivable to control by circadian-clock genes. While food is available only to the day-time in murine, circadian-clock genes expression in the liver becomes phase-shift relative to that in animals fed *ad libitum*. The main goal in this study is to investigate whether the circadian-clock genes expression in colon had daily patterns and whether this pattern was influenced by day-time feeding. RT-qPCR was used to examine the expression levels of *Per1-3*, *Cry1-2*, *Bmal1*, *Clock*, *Dbp*, *Rev-erα* and *Id2* genes in male adult Spraque-Dawley rats. We found that expression of *Per1-3*, *Cry1-2*, *Bmal1*, *Dbp*, *Rev-erα*, and *Id2* exhibited daily rhythm in the colon. Day-time feeding rested the expression patterns of *Per2*, *Cry1-2*, *Bmal1*, *Dbp* and *Id2* genes in colon. These results indicate that colonic circadian-clock genes and their related genes expression have the daily rhythmic patterns but only parts of them are affected by day-time feeding, this important time cue.

P5AM-20-3

ELEVATION OF CIRCADIAN-CLOCK GENES EXPRESSION IN LIVER AND KIDNEY BY STREPTOZOTOCIN-INDUCED DIABETES MELLITUS IN RATS

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Circadian-clock genes exhibit not only in the central nervous system but also in the peripheral organs. More and more studies found that circadian-clock genes involved in the regulation of not only circadian rhythm but also energy balance. In this study we used adult male Sprague-Dawley rats and injected streptozotocin (STZ; 60 mg/kg) to examine the roles of circadian-clock genes in livers and kidneys after diabetes mellitus (DM). Relative genes expression determined by RT-qPCR. The expression of circadian-clock genes, *Period1* (*Per1*), *Per2* and *Per3*, showed the diurnal rhythm in the liver, and kidney of control groups. The expression levels of *Per1*, *Per2* and *Per3* in liver were elevated in the afternoon in the STZ-DM groups. Interestingly, these genes expression in liver also were raised by STZ-non DM groups, but these were still lower than those in the STZ-DM groups. This implicated that circadian-clock genes expression was also sensitized to STZ. Moreover, the expression levels of *Per1*, *Per2* and *Per3* in kidney were elevated in the morning in the STZ-DM groups, but not in the STZ-non DM groups. In conclusion, the circadian-clock genes expression in peripheral tissues is related to the functioning or daily pattern of metabolic processes.

P5AM-20-4

UNUSUAL CIRCADIAN BEHAVIOR AND DIABETES MELLITUS IN MUTANT CRY1 TRANSGENIC MICE

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Cryptochrome proteins (CRYs) play indispensable roles as inhibitive components of the transcriptional- translational negative feedback loop in the molecular model underlying mammalian circadian rhythm. In order to clarify yet uncovered aspects of mammalian CRYs *in vivo*, we generated transgenic (Tg) mice ubiquitously expressing CRY1 having a mutation in the dipeptide motif of cysteine and proline that is conserved beyond evolutional divergence among animal CRYs: cystein414 of the motif was replaced with alanine (CRY1-AP). Mice overexpressing CRY1-AP (CRY1-AP Tg) displayed a unique circadian phenotype. Their locomotor free-running periods were very long (around 28 h) with rhythm splitting. Moreover, CRY1-AP Tg mice displayed abnormal entrainment behavior. In addition, we found that CRY1-AP Tg mice showed symptoms characteristic of diabetes mellitus: they exhibited polydipsia, polyuria, and glycosuria. The incidence of diabetes showed discernible sex-dependence: male animals were prone to show the symptoms. Furthermore, diabetic mice did not exhibit obesity. These results indicate that the motif of CRY1 is crucial not only to the mammalian clock system but also to the metabolic control. More detailed analyses of the pathogenesis in CRY1-AP Tg mice are now underway.

P5AM-20-5

TIME-DEPENDENT CHANGES IN THERMOREGULATION DURING COLD EXPOSURE IN FASTED MICE

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Fasting decreases body core temperature (T_b) in mice, which shows time-dependency, greater in the inactive phase and smaller in the active phase. The purpose of the present study was to clarify the mechanism involved in the change of T_b rhythm during fasting. Male ICR wild-type (WT) and *Clock* mutant (CM) mice, housed at 27°C in a 12:12-h light-dark cycle were exposed to the cold at 20°C after 48-h fasting, corresponding to either in the light phase (LP) or dark phase (DP). T_b decreased in the cold in all the groups. In WT, the reduction was greater in LP than in DP; however, the reduction was similar in both the phases in CM. Oxygen consumption and UCP1 mRNA increased in the cold in DP of WT and both the phases of CM, whereas remained unchanged in LP of WT. After the cold exposure, counts of Fos-IR cells increased in the medial preoptic area and paraventricular nucleus in all the groups, which were greater in DP of WT and both phases of CM than in LP of WT. These results indicate that fasting attenuates thermoregulatory responses to the cold, depending on time of the day. Neuronal activities in the hypothalamus during the cold exposure may be associated with the thermoregulatory response and its time-dependency, for which *Clock* gene plays an important role.

P5AM-20-6

EFFECTS OF AMBIENT TEMPERATURE DURING MATERNAL DEPRIVATION ON CIRCADIAN RHYTHMS OF NEONATAL RATS

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Light is the most potent synchronizer to the mammalian circadian pacemaker in the suprachiasmatic nucleus (SCN). However, circadian rhythms of neonatally blinded pups are entrained by nursing mothers, and periodic deprivation of the mother (MD) phase-dependently shifts the circadian rhythms of blinded pups. A lack of maternal care and change in ambient temperature are plausible synchronizers for pups. In the present study, ambient temperature during MD was kept 10, 20 or 30 °C to evaluate the effect of temperature as a synchronizer. Blind pups were experienced MD daily for 6h between postnatal day 1 (P1) and P5, and their locomotor activity rhythms were recorded after weaning. *Period2* expression rhythm of pups was measured from cultured SCN slice prepared from *Perod2-luciferase* (*Per2-luc*) transgenic rats at P6, P21 and P31. Ambient temperature dependent phase shifts were observed in locomotor activity rhythm at weaning and in the peak phase of *Per2-luc* rhythm at all post-natal ages examined. Phase relationship of *Per2-luc* rhythms among 3 MD groups of different temperature at P6 was not maintained at P21. Our results indicate that MD shifts the phase of circadian rhythm of blind pups through the circadian pacemaker in the SCN, and the effects are temperature dependent.

P5AM-20-7

RE-ENTRAINMENT OF CIRCADIAN RHYTHMS AFTER 8 H ADVANCED LIGHT-DARK CYCLES DEPENDS ON THE TIMING OF EXPOSURES TO NOVEL ENVIRONMENT IN MICE

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In this study, we examined effects of timing of scheduled exposures to novel environment with a running-wheel on re-entrainment of circadian rhythms to an 8 h advanced light-dark (LD) cycles in mice. We used adult male wild type (WT) and transgenic mice carrying a luciferase reporter of *Per1* expression (*Per1-luc* mice). On the day of 8 h advance shift of LD cycles, scheduled exposure either at ZT12-15 or ZT21-24 (ZT12 = dark onset of new LD cycles) was started. After 4 exposures, WT mice were released into constant darkness to evaluate phase-shifts of behavioral rhythms, while *Per1-luc* mice were killed for culture preparation. *Per1-luc* rhythm in the cultured SCN and peripheral tissues were measured. The circadian behavioral rhythm in the ZT12-15 mice completely re-entrained to the new LD whereas that in the ZT21-24 mice did not. The *Per1-luc* rhythms in the cultured SCN almost re-entrained regardless of the timing of exposures. In contrast, the exposures phase-dependently accelerated the phase-shift of circadian rhythms in the lung and skeletal muscle. Exposure at ZT12-15 completely re-entrained the rhythms whereas that ZT21-24 did not. Thus, acceleration of re-entrainment by novel exposures to an 8h shifted LD cycle depends on timing of exposure in different tissues and functions.

P5AM-20-8

DIFFERENTIAL RESPONSES OF THE CIRCADIAN RHYTHMS IN MOUSE SUPRACHIASMATIC NUCLEUS CELLS TO PROTEIN SYNTHESIS INHIBITOR, CYCLOHEXIMIDE

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In mammals, the suprachiasmatic nucleus (SCN) of the hypothalamus is the site of the central circadian clock. At the molecular level, circadian rhythms are generated by auto-regulatory transcription and translation feedback loops consisting of several clock genes and their protein products. In this study, we applied a protein synthesis inhibitor, Cycloheximide (CHX), into the cultured SCN and analyzed the clock gene, *Per1*, expression rhythms to assess the critical phase and duration of protein synthesis for molecular clockworks to function.
We treated organotypic coronal SCN slices from *Per1-Luc* transgenic mice with CHX on the day 3-4 of culture for 6 h up to 48 h. Bioluminescence from the SCN was monitored either by a photomultiplier tube (PMT) for the tissue rhythm monitoring or by a CCD camera for single cell imaging.
The *Per1-Luc* rhythms restarted from the same phase after CHX withdrawal for more than 18 h, suggesting the termination of oscillation. Surprisingly, after CHX 6 h, bimodal pattern was detected for the first several cycles. Then we measured Per1-Luc rhythms from individual SCN cells and found that the bimodality reflected differently phased two groups of SCN cells. We further examine the localization and functional differences between two groups of SCN cells.

P5AM-21-1

ADRENERGIC STIMULATION OF L-TYPE Ca^{2+} -CURRENT AND CICR IN TROUT CARDIOMYOCYTES: EFFECTS OF TISSUE AND TEMPERATURE

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In cardiomyocytes, Ca^{2+}-induced Ca^{2+}-release (CICR) refers to the release of Ca^{2+} from sarcoplasmic reticulum (SR). This is triggered by the transsarcolemmal Ca^{2+}-entry via L-type Ca^{2+}-channels and reverse mode Na^{+}/Ca^{2+}-exchange that occurs with membrane depolarization. The rise in intracellular Ca^{2+} activates contraction. Previous studies in rainbow trout have shown that despite a sizable SR Ca^{2+}-load, little of it actually contributes to the Ca^{2+}-transient under basal conditions. In this study, we used simultaneous whole-cell patch clamp and Ca^{2+}-imaging in isolated rainbow trout ventricular and atrial cells to test whether CICR increases during adrenergic stimulation. We also tested the effect of temperature (7, 14, and 21 degrees C) on this interaction. Our results show that atrial cells have larger L-type Ca^{2+}-current, and CICR contributes more to the Ca^{2+}-transient. Ventricular cells show greater sensitivity to adrenergic stimulation, which induces CICR at warm temperatures. These data support the hypothesis that the ventricular SR Ca^{2+}-store is important for enhancing ventricular function during stress in rainbow trout.

P5AM-21-2

THE ROLE OF REVERSE-MODE NCX IN THE NEGATIVE FORCE-FREQUENCY RELATIONSHIP IN THE RAINBOW TROUT HEART

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The Ca^{2+} that initiates contraction in the rainbow trout heart arrives at the myofilaments primarily through L-type Ca^{2+} channels in the sarcolemmal membrane. However, Ca^{2+} can also be brought across the sarcolemma by the Na-Ca exchanger (NCX) operating in reverse-mode (revNCX). The relative importance of this route of Ca^{2+} influx was assessed in single isolated cardiac myocytes from rainbow trout using the specific inhibitor of revNCX, KB-R7943. In isolated myocytes, KB-R7943 significantly reduce cell shortening. This reduction is due to a decrease in intracellular Ca^{2+} concentration as 5 μM KB-R7943 reduces the amplitude of the Ca^{2+} -transient by approximately 50 %. The relative importance of revNCX decreased as contraction frequency was increased (from 0.2 to 0.8 Hz) in both atrial and ventricular cells. We attribute this to a frequency-induced shortening of action potential duration which reduces the opportunity for Ca^{2+} to enter the cell on revNCX. We conclude that revNCX is an important Ca^{2+} influx pathway in trout myocytes and a reduction in Ca^{2+} influx on this pathway contributes to the negative force-frequency response.

P5AM-21-3

INTRACELLULAR DIFFUSION RESTRICTIONS IN TROUT CARDIAC FIBERS AND CELLS

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Cardiomyocytes are compartmentalized by barriers restricting intracellular diffusion of adenine nucleotides. Despite extensive studies on rat cardiomyocytes, the exact localization of these diffusion barriers is unknown. Possible candidates are t-tubules, sarcoplasmic reticulum (SR) and the outer mitochondrial membrane. Rainbow trout cardiomyocytes have a simpler cytological architecture than rat cardiomyocytes. They have smaller diameter, lower density of SR and lack t-tubules. We took advantage of the structural differences between rat and trout cardiomyocytes to study intracellular diffusion restrictions further. We measured the apparent ADP-affinity of trout skinned ventricular fibres and isolated cardiomyocytes at three different temperatures. Our results showed that trout fibers had a low ADP-affinity that increased with temperature. Isolated cardiomyocytes had a higher affinity that decreased with temperature, but it was still lower than expected for isolated mitochondria. This suggests that diffusion restrictions also exist in trout cardiomyocytes, which are a useful model for further studies of diffusion restrictions. We discuss the difference between fibres and cardiomyocytes, the effect of temperature and the physiological importance of diffusion restrictions.

P5AM-21-4

COMPARISON OF LENGTH-DEPENDENT CALCIUM ACTIVATION OF CARDIAC MYOFILAMENTS BETWEEN THE RAT AND THE RAINBOW TROUT

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Most fish regulate cardiac output via changes in stroke volume whereas most mammals regulate cardiac output via changes in heart rate. We hypothesized that this change in mechanism of regulation would coincide with a change in the myocardial response to stretch. This possibility was tested in permeabilized cardiomyocytes prepared from rat and rainbow trout ventricles, where both sarcomere length (SL) and degree of Ca^{2+} activation could be controlled. Myofilament Ca^{2+} sensitivity activation was higher in trout than in rat at each SL tested (2.0, 2.3, 2.5 and 2.7 μm). We also found that permeabilized trout myocytes produce greater passive tension at any given SL than rat. This was surprising as the trout heart is known to be more compliant. Interestingly, addition of phosphatase inhibitors in the permeabilizing solution reduced passive tension in the trout cells suggesting that phosphorylation of titin may be important in determining passive tension in particular in trout heart. In conclusion contractile properties in trout seem highly sensitive to the length-dependent modulation.

P5AM-21-5

EFFICIENT PHENOTYPIC CHANGES OF BRANCHIAL CHLORIDE CELLS IN THE MOZAMBIQUE TILAPIA TRANSFERRED FROM HYPOTONIC TO HYPERTONIC ENVIRONMENTS

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Euryhaline tilapia (*Oreochromis mossambicus*) survived in brackish water (BW; 20‰) but died in seawater (SW; 35‰) within 6 hrs when transferred directly from fresh water (FW). The epithelial chloride cells are the main sites for active ion transport in gills. Three subtypes of chloride cells with different apical surfaces, i.e., wavy-convex (subtype I), shallow-basin (subtype II), and deep-hole (subtype III), were found in hypotonic acclimated tilapia, while eventually only subtype III existed and subtype I and II gradually disappeared when acclimated to hypertonic environments. The process of pre-acclimation in BW is necessary for tilapia to develop the subtypes III chloride cells when transferred from FW to SW. This study investigated the phenotypic changes of chloride cells in the gills of Mozambique tilapia transferred from hypotonic to hypertonic environments and evaluated the time course leading to modification of chloride cell phenotypes for successful survival after transfer. Meanwhile, the densities and sizes of chloride cells were evaluated by scanning electron microscope. In addition to the phenotype observation, this study is the first finding and investigation of Mozambique tilapia surviving when experience such a drastic salinity changes.

P5AM-21-6

LOCALIZATION OF CARBONIC ANHYDRASES IN MITOCHONDRIA-RICH CELLS OF ZEBRAFISH

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Carbonic anhydrases (CAs) catalyze the reversible hydration of CO_2 to HCO_3^- and are known to regulate ion transport across the plasma membrane by interacting with ion transporters. Here we focus on freshwater fish and analyzed the roles of CAs in the ion homeostasis. Freshwater fish maintains its ion homeostasis by active absorption of ions through the mitochondria-rich (MR) cells which are localized in the skin and gill. Involvement of CAs in the MR cell function has long been suggested by experiments using CA inhibitors. However, the molecular mechanism of CAs in ion absorption remains to be solved. In this work, we used zebrafish (*Danio rerio*) as a model of freshwater fish and analyzed the function of CAs in the MR cells. Whole mount *in-situ* hybridization against zebrafish larvae confirmed that two *ca* genes are expressed in the vacuolar H^+-ATPase-rich MR cells (v-type MR cells), which function in Na^+ uptake. Immunostaining with polyclonal antibodies demonstrated the concentrated localization of CAs in the apical region of MR cells. The influence of CAs to Na^+ uptake was observed by knocking down the CAs. These results suggest that CAs have an important role in Na^+ uptake from the environmental freshwater.

P5AM-21-7

GILL TRANSCRIPTOME CHANGES UNDER DIFFERENT CONDITIONS OF WATER CALCIUM AVAILABILITY IN TETRAODON NIGROVIRIDIS

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The gill is one of the main organs regulating calcium uptake in fish. This study aimed at unraveling the gene networks controlling the responses of green puffer fish *Tetraodon nigroviridis* to changes in the water calcium ion availability. Green puffer fish were placed in 12 ppt water containing 0.01 (low group), 2.9 (control) or 10mM (group high) Ca^{2+} and sacrificed at 2 and 12h after transfer. SuperSAGE (serial analysis of gene expression) gill libraries from these samples were massively parallel (454) pyrosequenced yielding 79,367 unique tags (small transcript identifiers, 26bp). Of these 1,426 were differentially expressed between control and low/high Ca^{2+}, with 1,006 regulated more than 5-fold. 66.5% of unitags could be annotated to *T. nigroviridis* genes or cDNAs, while 41.4% could be assigned to proteins in the Swiss-Prot database. There was a strong positive linear correlation between the differential expression in gill determined by SuperSAGE and real-time quantitative PCR for a group of selected genes. Detailed analysis revealed features that could provide novel insights into regulatory mechanisms in response to rapid changes in environmental calcium.

P5AM-21-8

EXCRETION OF BORATE BY THE KIDNEY OF SEAWATER FISH

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Boron is known as a vital micronutrient in plant and animal, but is toxic at high concentrations. Although seawater (SW) contains a relatively high concentration of borate (0.4 mM) and SW fishes drink large amount of SW, little is known about how the excess borate is eliminated. To address this issue, we tried to identify borate transporters of mefugu (*Takifugu obscurus*), a euryhaline pufferfish which can survive both in freshwater and SW. The concentrations of borate in bladder urine of freshwater- and SW-acclimated mefugu were 0 and 27 mM, respectively, suggesting the presence of excretory system in the kidney of SW mefugu. Through data mining of the fugu genome sequence, we identified two homologous genes for Na^+-coupled borate transporter (solute carrier family 4 member 11, SLC4a11) and named them NaBC1A and NaBC1B. In the kidneys of mefugu, the expression of NaBC1A was upregulated after SW acclimation. Immunohistochemistry showed that NaBC1A is expressed in apical membrane of renal proximal tubules. These observations suggest that SW fish excrete excessive borate into the urine by NaBC1A to avoid its toxic effects.

P5AM-21-9

THE EFFECT OF INFECTION BY VIBRIO VULNIFICUS ON THE CARDIAC ACTIVITY AND PRO-INFLAMMATORY GENE IN ZEBRAFISH

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The activation of sympathetic and parasympathetic nervous system modulates the heart rate (HR). The balance of sympathetic and parasympathetic tone changes the intervals between the two neighboring heart beats and induced the heart rate variability (HRV). It has been indicated that the HRV was a useful tool for screening the physiological state of the subject under certain pathological condition. This study investigated the change of HR, HRV and pro-inflammatory genes induced by the infection of *Vibrio vulnificus* (Vv) in zebrafish. The electrocardiogram of zebrafish (ECG) was recorded before and after infection for several time points, and the changes in HR and HRV were analyzed. In addition, the expressions of pro-inflammatory genes were also monitored with RT-PCR technique. The zebrafish were immersed in the Vv-contained solution. The HR increased with the time of infection and would be significantly higher than the control after 3 hours infection. The mRNA level of TNF-α, COX-2, and IL1-β were also enhanced with the infection time. The expression of pro-inflammatory genes correlated to the change of HR and HRV (NN50 and pNN50). These results suggest that HRV is also a convenient method for monitor the physiological state in zebrafish.

P5AM-21-10

THE COMPARATIVE STUDY OF SOME NEW ACYL-HYDRAZONES AND 1,3,4-OXADIAZOLINES ON INFLAMMATION-INDUCED NITRIC OXIDE SYNTHESIS

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Flavones are a class of compounds widely present in nature.The literature reports some data regarding the importance of the azole heterocycles for the antimicrobial and antiphlogistic activity.

The aim of the study was to evaluate the effect of some newly synthesized polyheterocyclic systems containing the chromone and 1,3,4-oxadiazoline nucleous and the intermediate acyl-hydrazones compounds on phagocytes and inflammation-induced nitric oxide synthesis.The positive control group of inflammation, and those treated with the synthesized 7-Arylidenhydrazinocarbonyl-methylen-oxy-flavones and 2-(2'-phenyl-7'-oxymethyl-croman-4'-on)-4-N-acetyl-5-aryl-D2-1,3,4-oxadiazolines were also compared with a group treated with diclofenac.After 24 hours from turpentine administration blood samples were harvested for the in vitro phagocytosis test, total leukocyte count, differential leukocyte count expressed as percentage, and serum nitrite/nitrates determination (Griess).

All tested compounds reduced nitric oxide synthesis, total leukocyte count, phagocytes percentages and phagocytes activity. The antiinflammatory effect was lower then diclofenac effect. By cyclisation of the acyldrazones to the 1,3,4-oxadiazolines compounds the effect increased.

P5AM-21-11

THE COMPARATIVE STUDY OF SOME NEW ACYL-HYDRAZONES AND 1,3,4-OXADIAZOLINES ON INFLAMMATION-INDUCED OXIDATIVE STRESS

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Flavonoids represent a major group of natural compounds with beneficial pharmacological effects, including anti-inflammatory, anti-allergic, antiviral, anticancer inhibitory activities on several enzymes and antioxidative properties.

The literature reports some data regarding the importance of the heterocyclic systems like D2-1,3,4-oxadiazoline nucleous for the antibacterial, antifungal and antiflogistic activity. Also, it has been cited that the acyl-hydrazone moiety is able to form the chelats with the transition metal ions.

The aim of this study was to evaluate comparative the oxidative balance in turpentine-induced acute experimental inflammation after a treatment with some new synthesized systems containing the chromone and 1,3,4-oxadiazoline nucleous and the intermediates acyl-hydrazones.The positive control group of inflammation, and those treated with the 7-Arylidenhydrazinocarbonyl-methylen-oxy-flavones and 2-(2'-phenyl-7'-oxymethyl-croman-4'-on)-4-N-acetyl-5-aryl-D2-1,3,4-oxadiazolines were compared with a group treated with diclofenac. After 24 hours from turpentine administration we measured TOS and TAR. We concluded that the tested compounds reduced the oxidative stress by decreasing TOS and increasing TAC. The antioxidative effect was better than of diclofenac.

P5AM-21-12

STUDIES ON ANTI-INFLAMMTORY ACTIVITIES OF HEXANE FRACTION OF ARDISIA CRISPA EXTRACT

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Ardisia crispa has been claimed by local villagers to have medicinal properties, which is widely used in treating dysmenorrhea, rheumatism, orchitis, skin problem, coughs, fractured bones, sprains and treatment for women afterbirth. Hexane fraction of ethanolic extract of Ardisia crispa (ACHE) was used in this study. For anti-inflammatory activity, 12-O-tetradecanoylphorbol-13-acetate (TPA) was applied to ear of mice to induce oedema and treated with 0.5,1 and 2mg/ear of ACHE topically. In Cotton-pellet granuloma test, treated groups were received 3, 10, 30 and 100mg/kg of hexane extract orally for 7 days. For antipyretic activity, mice were injected brewer's yeast to induce fever and then given 10, 30,100 and 300mg/kg of ACHE orally. The results demonstrated that 1 and 2mg/ear of ACHE produced significant suppression of 19.9%, 20.2% on oedema in dose dependent manner excepted the lowest dose of ACHE showed no significant compared to that of control. 30, 100 and 300mg/kg of ACHE in antipyretic studied are exhibited inhibition effect higher ($P<0.01$) than that of acetaminophen. ACHE also elicited a significant ($P<0.05$) inhibition of granuloma tissue and exudate formation. Thus, it can be concluded that *Ardisia crispa* has significant anti-inflammatory and antipyretic effects.

P5AM-21-13

OXIDATIVE STRESS AND NO-DEFICIENCY CONTRIBUTE TO HYPERTENSION FOLLOWING REDUCTION IN NEPHRON NUMBER OR HIGH SALT-INTAKE

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Aim: In the present study we investigated the effect of nitric oxide stimulation or antioxidative treatment on blood pressure and tubuloglomerular feedback (TGF) response in hypertensive animals with reduced nephron number and chronic salt treatment.

Methods: Rats were uninephrectomized (UNX) or sham-operated at 3-weeks of age and then subjected to high salt diet (HS) or normal salt diet (NS), together with normal, L-arginine or tempol supplemented drinking water for 6-8 weeks, followed by blood pressure and TGF measurements.

Results: Chronic salt-treatment caused hypertension in sham-operated controls (110±4 mmHg) which was augmented in UNX animals (144±8 mmHg), compared with non-treated controls (98±2 mmHg). L-arginine or tempol supplementation reduced blood pressure in both hypertensive groups, but had no significant effects in the controls. UNX+HS treated animals demonstrated an increased TGF-response, compared with the controls. Both L-arginine and tempol normalized the TGF-response, but had no effect in the controls.

Conclusions: Reduced nephron number and chronic salt loading cause hypertension. Increased oxidative stress and nitric oxide deficiency may increase the TGF-response and thus play an important role in the development of hypertension.

P5AM-21-14

NOX4 AND CU-ZN SOD ARE INCREASED AFTER PROLONGED FASTING IN NORTHERN ELEPHANT SEALS

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Northern elephant seals I(*Mirounga angustirostris*)I are naturally adapted to prolonged periods (2-3 mo) of food and water deprivation. Similar fasting periods in other mammalian species would have deleterious effects on cellular metabolism by increasing oxidative stress. However, the effects of prolonged fasting on oxidative stress in these "fasting-tolerant" mammals are not been described. To test the hypothesis that prolonged fasting in seals is not associated with increased oxidative stress and inflammation, blood and muscle biopsy samples (n= 19) were compared between early and late fasted seals. Fasting induced a 98% increase in NOX4 protein expression associated with a 57% increase in Cu-Zn superoxide dismutase (SOD) protein expression. Indices of lipid peroxidation, TBARS, and 8-isoprostane were not significantly increased after fasting. Inflammatory markers, TNF- α and CRP were also not significantly increased. This observed in increase in NOX4 and SOD suggests that the pro- and anti-oxidative balance is maintained (at least for superoxide) resulting in the lack of observed increase in oxidative stress and inflammation. The data suggest that elephant seals have evolved robust mechanisms that have allowed them to adapt to their potentially detrimental behaviors such as prolonged fasting.

P5AM-21-15

AGE AFFECTS THE COLLOID OSMOTIC PRESSURE OF BLOOD AND INTERSTITIAL FLUID OF CHICKENS

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Building only a small fraction of solutions total osmotic pressure and colligative properties, protein osmotic pressure (COP) is of sufficient magnitude to influence Starling equilibrium, water movements and tissue hydration. In many species, these functional elements are influenced by age, but evidence of such influence in avians is sparse. The COP of serum (COP_S) and suction blister fluid (COP_{IF}) were determined in anaesthetized female and male chickens in four successive stages: juvenile, early and full maturity, and sexual senescence. For calculating COP, an equation by Ahlqvist (2004) that formulates the effects of each protein fraction on COP_S, was applied. It was shown that COP_S values increased by age and were similar in both genders. The mean value in females was 14±0.83 mmHg and 15±0.83 mmHg in males. In contrast, COP_{IF} slowly decreased by age in females, but did not change significantly in males. The mean COP_{IF} value in females was 4.8±0.74 mmHg and 5.6±0.21 mmHg in males. In conclusion, the COP values around the vessel wall are affected by aging in chickens. The increase in COP_S and decrease in COP_{IF} favour reabsorption and maintaining intravascular volume. Females seem to be more prone to tissue dehydration due to the loss of tissue's counteracting force for COP_S.

P5AM-21-16

EFFECTS OF GINGER (*ZINGIBER OFFICINALE*) ON HEPATORENAL FUNCTIONS AND HEMATOLOGICAL PARAMETERS IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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An evaluation of the effects of aqueous Ginger extract on the hepatorenal functions and hematological parameters of streptozotocin (STZ) induced diabetic rats (n = 7 for each group) was carried out. Graded doses (100, 300, 500 mg/kg) of *Z. officinale* were administered to the animals orally for 30 days. Blood glucose level and body weights were measured weekly. Biochemical analysis of hepatorenal functions and hematological parameters were carried out. Results showed a significant (p<0.01) reduction in the blood glucose level at all the doses tested. Attenuated hematological parameters (RBC, WBC, Neutrophils, Lymphocytes and PCV) were also significantly (p<0.01) restored at the high dose in treated group when compared to normal control. Administration of *Z. officinale* (500 mg/kg) (p<0.001) and (p<0.05) significantly reduced STZ induced elevation of serum ALT, AST, ALP, Creatinine, Urea and increase body weight respectively. The present study reveals that the high dose (500 mg/kg) of aqueous extract of *Z. officinale* ameliorate the blood glucose level, hepatorenal damage, and restored altered hematological parameters of STZ induced diabetic rats, which suggest its potential as a phytomedicine.

P5AM-21-17

MR T2* MEASUREMENTS OF OXYGEN SATURATION IN ADULT HUMAN AND SHEEP BLOOD IN-VITRO

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T2* may be used to measure oxygen saturation (SO_2) in blood non-invasively, e.g. in human fetuses. To obtain standard measurements, heparinized blood from sheep and from human volunteers was oxygenated (SO_2 10 to 100 %; OSM3), and hematocrit was 43% (human) or varied from 26 to 51 % (sheep). The samples (11 mm diameter test tubes) were imaged with a 12-channel headcoil at 3T (Tim/Trio, Siemens Medical Solutions). To calculate T2*, a multiecho, spoiled, gradient recalled echo acquisition was used with 7 different echo delay times TE (5-40 msec at 5 msec increments), TR=400 msec, 10 mm thick, 0.5 x 0.5 mm in-plane resolution. 1/T2* (sec^{-1}) was derived from the slope of log signal intensity versus TE.
In human, 1/T2* depended on SO_2 as 1/T2*=331-3.3 SO_2 (r^2=0.97), and in sheep the bivariate relation was 1/T2*=53-0.66 $SO_2$2+0.48 hematocrit (r^2=0.78). The estimated (both r=0.87) constants $R2_0$ (sec^{-1}) and K of the Luz-Meiboom approximation ($1/T2^*=R2_0+K(1-SO_2/100)^2$) were 10.6 sec^{-1} [95%CI: 6.8-14.4] and 85.8 [71.7-99.8] in sheep (all hematocrits), and 38.4 sec^{-1} [18.0-58.8] and 370.7 [311.5-429.9] in humans, respectively.
Animal experiments on the feasibilty of measuring oxygen saturation in the fetus with MRI will have to take the difference in T2* of blood between species into account.

P5AM-21-18

ROLE OF VIP IN THE REGULATION OF OMASAL MOTILITY IN SHEEP

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Relaxation of the reticulo-omasal orifice (ROO) in the ruminant forestomach has been shown to be regulated by VIP, however, it remains unknown whether the omasal body is also regulated by VIP. Thus, the present study examined the effect of intravenous infusion of VIP on omasal motility in sheep and mRNA expression of PACAP/VIP receptor subtypes in the ovine omasum. Under halothane anesthesia, five Suffolk-strain mature sheep were equipped with a cannula in the dorsal sac of the rumen and bipolar electrodes in the greater curvature of the omasum (GC). Ruminal and omasal motility was recorded by manometry and electromyogram (EMG) in conscious sheep, respectively. Intravenous injection of porcine VIP at 3-100 pmol/kg significantly and dose-dependently decreased number of spike activities of omasal EMG. After the in vivo experiment, animals were euthanized under anesthesia, and muscle layers of the ROO, omasal canal (OC), and GC were excised. Real-time PCR on the muscle layer-extracts demonstrated 100-times higher mRNA expression ratio of VPAC2 receptor than that of PAC1 and VPAC1 receptors in the ROO, OC, and GC of the omasum. The data suggests that VIP and VPAC2 receptor play crucial roles in the regulation of relaxation of not only ROO but also the omasal body in sheep.

P5AM-21-19

THE COMPARISON OF THE IN VITRO HISTAMINE INDUCED AIRWAYS SMOOTH MUSCLE RESPONSE IN HUMAN BRONCHI AND RAT TRACHEA

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The contractile response on histamine was registered with or without addition of an inhibitor of nitric oxide (NO) synthesis, N-nitro-L-arginine (L-NA). We used human bronchi from 4 patients undergoing resection for pulmonary carcinoma and tracheal spirals from 5 healthy rats (Sprague Dowley) and 5 ovalbumin sensitised rats. The airways were mounted in an organ-bath system and isometric contraction was recorded using a computerized data acquisition system with specific software.
Results: For human bronchi, the dose-response curve to histamine indicates an increase contractile response without changing the range of efficient contractile doses. For normal rat tracheal spirals, contraction at 0.00001 M histamine was 0.34 +/- 0.2 g without L-NA and 0.37 +/-0.4 g after L-NA addition. In ovalbumin sensitized rats L-NA modified histamine contraction from 0.30 +/- 0.13 g at 0.36 +/- 0.15 g (p<0.01).
We concluded that local NO production is involved in maintaining of smooth muscle tracheo-bronchial tone in both rat and human in vitro preparatus. The ovalbumin sensitized airways are more responsive to NO inhibition. Despite the force difference between species, both models may be reliable for study of asthmatic reactivity

P5AM-21-20

RESPIRATORY RHYTHM GENERATION IS PRESENT BUT NOT ALWAYS EXPRESSED IN NEWBORN FAT-TAILED DUNNARTS (*MARSUPIALIA: SMINTHOPSIS CRASSICAUDATA*)

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The fat-tailed dunnart produces one of the smallest newborn mammals (~4mm long weighing 13-15mg). They typically do not breathe until 3-4 days postpartum (P) achieving gas exchange across the skin (Mortola et al., *Nature*, 1999). Even exposure to hypercapnia (5% CO_2) fails to promote breathing on the day of birth (P0) but does suppress metabolism. As dunnarts age, breathing begins to occur spontaneously and hypercapnia increases ventilation via increases in tidal volume. Interestingly, 100% of *in vitro* brainstem-spinal cord preparations from P0 dunnarts produce episodic bursting (fictive breathing) under basal conditions (5% CO_2, pH 7.4). Furthermore, high CO_2 (8% CO_2, pH 7.2) increases burst frequency and stabilises the rhythm of *in vitro* P0 preparations. *In vitro* preparations from older dunnarts continue to respond to high CO_2 by increases in burst frequency. These data suggest that P0 dunnarts can produce respiratory rhythm at birth even though this rhythm usually is not expressed. This may reflect a strong inhibitory mechanism *in vivo* that overrides the high central neural drive seen *in vitro*. It may also reflect the change from a high chest wall compliance that constrains breathing at birth to a lower compliance that permits breathing and increases in tidal volume with age.

P5AM-21-21

VENTRAL BRAINSTEM NEAR THE VAGAL NERVE IS IMPORTANT IN AMPHIBIAN VENTILATORY DRIVE

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Bullfrog ventilation consists of two rhythms utilizing buccal musculature, one consisting of regular buccal undulations which ventilate the buccal cavity of adult bullfrogs and gills of larval stages; the other consisting of large buccal contractions that ventilate the lungs. Each rhythm is generated by a separate oscillator in the brainstem; the gill/buccal oscillator is located near the vagal nerve and the lung oscillator is located near the facial nerve. We assessed the location and relative contribution of CO2-sensitive sites in the bullfrog brainstem. We identified two CO2-sensitive sites that contribute to the CO2 respiratory drive near each ventilatory oscillator. Results indicate the CO2-sensitive site associated with the gill/buccal oscillator is equal in importance and perhaps dominant to the CO2-sensitive site associated with the lung. Activity-sensitive dye (sulforhodamine 101) was used to image active neuronal populations, including autorhythmic neurons and those sensitive to CO2. A high degree of activity was apparent near the vagal nerve and persisted despite application of high Mg2+ as a synaptic blockade. These results indicate a significant role in neuroventilatory control and chemosensitivity in the vagal nerve region of the amphibian brainstem.

P5AM-21-22

NEURAL REGULATION OF SWALLOWING IN EEL: WITH REFERENCE TO THE PHARYNGEAL CONTRACTION AND THE UPPER ESOPHAGEAL RELAXATION

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To ingest foods or water, swallowing is essential for all vertebrates. However, the central regulation system in mammals has not been clarified sufficiently because of their complication of neuronal networks and of actions of swallowing-associated muscles. We have investigated the regulation system of drinking behavior using eel, whose swallowing is completed simply with the contraction of the pharyngeal muscle (PhrM) together with the relaxation of the upper esophageal sphincter (UES). These muscles are innervated by cholinergic neurons in the glossopharyngeal-vagal motor complex (GVC) in the medulla. Thus, the activation of the GVC constricts peripheral muscles while the inactivation induces relaxation. Here we show that the neurons controlling the GVC localize in the commissural nucleus of Cajal (NCC). When NCC was stimulated, motoneurons innervating the PhrM were activated, while the spontaneous firing of motoneurons of the UES was suppressed immediately. The inhibitory effect was mediated, in part, with catecholamines (CAs) since the effect was suppressed under presence of the CA receptor antagonists. This may be basic model to understand the central swallowing regulation in mammal since these components are comparable to the mammalian nuclei involved in the swallowing.

P5AM-21-23

ACIDIC SECRETED PROTEIN IN PITUITARY (ASPIP) BIOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION OF A NOVEL PITUITARY PROTEIN

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The pituitary gland is structurally and functionally the most complex endocrine gland in vertebrates. A novel transcript was recently isolated from the pituitary gland of the sea bream Sparus aurata and called Acidic Secreted Protein in Pituitary (ASPIP) because it shares high amino acid identity (N-terminal and mid-region) with Acidic Secreted Protein in Cartilage (ASPIC), an extracellular matrix marker for chondrocytes. In silico analysis of public databases reveal ASPIP is present in cyanobacteria, moss, invertebrates and fish, while ASPIC is present in fish and other vertebrates. No clear function has been assigned to either ASPIP or ASPIC. To establish the biological and physiological function of ASPIP a recombinant His-tag fusion protein was generated and used to generate polyclonal antisera and carry out biophysical studies. Immunohistochemistry and Western blot revealed that ASPIP is abundantly expressed in nervous tissue, liver and kidney where it is principally localised in the cytosolic cell compartment.

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P5AM-22-1

THE FUNCTIONAL RELATIONSHIP OF THE TANDEM REPEATS IN MITOCHONDRIAL DNA CONTROL REGION TO ENVIRONMENTAL ADAPTATION IN MEDAKA *ORYZIAS LATIPES*

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It has been reported that Medaka *Oryzias latipes* has a large geographic distribution and a distinct intraspecific genetic variation. We compared the whole mitochondrial DNA (mtDNA) sequences of Medaka among 8 local stocks and 4 inbred strains. The genetic distances among geographically distinct groups were highly diverged (10-14%). Phylogenetic trees and nonsynonymous/synonymous substitution rate ratios of mitochondrial protein-coding genes indicated that a few mutations might be retained by adaptive selection. The number of tandemly repeated 11 nucleotide units (TR) in mtDNA control region (CR) was highly varied within this species, while the two other *Oryzias* species, inhabiting tropical regions, had no repeats. The TR number and meteorological data in corresponding habitat indicated that the TR number correlated to the thermal environment in the original habitats. We found that the induction of transcriptional level of the mtDNA-encoded cytochrome oxidase subunit I gene by cold temperature seemed to be correlated with the TR number. This is the first study to suggest the functional relationship of the tandem repeats in mtDNA CR to environmental adaptation.

P5AM-22-2

INVOLVEMENT OF THIOREDOXIN SUPERFAMILY IN THE PROSTAGLANDIN SYNTHESIS PATHWAY

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Prostaglandin (PG)s are essential fatty acids, which function in inflammation, blood clotting, and sleep induction, etc. These bioactive compounds are synthesized from arachidonic acid via PGH_2, when cells are stimulated with some proinflammatory factors. Out of them, PGD_2, PGE_2 and $PGF_{2\alpha}$ are generated by various enzymes, which are derived from different ancestors.

Recently, we identified new enzymes to generate PGE_2 and $PGF_{2\alpha}$, mPGES-2 and tPGFS. Both enzymes include the thioredoxin (Trx)-like domain with a Trx-type active site (CXXC motif). They are first enzymes whose Trx-like domains are directly involved in PG syntheses. Trx superfamily proteins are considered to be reduction-oxidation enzymes involved in the cellular regulation, whereas mPGES-2 and tPGFS are synthases related to the catabolic reaction of PG. In addition to those enzymes, hematopoietic PGD synthase also has the Trx-like domain, but it does not have the CXXC motif. Instead, glutathione is involved in the catalytic reaction as a cofactor.

To investigate the significance of the Trx superfamily proteins in the PG synthesis pathway and the functional divergence of these proteins, we examined the evolutionary relationship of the enzymes utilizing computational studies.

P5AM-22-3

THE COMPOUND EYE OF THE MINUTE MOTH *ECTOEDEMIA ARGYROPEZA*

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There are two basic types of insect compound eye distinguished by the mechanism of image formation: apposition and superposition. A minimum size for superposition optics to function has theoretically been determined and it was concluded that superposition cannot effectively improve vision below a certain eye size. Moths, being mostly nocturnal, characteristically have relatively large compound eyes, which are of the superposition type. However, the parthenogenetic leaf-miner moth Ectoedemia argyropeza (Zeller 1839), Nepticulidae, with a wingspan of 5.0-7.0 mm and a body length of 2 mm, has eyes smaller than the predicted minimum size required for superposition. As a flying insect, E. argyropeza must be able to see, e.g. to avoid obstacles. Therefore modifications to the superposition eye of larger moths could be expected and, indeed, were found: structurally the eyes of E. argyropeza resemble apposition eyes with a tiered retinal arrangement. The objective of this study was to determine the ultra- and gross structural organisation of the E. argyropeza eye using scanning and transmission electron as well as light microscopy, and to describe the changes that allowed the moth superposition eye to become a photoreceptor that could serve one of the tiniest members of the moth taxon.

P5AM-22-4

CALCIUM CYCLING IN SCOMBRID CARDIAC MYOCYTES: A PHYLOGENETIC COMPARISON

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The diversity in cardiovascular anatomy and function within the family Scombridae provides an excellent opportunity to characterize potential specializations in excitation-contraction coupling (ECC) from a phylogenetic viewpoint. This study investigated the effects of acute temperature change on Ca^{2+} cycling in ventricular myocytes from bluefin tuna (*Thunnus thunnus*), yellowfin tuna (*Thunnus albacares*), Pacific mackerel (*Scomber japonicus*), and Pacific bonito (*Sarda chiliensis*). We hypothesised that tuna myocytes would have enhanced Ca^{2+} cycling over a range of temperatures when compared to the Pacific bonito and mackerel. Effects of experimental temperature (15, 19 and 23 ^{o}C) on the sarcoplasmic reticulum Ca^{2+} load (*SRload*) and L-type Ca^{2+} channel current (*ICa*) density and kinetics was assessed using whole-cell voltage-clamp. Our data suggest the bluefin tuna ventricle possesses the highest *SRloads* among the scombrids we tested, suggesting a more "mammalian-like" form of ECC. Surprisingly, the Pacific mackerel had *SRloads* and *ICa* densities that were similar to the bluefin, while the yellowfin tuna appeared less SR dependent. The bonito demonstrated an extremely small *ICa* and *SRload*, suggesting they may utilize a different ECC strategy than other scombrids.

P5AM-22-5

CALCIUM CHANGES WITH VASODILATION OF SPINDLE-SHAPED CONTRACTILE CELLS IN THE BLOOD VESSEL WALLS OF THE CRAYFISH, *PROCAMBARUS CLARKII* (*Pc*)

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Procambarus clarkii (*Pc*) redistribute arterial flows when exposed to hypoxic water. With hypoxia, blood flow increases in the anterior aorta and decreases in the posterior and sternal arteries, shifting more hemolymph flow to the head. Previous studies found neural regulation of cardioarterial valves, but no evidence of arterial muscle. This investigation of *Pc* blood vessels examined resistance to blood flow by vasoconstriction. Arteries were isolated and isometric force was measured on a wire myograph containing bicarbonate-buffered crayfish saline bubbled with carbon dioxide. No contraction of arteries was found in crayfish saline in response to high KCl membrane depolarization, but a calcium-free solution containing 0.1 mM EGTA caused a decline in contractile force. After relaxation, depolarization with high KCl in 0 Ca^{2+} produced a small and transient contraction. In *Pc* arterial rings loaded with fura-2, spindle-shaped cells were found in the blood vessel walls and intracellular Ca^{2+} changed during contractions. Findings suggest that crayfish blood vessels isolated in crayfish saline have contractile tone. Changes in arterial resistance may be regulated by vasodilation or Ca^{2+}-dependent vasoconstriction, producing important effects on resistance to blood flow.

P5AM-22-6

REPRODUCIBLE PATTERNS OF HEART RATE AND STROKE VOLUME RESPONSES TO COMBINED HEAD UP TILT AND LOWER BODY NEGATIVE PRESSURE

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Aims: We studied whether the hemodynamic response to augmented orthostatic stress was reproducible within subjects and whether characteristic patterns in the hemodynamic response could be distinguished between different subjects. Material and methods: Ten healthy young males were subjected to graded Lower Body Negative Pressure to achieve a pre-syncopal end-point in four tests each separated by two weeks. Beat to beat continuous hemodynamic variables were measured and analyzed by statistical models. Results: From supine control to presyncope, heart rate, mean arterial as well ablood pressure, pulse pressure and stroke index showed the expected responses (all changes $p<0.05$). The time courses of heart rate, stroke volume and orthostatic tolerance times (15±6 to 18±7 min, n.s) were highly reproducible between trials done in the same subject but different between trials done in different subjects. Conclusion: The difference observed between subjects indicates preferred activation of selected pathways of the various components of blood pressure These characteristics are a prerequisite control in different individuals while at the same time the high reproducibility measured within the same subject shows that preferential mechanisms are highly conserved within the same individual.

P5AM-22-7

NEURAL CONTROL OF ARTERIAL BLOOD PRESSURE DURING HEAD DOWN ROTATIONS IN ANESTHETIZED RABBITS

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We previously reported that head down rotation (HDR) induced a transient drop of arterial blood pressure (ABP) at 6.2 ± 0.4 s from the HDR onset. HDR induces a head-ward fluid shift which activates baroreceptors. HDR also stimulates vestibular organs. This study was undertaken to elucidate control of the ABP during HDR. Experiments were carried out on urethane-anesthetized rabbits. The animal was tilted to 45 degree head-down in 5 s and kept at the position for 5 minutes. We examined the renal sympathetic nerve activity (RSNA), cervical sympathetic nerve activity (CSNA), aortic depressor nerve activity (ADNA), central venous pressure (CVP) and ABP. RSNA decreased after 1.6 ± 0.2 s from the onset of HDR which was followed by the ABP drop, suggesting that the drop of ABP is possibly induced by inhibition of the SNA. The suppression of RSNA was larger than that of CSNA.The CVP did not change significantly. A peak of ADNA increase was 3.2 ± 0.5 s after the onset. Therefore, the suppression seems to be induced mainly by an quicker mechanism than baroreceptor reflex. RSNA, CSNA and ABP did not change significantly during HDR in vestibular-lesioned rabbits. The results suggest that vestibular organs are involved in the transient drop in ABP during HDR.

P5AM-22-8

GLUCAGON LIKE PEPTIDE-2 DO NOT ALTER SEVERITY OF CARRAGENEAN INDUCED KNEE ARTHRITIS BUT HASTENS ACCOMPANYING INTESTINAL INFLAMMATION IN RATS

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Mechanisms of inflammation induced remote organ injury involve oxidant generation. In this study the possible anti-inflammatory effects of GLP-2 were evaluated in a rat model of carragenean induced chronic arthritis. Rats divided into 1.control, 2.GLP-2, 3.arthritis, 4.treatment groups. 0.1 ml of 3% of carragenean/saline were injected into knee joint of the rats in the groups 3,4 and 1,2 respectively. GLP-2/vehicle were administered intraperitoneally 5 mcg /rat once daily to the groups 2,4 and 1,3 for 4 weeks. Intestinal permeability was assessed using the blood-to-lumen clearance of 51Cr-EDTA at the end. Rats were decapitated, severity of arthritis was determined histologically. Myeloperoxidase activity (MPO), lipid peroxidation products (LP) and glutathione (GSH) levels were determined in intestinal samples. Carragenean caused chronic inflammation in the knee joint, increased intestinal MPO activity, and LP levels. It depleted tissue glutathione but has no effect on intestinal permeability. GLP-2 treatment did not alter severity of arthritis. GLP-2 decreased neutrophil infiltration and restored glutathione levels in the intestine but intestinal permeability did not change.Anti-inflammatory actions of GLP may only involve gut specific mechanisms.

P5AM-22-9

IMMUNOHISTOCHEMICAL LOCALIZATION OF TRANSIENT RECEPTOR POTENTIAL (TRP) ION CHANNELS IN SNAKE INFRARED SENSORY ORGANS

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For poikilothermic animals, the ability to detect environmental temperature change is vital to survival. Cutaneous thermosensors occur in diverse species, but snakes from two distantly related families, the ancient Boidae (boas, pythons) and the highly derived Crotalinae (pit vipers), possess extraordinarily sensitive, facial pit organs, believed to function in prey detection and habitat selection. These unique sensory organs are part of a complex thermal imaging system, which maps thermosensory information in a spatially-conserved manner in the brain.

Neither the candidate signaling molecules nor the transducing cells have been identified. Thermosensitive TRP channels such as TRPV3 and TRPV4 can be activated by physiologically warm temperatures, and are expressed in sensory neurons as well as non-neural tissues. We have found evidence for the expression of TRPV3 and TRPV4 ion channels in different cell populations within the pit organs of several evolutionarily distinct snake species. These results indicate, for the first time, the presence of TRPV3 and TRPV4 homologs in the pit organ of snakes, suggesting a relationship to a common thermosensor, with a potentially novel TRP channel contributing to the extreme thermosensitivity of this unparalleled infrared sensory organ.

P5AM-22-10

USABILBITY OF GAS-PRESSURIZED ELASTIC GLOVE AND SLEEVE FOR EXTRAVEHICULAR ACTIVITY

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Current U.S. extravehicular mobility unit (EMU) is pressurized at 220 mmHg. The suit expands due to pressure differential between inside and outside of the suit. Significant work is required to move due to the expansion. However, if the suit is constructed by elastic material unlike current EMU, working efficiency might be improved. In this study, we compared physiological and ergonomic effects between elastic- and non-elastic glove and sleeve with the same pressure differential as the current EMU.

Skin blood flow and temperature on the hand and the arm did not change by elasticity. Range of motion of the middle finger with the elastic glove was significantly larger than that of the non-elastic glove. Maximum grip strength and the endurance time with the elastic glove were higher than those of the non-elastic glove.

Amplitudes of electromyography of the flexor carpi radialis muscle during grip and biceps brachii muscle during 90 degree-bending of the elbow with elastic glove and sleeve were significantly smaller than those with the non-elastic glove and sleeve.

These results suggest that the elastic glove and sleeve have better mobility and power generation compared to non-elastic glove and sleeve like current EMU.

P5AM-22-11

Ki-67 EXPRESSION CORRELATED WITH LYMPHOVASCULAR INVASION IN BREAST CANCER

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The Ki-67 index is a physiological parameter involving in cell proliferation. Though not recommended as an independent prognostic factor in breast cancer, it is a useful marker to evaluate the tumor growth fraction. The study is to evaluate the correlation of Ki-67 expression with the conventional pathologic assessment. Between Jun., 2007 and Dec., 2008, 35 breast cancer specimens were obtained from 34 consecutive women who underwent definite surgery at our hospital. The pathology was investigated for the cell type, nuclear grading, estrogen receptor, progesterone receptor, Her-2/neu score, lymphovascular invasion, tumor hemorrhage & necrosis, perineural invasion and lymphoplasmic infiltration. The Ki-67 antigen was stained by the IHC method. The data was analyzed with Chi-Square methods. Significant correlation was found between lymphovascular invasion status and nuclear Ki-67 expression. The presence of lymphovascular invasion in breast cancer has been found to be a poor prognostic factor. In NSABP-6 clinical trial, higher tumor local recurrence correlated with lymphovascular invasion. Our results demonstrated Ki-67 over-expression correlated to lymphovascular invasion. Prognosis of above patients is expected in the future.

P5AM-22-12

STRESS AND ADAPTATION STRATEGY

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Many studies have established the routes by which mammals use two types of adaptation strategy under unfavorable conditions. The resistant strategy permits the organism to recover from or avoid the different effects of stressors. On the contrary, the tolerant strategy permits passive resistance to inescapable stressors . The polar strategies display drastically different metabolic patterns. Firstly the resistant strategy allows the development of hypermetabolism shifts. It is manifested in enhanced O_2 consumption as well as glucose and free fatty acid utilization. The tolerant strategy is characterized by metabolic arrest by means of a reversed or negative Pasteur Effect (reduced glycolytic flux at reduced O_2 availability), while coupling of metabolic and membrane function is achievable in spite of the lower energy turnover rates by maintaining membranes of low permeability.Our data provide evidence that immune-endocrine interactions modulate the strategy of adaptation under stress conditions through changing the sensitivity to pro-inflammatory cytokines.When the “tolerant strategy” of adaptation is dominant, sensitization to IL-1 occurs. When the “resistant strategy” of adaptation is observed, CYP monooxygenases become desensitized to pro-inflammatory cytokines.

P5AM-22-13

CORRELATED RESPONSES TO A MULTIDIRECTIONAL ARTIFICIAL SELECTION IN THE BANK VOLE: ACTIVITY, METABOLISM, AND FOOD CONSUMPTION

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Phylogenetically based comparative methods have become a standard tool in studies on evolution of physiological adaptations. "Experimental evolution" offers a complementary approach to the same questions. We asked how the level of metabolism and activity of a wild rodent, the bank vole (*Myodes glareolus*), would change in response to selection in three directions: the ability of voles to grow on a low-quality herbivorous diet (H); high swim-induced aerobic metabolism (A); and intensity of predatory behavior towards crickets (P). Four replicate lines are maintained for each of the directions and an unselected control (C). After 3 generations direct responses were significant for all three directions of the selection. In subsequent generations voles from A and P lines were more active than those from H and C lines. The basal metabolic rate (BMR) was higher in A than in C or H lines and it was intermediate in P lines. Food consumption was higher in A than in H lines, and it was intermediate in C and P lines. The maximum forced-exercise and cold-induced metabolic rates were higher in A compared to C lines. The lines of voles will provide a unique model to study biochemical and molecular factors underlining the experimental evolution of behavioral and physiological traits.