Poster Presentations

Day 2

（March 22, 12:45～14:00）

P2-001～P2-057	Embryology, Regenerative Medicine, Development, Growth, Aging
P2-058～P2-087	Cartilage, Bone, Connective tissue
P2-088～P2-115	Muscle
P2-116～P2-138	Digestion, Digestive system
P2-139～P2-172	Oral physiology, Tooth, Salivary gland
P2-173～P2-188	Blood, Lymph, Immunity
P2-189～P2-246	Circulation
P2-247～P2-256	Respiration
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P2-270～P2-300	Reproduction, Genital organ
P2-301～P2-336	Endocrine
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P2-366～P2-405	Nutritional and metabolic physiology, Thermoregulation

P2-001

Epigenetic regulation on histone H3K27 is involved in redifferentiation process during leg regeneration in the cricket *Gryllus bimaculatus*

Bando, Tetsuya[1]; Hamada, Yoshimasa[2]; Mito, Taro[3]; Noji, Sumihare[3]; Tomioka, Kenji[2]; Ohuchi, Hideyo[1] (*[1]Dept. of Cytol. & Histol., Okayama Univ. Grad. Sch. Med. Dent. Pharma. Sci., Okayama, Japan; [2]Dept. of Biol. Sci., Okayama Univ. Grad. Sch. Nat. Sci. Tech., Okayama, Japan; [3]Inst. Tech. Sci., Univ. of Tokushima, Tokushima, Japan*)

Hemimetabolous insect such as cricket *Gryllus bimaculatus* has remarkable regenerative capacity. When cricket lost a part of leg, distal missing part of leg is regenerated from blastema, which is a population of proliferating multipotent cells. Blastema cells are dedifferentiated from differentiated cells and redifferentiate to several types of differentiated cells to regenerate the lost part. To know whether gene expressions could be epigenetically changed in the blastema cells, we performed transcriptome analysis on blastema cells. Our analysis showed that expression of *Enhancer of zeste* (*E(z)*), which encodes methyltransferase for histone H3K27, was upregulated in the blastema of regenerating leg compared with normal leg. We analyzed the functions of *E(z)* on regeneration process of cricket leg using RNA interference (RNAi). In the *E(z)*(RNAi) cricket, methylated histone H3K27 was diminished as revealed by immunostaining and an extra leg segment was formed between tibia and tarsus of the regenerated leg. Expression domain of a leg patterning gene *dachshund*, which promotes differentiation to tibia, was expanded in tarsus of the *E(z)*(RNAi) regenerating leg. These results suggest that epigenetic regulation on histone H3K27 is crucial for redifferentiation process during leg regeneration in the cricket.

(COI: No)

P2-002

Postnatal growth of hindlimb bones are restricted by undernutrition during early embryonic period

Kimura, Tomoko[1]; Hino, Kodai[1]; Kono, Tadaaki[1]; Kaneko, Shunya[1]; Tamagawa, Toshihiro[1]; Morita, Wataru[2]; Nakatukasa, Masato[2]; Daigo, Yataro[1]; Takano, Atsushi[1]; Nitta, Norihisa[1]; Ushio, Noritoshi[1]; Udagawa, Jun[1]; Kudo, Motoi[1] (*[1]Grad. Sch. Med. Shiga Univ., Shiga, Japan; [2]Grad. Sch. Sci. Kyoto Univ., Kyoto, Japan*)

Epidemiological studies have revealed that maternal undernutrition increases the risk of cardiovascular diseases, diabetes and osteoporosis. We previously reported that maternal 50% dietary restriction during early pregnancy restricts postnatal growth of hindlimb bones in female offspring. We analyzed the effect of 40% dietary restriction during early pregnancy on the growth of the trunk, femur and tibia in rats. Pregnant Wistar rats were divided into two groups. The undernourished (UN) group underwent 40% dietary restriction from E5.5 to E11.5, whereas the control group was fed AIN-93G ad libitum. In female neonates, the trunk and tibia were significantly longer in the UN group offspring than in controls. However, the length of tibia was smaller in UN offspring than in controls at 16 weeks of age. In the mesenchyme of the posterior limb bud at E13.5, the expression level of Grem1, which contributes to maintain limb outgrowth, significantly decreased in UN offspring than in control offspring. These results suggest that undernutrition during early fetal period affects epigenetics of the presumptive limb area and inhibits the postnatal growth of the tibia.

(COI: No)

P2-003

Comparison of early neuronal developmental stages between human iPSCs-derived neurons and rat primary cultured neurons

Roppongi, Reiko T[1]; Ohara, Yuki[1]; Yamazaki, Hiroyuki[1]; Koganezawa, Noriko[1]; Ootsu, Mao[1]; Sato, Kaoru[2]; Sekino, Yuko[2]; Sirao, Tomoaki[1] (*[1]Dept Neurobio & behav, Grad Sch Med, Gunma Univ, Maebashi, Japan; [2]National Institute of Health Sciences, Tokyo, Japan*)

We analyzed neuronal development of hiPSCs-derived neurons (hiPS-Neuron) particularly focusing on their early development stages. We cultured two types of hiPS-Neuron, and compared their development with rat neurons by Dotti·s classification. At 2 days in vitro (DIV), both hiPS-Neuron and rat neurons showed three developmental stages 1 to 3. The percentage of neurons at each stage were different between hiPS-Neuron and rat neurons. Furthermore there were significant decreases in the neurite length and numbers, branching points, and axon length in hiPS-Neuron. It is suggested that hiPS-Neuron extends their neurites more slowly than rat primary cultured neurons. Ineteresstingly the axonal differentiation of iCell neurons (CDI) occurs more slowly than ReproCELL DA neurons and rat neurons. We then double-labeled the neuorns for drebrin and F-actin. We found that the localization patterns of F-actin and drebrin in growth cones of iCell Neuron were similar to those of rat neurons. To test whether there is a difference in the effect on F-actin severing and depolymerization drug Cytochalasin D on the growth cone. Cytochalasin D caused drebrin and F-actin to shift from the transitional zone to the distal edge of growth cone in rat neurons and iCell Neurons. These data suggest that although hiPS-Neuron extends their neurite more slowly, it might be useful for pharmacological evaluation.

(COI: No)

P2-004

Influence of endogenous Akt/β-catenin signaling in hypothalamic differentiation from mouse embryonic stem cells

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Mouse embryonic stem cells (mESCs) are reported to differentiate into Rax$^+$ hypothalamic progenitor cells when cultured as floating aggregates in a growth factor-free chemically defined medium (Wataya et al., 2008, PNAS). However, we found that proportion of induced Rax$^+$ cells varies between experimental trials (40-85%). The original study also showed that exogenous activation of Wnt or Akt signaling markedly suppresses hypothalamic differentiation from mESCs, raising the possibility that endogenous Wnt or Akt activity is involved in the variability of Rax$^+$ cell induction. To test this hypothesis, we conducted differentiation culture in the presence of Wnt or Akt inhibitor. Addition of a Wnt inhibitor (Dkk-1) had no effect on the proportion of Rax$^+$ cells, but an Akt inhibitor (AktiVIII) moved the proportion into a higher range. Similar effects were produced by a compound (XAV939) that stimulates degradation of β-catenin, a downstream effector for Akt as well as Wnt pathway. Moreover, we found a clear negative correlation between Rax$^+$ cell proportion and total cell number in the differentiated mESC aggregates. These data indicate that selective inhibition of endogenous Akt/β-catenin signaling enriches Rax$^+$ hypothalamic progenitors derived from mESCs, possibly by suppressing the proliferation of other lineage cells.

(COI: No)

P2-005

Improvement of the efficiency of differentiation of mouse embryonic stem cells into insulin-producing cells by lentivirus-mediated transduction of transcriptional factors enriched in mouse islets

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Cell replacement therapy for diabetes has become possible by artificially generated pancreatic β-cells from pluripotent stem cells. However, the yield and the functional maturation of insulin-producing cells differentiated from these cells are still poor. It is important to develop a method to facilitate the differentiation efficiency and the maturation of insulin-producing cell to bona fide pancreatic β-cells. In the present study, we compared the gene expression between the terminally differentiated cells and mouse islets by microarray analysis. And we found 86 genes that expressed in islets but not in differentiated cells from mouse embryonic stem cells(mESCs), and six genes including *Mesp1*, *Rfx6* and *Isl1* of them have function as "DNA binding" with Molecular Function Ontology. We examined tissue distribution of the six genes by quantitative PCR analysis and found that the expression of almost of the genes were highest in islets. We next prepared lentivirus carrying each gene and transduced into differentiating mESCs. In Mesp1-transduced cells, some β-cells specific gene expressions were increased compared with normally differentiated cells. *Rfx6* and *Isl1* are known to have important role in pancreatic development, whereas the role of *Mesp1* and other genes in pancreatic development have not been revealed. In conclusion, this study suggests possible candidate for unknown key factors for pancreatic development and differentiation.

(COI: No)

P2-006

Sbno1 is required for maintenance and differentiation of the neural stem cells

Katsuyama, Yu[1]; Nakamura, Ryuji[1]; Sugiyama, Taku[1,2]; Osumi, Noriko[1]; Abe, Takaya[3]; Aizawa, Shinichi[3]; Hibi, Masahiko[4]; Terashima, Toshio[5] (*[1]Grad. Sch. Med. Tohoku Univ., Sendai, Japan; [2]RIKEN BSI Saitama, Japan; [3]RIKEN CDB Kobe, Japan; [4]Nagoya Univ., Nagoya; [5]Grad. Sch. Med. Kobe Univ., Kobe, Japan*)

Sbno1 is included in strawberry notch (sbno) family protein, of which structure is similar to nucleic acid helicases. Drosophila mutants of sbno gene exhibit abnormal morphologies, which are common to mutants of Notch signal related genes, suggesting that sbno is involved in Notch signaling pathway. However, knockdown or knockout experiments utilizing other animals, such as nematode and fish, did not clearly indicate sbno function in Notch signaling pathway. Recent genome analysis of human suggested that SBNO1 is relevant to normal development and function of brain. Here, we examined function of Sbno1 in mouse embryogensis focusing on brain development. Our immunohistological observations utilizing anti-Sbno1 antibodies showed strong nuclear expression of the protein in the differentiating neurons, whereas the expression was weak in the zone facing the ventricle. We then constructed floxed Sbno1 transgenic mouse mutant line, and crossed it to Emx1-Cre driver line to achieve dorsal forebrain specific Sbno1 knockout out. The embryonic cortex of the mutant exhibited premature neuronal differentiation and robust cell death. When we observed an earlier stage of cortical development in the mutant embryos, we found ectopic expression of p53, the tumor-suppressor protein. These observations in the mutant suggest that Sbno1 is required for two aspects of stem cell function, cell cycle regulation and production of neurons.

(COI: No)

P2-007

Identification of a novel pluripotency factor that is conserved between planarian neoblasts and human Muse cells by using planarian neoblast-specific antibody

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Muse cells are human adult stem cells that are found in mesenchymal tissues. Muse cells can be differentiated into various types of cells of all three germ layers, and are not tumorigenic. Looking over across the animal kingdom, we can find similar types of multipotent adult stem cells in many evolutionally primitive animals. Among such animals, planarian has been used as a model organism for regeneration. The pluripotent stem cells in planarians are called neoblasts. We hypothesized that the evolutional origin of Muse cells might be neoblasts. Using newly-developed neoblast-specific antibodies (Abs), we performed immunoprecipitation (IP) and mass spectrometry analysis for the IP products, and identified specific IP products. Double-immunostaining experiments using the neoblast Ab, and the specific Ab against the identified factor, revealed that the staining pattern merged well in Muse cells. Furthermore, we found that some cells in connective tissues of human pancreas were specifically double-stained with the specific Ab and the Muse cell marker SSEA-3 Ab. Collectively, we identified strong candidates of common proteins in multipotent adult stem cells that were conserved from planarian to human.

(COI: No)

P2-008

A molecular mechanism underlying planar cell polarity orientation in Drosophila

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How individual cell polarity becomes aligned along a global axis within a tissue is a central question in developmental biology. In Drosophila, planar cell polarity (PCP) molecules such as Dachsous (Ds) and Four-jointed (Fj) may function as global directional cues orienting cellular asymmetry, which is manifested as polarized localization of PCP core proteins such as Frizzled (Fz). However, the relationship between the Ds/Fj gradients and Fz asymmetry in the eye is opposite to that in the wing, thereby causing controversy about how these two systems are connected. Here, we show that this relationship is determined by the ratio of two Prickle (Pk) isoforms, Pk and Spiny-legs (Sple). Pk and Sple have antagonistic functions and form different complexes with distinct subcellular localizations. In wings where a Pk:Sple ratio representative of the eye was artificially created, Sple-Dachs cooperation polarized Sple at the cell edge exhibiting the highest Ds level, leading to a reversal of PCP orientation. A mathematical model was used to demonstrate that Sple is the key regulator connecting the Ds/Fj gradients and the PCP core proteins. Our model may explain the previously noted discrepancies in terms of the differing relative amounts of Sple in the eye and wing.

(COI: No)

P2-009

Histological findings of the embryonic lung tissue in *Foxc2* knockout mice

Tsuji, Mayoko[1,2]; Morishima, Masae[1]; Shimizu, Kazuhiko[1]; Kondo, Mitsuko[2]; Tamaoki, Jun[2]; Kume, Tsutomu[3]; Ezaki, Taichi[1] (*[1]Tokyo Women's Univ. Sch. Med. Dept. Anat. Dev. Biol., Tokyo, Japan; [2]Tokyo Women's. Med. Univ. Med. First Dept. Med., Tokyo, Japan; [3]Northwestern Univ. Sch. Med.*)

Foxc2 gene is one of known genes for lymphedema-distichiasis syndrome. Moreover this gene is expressed in several embryonic tissues including endothelial and mesenchymal cells of developing cardiovascular system, and several cancer cells. During the investigation of cardiovascular anomalies in *Foxc2* mutants, we found these mutants also showed thicker alveolar septa than wild type fetuses, although heterozygotes could survive after birth. In this study, we characterized the lung tissues to clarify the possible improvement of *Foxc2* gene in the lung development. Mutant fetuses, at embryonic days 15.5-18.5, were obtained by mating between ICR-*Foxc2* heterozygotes (Control: wild type littermates, WT). To characterize tissue morphology, cryo-sections of the lung were made and stained either with hematoxylin-eosin or immunohistochemically. As markers for the lung epithelium differentiation efficacy, pro-SPC and podoplanin were used. CD31 and type4 collagen were used to estimate the lung maturity. Although *Foxc2* mutants, especially in null fetuses, showed cuboidal type1 alveolar cells and narrower lumens than WT, the immunostainings for CD31 or podoplanin in the mutants showed that the distance between vascular endothelial cells and type1 alveolar cells shortened enough compared with those in WT. These data may indicate that *Foxc2* gene can be involved in the maturation process of type1 alveolar cells.

(COI: No)

P2-010

Serotonin transporter during palate formation in mice

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Background and objectives: Palatogenesis is directed by epithelial-mesenchymal interactions and many factors may contribute to the formation of palate. Previous works suggested that serotonin (5-HT) plays an important part in craniofacial development. However, little information exists on the precise role of serotonin transporter (SERT) in palatogenesis. Here, we assessed the localization of SERT in developing mouse palate to determine whether SERT might function during palate formation.
Materials and Methods: Embryos recovered from timed pregnant C57/BL10 mice were used. We evaluated immunoreactivity for SERT during palate formation. We also examined the localization of Ki-67 and cytokeratin (CK) in order to identify the proliferating cells and epithelial cells, respectively.
Results: No reactivity for SERT was observed in the palatal selves oriented vertically at E13.5. The shelves were horizontally at E14.5 and some epithelial cells at the tip of the palatal shelves had SERT labeling. As palate formation progressed, no SERT labeling was observed in the palate at E15.5. CK showed a similar pattern to SERT, whereas Ki-67 was diffusely distributed in the palate mesenchyme. At postnatal day, Ki-67 was seen in the basal layer and CK was observed in the oral epithelium. In contrast, SERT was detected in the basal and middle layer of oral epithelium of the palate.
Conclusions: These findings suggest that SERT contributed to palate formation. SERT may control the differentiation of the palate epithelial cells.

(COI: No)

P2-011

Expression of Osterix in the cleft palate of A/J mouse embryo

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Cleft lip and/or palate, which are caused by aberrant palatal development, are known as one of the most numerous congenital anomalies in human. Palate development begins around embryonic day 13.5 (E13.5) in the mouse. At E14.5, the shelves elevate to a horizontal position above the dorsum of the tongue and the medial edge epithelium of the horizontal palatal shelves contact, adhere and fuse along their midline, forming a midline epithelial seam. At E17.5, the continuous palate formation separates the oral and nasal cavities for breathing and feeding at the same time. The process of palate development is conserved between humans and mice. The A/J mouse strain, in which the incidence of spontaneous cleft lip and palate is 8%-12.1%, has been used as an animal model for human cleft lip and palate. In the present study, we examined expression of osteogenic markers including Osterix and Runx2, and the chondrogenic marker Sox9, during palate development of the two mouse strains, ICR and A/J, using immunohistochemistry. We found that the palate of A/J mice at E16.5 was smaller when compared to ICR, and that the markers' expression in the developing palate was different between ICR and A/J strains and between individuals with and without cleft palate in A/J mice.

(COI: No)

P2-012

Effect of mesenchymal cells in skeletal muscle myoblast cells stratification

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PURPOSE: To investigate proliferation potency of myoblast cells to reveal mechanism of myoblast cells stratification in the creating rabbit oral three-layer lamination sheet inserted mesenchymal cell layer between epithelial cell and myloblast cell sheets.
METHODS: Skeletal myoblast cells and mesenchymal cells were obtained from rabbit oral mucosal tissue using enzymatic digestion. Skeletal myoblast cells were spread onto each of the 6well inserts and cultured with advanced-DMEM with 10% FCS. 3days later, collagen gels or collagen gels containing isolated mesenchymal cells were laminated onto a cultured skeletal myoblast cells. These laminated sheets were labeled with 10mM BrdU for 48 hours before end of the culture. 2 weeks later, we analyzed the samples for immunohistochemistry, reverse transcriptase-polymerase chain reaction (RT-PCR), and DNA cell cycle analysis by fowcytometry.
RESULTS: Myoblast cell sheets stratification was observed by co-cultured with collagen gels or collagen gels containing mesenchymal cells. As the BrdU corporation and cell cycle analysis shows, the proliferation potency fall tendency observed by co-culture with collagen gel, was not observed in co-culture with a mesenchymal cell.
CONCLUSION: Although the still more examination is required about stratification mechanism of skeletal muscle myoblast cells, it is possibility that the mesenchymal cellsinvolved in maintaining the proliferation potency of myoblast cells.

(COI: No)

P2-013

Role of Semaphorin-Rho signaling in ameloblast differentiation

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During tooth development, ameloblasts differentiate into highly polarized matrix-secreting ameloblasts from oral epithelial cells to form enamel. Recently, we reported Rho kinase regulated ameloblasts differentiation through actin polymerization and cell-cell adhesion. However, the up-stream signal cascade is unclear. Previous reports suggested that Semaphorins, originally identified as axon guidance factors, mediated Rho signaling. Thus, in this study, we explored whether semaphorin regulate ameloblasts differentiation through Rho signaling. Immunohistochemistry of mouse incisors showed that Semaphorin 4D (Sema4D) and its receptor Plexin B1 strongly expressed in the polarized secretory ameloblasts, whereas they weakly expressed in inner enamel epithelial cells. Sema4D recombinant protein increased activity of RhoA and actin polymerization in cultured dental epithelial cells. On the other hands, neutralized antibody of Sema4D resulted in morphologic degeneration and reduction of amelogenin expression. A knock down of PlexinB1 inhibited RhoA activation, actin polymerization and amelogenin expression. Furthermore, The polarization of ameloblasts in transgenic mice with ameloblasts-specific expression of RhoA dominant-negative form was hindered. Expression of amelogenin and polymerized actin were also inhibited in the ameloblasts. Together, these results demonstrated that Sema4D-PlexinB1 signal pathway regulated the polarity and matrix production during ameloblasts differentiation through RhoA activity.

(COI: No)

P2-014

Searching the genes regulating myoblast fusion by using perfusion marker

Kurisaki, Tomohiro[1]; Nakai, Yuji[2]; Nagashima, Masabumi[1] ([1]*Dept. Anat., Saitama Med. Univ., Saitama, Japan;* [2]*Inst. Food Sci, Hirosaki Univ.*)

Myoblast fusion is essential to form the multi-nucleated muscle fibers that provide the contractile strength of skeletal muscle. Myoblast fusion follows an ordered set of events: recognition, adhesion, and plasma membrane union, which results in syncitium formation. However, relatively little is yet known about the molecular mechanism of membrane union. Recently we established a monoclonal antibody reactive to prefusion myocyte that is a fusion-competent, mononucleated muscle cell. By using this antibody, we have created a list of highly expressed genes just before muscle cell fusion. This list includes Jam and Myomaker. It was reported recently that they were critical for myoblast fusion, respectively. However these gene products should associate with other partner molecule to function. We searched another molecule from the list. We found that a zymogen of the digestive enzyme may regulate muscle cell fusion. Besides, listed genes were clustered to several groups by functional annotation. Then we found that the several genes involved in the formation of the tight junction were upregulated in the prefusion myocytes. We will report on the results of analysis of these genes.

(COI: No)

P2-015

5-HT4 receptor-mediated facilitation of neurogenesis of enteric neurons from transplanted brain-derived neural stem cells in the deep tissue of mouse small intestine underwent transection and anastomosis

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Two photon-excited fluorescence microscopy (2PM), can provide deeper optical penetration (several hundred μm) in in vivo preparations. We have used this approach in Thy1-promoter YFP mouse after gut transection and anastomosis. The fetal brain-derived neural stem cell (NSC) transplantation from the tail vein was performed after treatment with red fluorescent cell linker, PKH26. We obtained clear three-dimensional imaging of newborn enteric neurons generated from enteric neural progenitors (enteric NSC; green fluorescence) and those from transplanted NSC derived from the fetal brain (red fluorescence). Number of new neurons from the transplanted NSC was much smaller (approximately 10%) than that from enteric NSC. Neurogenesis was promoted by application of a 5-HT_4-receptor agonist, mosapride citrate (MOS: 100 μM) and this promotion was inhibited by simultaneous application of a 5-HT_4-receptor antagonist, SB-207266 (50 μM). After in vivo imaging, immunohistochemical studies were performed and PGP9.5 positive cells (neurons), and red fluorescence and green fluorescence positive cells were compared by confocal microscope. New enteric neurons overlapped with red fluorescence positive fetal brain-derived NSC and green fluorescence positive enteric NSC in the deep tissue of mouse small intestine.

(COI: No)

P2-016

Ectopic expression of Sema3A in the forebrain impairs the migration of GnRH neurons

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Gonadotropin-releasing hormone (GnRH)-producing neurons that play a role in regulating the reproductive system originate in the olfactory placode and migrate to the hypothalamus. Once GnRH neurons enter the medial forebrain at the site slightly caudal to the olfactory bulb, they undergo axophilic migration in association with a subset of olfactory fibers in a dorsocaudal direction. Class 3 semaphorin (Sema3A) is a secreted protein that functions in repulsive axon guidance and cell migration. We previously showed that chick Sema3A mRNA was expressed in the olfactory bulb and the restricted region of dorsal septum to which GnRH neurons tend to avoid approaching. Most migrating GnRH neurons expressed mRNA of neuropilin-1, a Sema3A receptor. To examine whether Sema3A contributes to the migration of GnRH neurons in the brain, the Sema3A-expression vector was introduced into the medial forebrain of embryonic days 3.5 chick embryos by in ovo electroporation. When misexpression of Sema3A was observed in the rostral part of the medial forebrain 3 days after the treatment, GnRH neurons migrated in a short distance along the medial forebrain surface, but could not proceed to the dorsal septum. In another case, many GnRH neurons were clustered at the entry point of the medial forebrain. These results suggest that Sema3A plays a chemorepulsive role in the migration of GnRH neurons in the brain.

(COI: No)

P2-017

Surrounding cells affect gene expression pattern of human beta-defensins and the shape of population in squamous cell carcinoma cells *in vitro*

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This study aimed to analyze the variation in gene expression levels of human beta-defensins (*hBDs*) in human oral squamous cell carcinoma cells (OSCC) under the co-culture with murine cells. Cell lines of OSCC (HSC-3, HSC-4) were co-cultured with NIH/3T3 or ATDC5 for 1.5 days. The gene expression pattern of the *hBDs* was investigated by a real-time RT-PCR. The expression patterns of *hBDs* of OSCC under co-culture were different from those of OSCC cultured on themselves. The expression of *hBD1* increased significantly when co-cultured with NIH/3T3, however, decreased significantly when co-cultured with ATDC5. Expression of *hBD2* and *hBD4* tended to decrease in co-culture. In a microscopy, small colonies of OSCC surrounded by NIH/3T3 cells were found at 1.5 days. Whereas, no obvious colonization of OSCC was found in the co-culture with ATDC5. Positive signals for anti-HBD1 antibody were found in OSCC aggregations co-cultured with NIH/3T3, however, the weak signals were found in OSCC cells co-cultured with ATDC5. These results suggested that the expression pattern of *hBDs* of OSCC is dependent on the co-culture partner. The different expression of *hBD1* may cause under the different morphology of OSCC population.

(COI: No)

P2-018

Mechanisms and roles of autonomous cell movements in embryonic cells during amphibian gastrulation

Takano, Kazuhiro[1]; Obata, Shuichi[2]; Masumoto, Mika[2]; Asashima, Makoto[3]; Nagashima, Masabumi[1] ([1]*Dept. Anat. Fac. Med. Saitama Medical Univ., Saitama, Japan;* [2]*Div. Biol. Coll. Lib. Arts and Sci. Kitasato Univ., Kanagawa, Japan;* [3]*Res. Cent. Stem Cell Eng. AIST, Ibaraki, Japan*)

Gastrulation is one of important steps for morphogenesis during multicellular animal embryogenesis. Morphogenetic movements during gastrulation relocate embryonic cells to form three germinal layers (ectoderm, mesoderm and endoderm). Unique autonomous cell movements are known in isolated embryonic cells from amphibian gastrula. However, little is known about their mechanisms. In this study, we investigated the mechanisms and roles of autonomous cell movements in embryonic cells isolated from amphibian gastrula of Japanese newt. Histochemical experiments and live cell imaging using Ca^{2+} cannel activators or inhibitors revealed new findings in relation to embryonic cell movements. Isolated presumptive ectodermal cells carried out mainly circus movement that is based on plasma membrane blebbing. Isolated presumptive mesodermal and endodermal cells, on the other hand, carried out mainly vermiform movement that is based on elongation of cellular body. Their two types of autonomous cell movements in the isolated gastrula cells are regulated by different intracellular Ca^{2+} signaling systems and localized actin polymerization. These findings suggest that development and formation of Ca^{2+} signaling mechanisms depending on a type of germinal layers play an important role in the initiation and execution of morphogenetic cell movements during gastrulation.

(COI: No)

P2-019

Impaired development of left anterior heart field by ectopic retinoic acid causes transposition of the great arteries in the chick embryonic heart

Nakajima, Yuji; Naramatsu, Mayu; Kamimura, Tatsuya (*Grad. Sch. Med. Osaka City Univ., Osaka, Japan*)

Background: Transposition of the great arteries (TGA) is one of the most often diagnosed cyanotic congenital heart defects at birth. One of the etiologies causing TGA morphology is the disruption of the left-right axis development. The anterior heart field (AHF) resides in the anterior pharyngeal arches and secondary heart field (SHF) in the coelomic mesoderm dorsal to the heart outflow tract migrate to form right ventricle as well as conotruncus. We previously reported that each heart field contributes to form distinct conotruncal region (Dev Dyn 241:284-293, 2012). The aim of this study is to find the responsible AHF/SHF region, of which abnormal development causes TGA.
Results: We placed a retinoic acid (RA)-soaked bead to the left, right or both sides of AHF of the first and second pharyngeal arches (or SHF) at stage 12 to 14 (embryonic day 2) chick embryos and examined the conotruncal heart defect at stage 34 (ED 8). TGA was diagnosed in embryos, to which RA-soaked bead had been placed on the both sides of AHF or left AHF at stage 12. AHF exposed to RA showed a reduced expression of isl1 and failed to migrate to the conotruncus leading its truncation. In cultured AHF, RA suppressed the expansion and differentiation of cardiomyocytes.
Conclusion: Left AHF in the anterior pharyngeal arches of stage 12 chick embryo is the responsible region of which impediment causes TGA morphology.

(COI: No)

P2-020

Remodeling from hemangioblasts to endocardial cells in the chick embryo

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The development of blood vessels occurs by two different processes: vasculogenesis and angiogenesis. Recent studies have suggested the endocardial cells would arise via the process similar to vasculogenesis during cardio-genesis. However, a detailed morphological analysis on the differentiation of hemangioblasts into endocardial cells has not been confirmed until now. In the present study, we report the morphological process on the differentiation of hemangioblasts into endocardium cells. Chick embryos, 4-4 1/2 days (HH stages; 23-24) were mainly used for this study. Samples were fixed with Zambonin's or Karnovsky's fixative solution for a light microscopy and for eletron microscopic analysis, respectively. Furthermore, immunohistochemistry for FLK-1 was visualized with SAB method. Some of FLK-1 positive cells were observed near the endocardium covering the cardiac lumen. Various differentiated precursor cells from hemangioblasts into endocardial cells were observed at electron-microscopic levels. Some cells had granular reticulum and Glogi-complex associated with large vacuoles containing fine-filamentous substance in their lucid cytoplasms. These cells appeared to be balloon-shaped, adhering on the luminal surface of endocardial cells. Some cells labeled with anti-FLK-1 antibodies might be finally differentiated into endocardial cells. The present results support the hypothesis that endocardium might be constructed via the vasculogenesis during cardio-genesis.

(COI: No)

P2-021

Immunohistochemical observation of the aorta and heart outflow tract during the initial formation of proximal coronary arteries

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Morphological mechanism that establishes the proximal coronary arteries is largely unknown. Using quail embryonic hearts we investigated the wall structure of the aorta and ventricular outlet during the formation of the proximal coronary arteries. Immunohistochemistry showed that tropoelastin (TELN) and fibrillin-2 (FBN2) were already deposited in the distal region of the aorta and inner mesenchyme of the myocardial sleeve prior to the formation of the primitive coronaries at 120-128 hours incubation. Smooth muscle α-actin (SMA) was accumulated in the aortico-pulmonary (AP) septum of the myocardial sleeve. Later at 132-134 hours incubation, primitive coronary arteries began to develop in the right and left coronary sinuses. At this time, SMA positive cells were diminished not only in the AP septum but also in the proximal aorta. Elastic fibers developed in the aortic wall did not extend to the aortic bulb (mesenchymal gap), which were located between the aorta and myocardial sleeve. At 138-152 hours incubation, SMA was accumulated again in the AP septum; and TELN and FBN2 were distributed over the aortic media and the inner mesenchyme of myocardial sleeve. Aortic elastic fibers were extended to the inner mesenchyme of myocardial sleeve. These observations suggest that mesenchymal gap between the aorta and the myocardial sleeve may play a role in the initial formation of the proximal coronary arteries. (This work was supported by Saitama Prefectural University Research Grant.)

(COI: No)

P2-022

Effects of FoxO1 on angiogenesis in the retina

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FoxO1 is a mammalian homolog of Daf-16, known as a longevity gene in C.elegans. FoxO1 is a transcription factor which controls cell cycle arrest, apoptosis, stress resistance, and energy metabolism. Since KO mice die around embryonic day 11 due to impaired angiogenesis, FoxO1 is thought to have an important role in vascular development. However, its mechanism has not completely been clarified. In order to elucidate its function in vascular development, we focused on its role in the endothelial cells during the postnatal angiogenesis in the retina. First we revealed the distinct localization pattern of FoxO1 protein in endothelial tip cell, which exists in the developing vascular front, in the WT mice retina. Secondly we examined which of the upstream signals control the localization by immunohistocmistry with antibodies against phosporylated Akt, ERK and JNK. Furthermore we examined impaired angiogenesis in detail in endothelial-cell specific FoxO1 KO mice. The length of the newly formed vessel was reduced and the branch points and the number of tip cells were increased in FoxO1 KO mice. Finally, to find out genes responsible to the abnormalities, we applied in situ hybridization histochemistry for some genes rich in the tip cells, such as PDGF-B, Ang2 and ESM, to WT and KO mice and identified some candidate genes. Taken together, FoxO1 was suggested to modify angiogenesis through transcriptional regulation of some genes in the endothelial tip cells.

(COI: No)

P2-023

Early life stress reduces BDNF expression and its related factors in rat hippocampus during brain development

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Early life stress interrupts brain development through the disturbance of various neurotransmitter and neurotrophic factor activities, but the details remain unclear. Brain-derived neurotrophic factor (BDNF) is one of the important trophic factors involved in neuronal growth and synaptic connection during early postnatal period. Some previous studies indicated that stress suppresses BDNF-stimulated signal pathway. We examined that how maternal separation (MS) stress influences BDNF expression and its related factors between neonatal and weaning period. The SD rats were individually separated from their dams for 3h twice-daily during postnatal days (PDs) 2-20, and the hippocampus on PDs 7, 10, 14, and 21 were analyzed using real-time RT-PCR and western blot. MS decreased mRNA and protein levels of BDNF on PD7, but did not affect on PD10, 14, and 21. In addition, MS decreased expression and/or activation of BDNF-related factors such as ERK signaling, GABA synthetic enzymes, and cholesterol synthetic enzymes on PD7. Given functional synapses are commenced to form and GABAergic inhibition is recruited around PD7, these alternations potentially disrupt normal balance of brain development.

(COI: No)

P2-024

DINE functions as a protease required for the motor nerve terminal arborization and neuromuscular junction formation

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Damage-induced neuronal endopeptidase (DINE) is a unique membrane-bound metalloprotease that we identified as a nerve injury-inducible gene. DINE-deficient mice (DINE KO) die of respiratory failure immediately after birth, because the phrenic motor nerve fails to arborize and form neuromuscular junction (NMJ) in diaphragm. This suggests that DINE plays a crucial role for motor neuron (MN) development as well as NMJ formation, although the physiological function of DINE remains unclear. Recent reports have shown that some metalloprotease family members function independent of their protease activity. To clarify the significance of DINE protease activity in developing MN, we performed a rescue experiment of DINE KO phenotype by crossing transgenic mice (Tg) overexpressing either wild type (WT) DINE or protease active site-deleted (mut) DINE specifically in MN. The overexpression of WT DINE rescued the abnormal nerve arborization and NMJ formation in DINE KO, while that of mut DINE failed. In addition, more detailed histological analysis of DINE KO revealed that the immature Schwann cells (SCs) along the axons of DINE KO showed abnormal morphology and alignment. Consistent with this finding, the expression of SC differentiation marker Oct6 decreased in DINE KO. SCs co-cultured with DINE-deficient MN were not capable of making ordinary association and alignment to the axons. These findings suggest that axonal DINE has a protease activity which may influence the axon-SCs interaction and thereby form proper nerve arborization and NMJ.

(COI: No)

P2-025

What is the crucial factor that governs the regenerative capacity of the spinal cord after injury in Xenopus?

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In mammals including human, anatomical reconstruction after spinal cord injury (SCI) is limited thus functional recovery hardly occurs. Although so many attempts have been done to develop the treatment for SCI, we have not reached the drastic treatment that enables the functional recovery. To elucidate the really fundamental factor that determines the regeneration capacity after SCI, we utilize Xenopus as the animal for studying the regeneration capacity after SCI. Because Xenopus tadpole is known to have the great capacity of regeneration, in which the spinal cord spontaneously regenerate to achieve the almost complete functional recovery even after complete transection, and to gradually loose its regenerative capacity through the metamorphosis, fascinating us to compare the reaction after SCI between the regenerative and no-regenerative stages. To elucidate the basic capacity of stem/progenitor cells existing in the normal spinal cord of Xenopus tadpole, we analyzed the expression patterns of transcription factors regulating the cell-linage specification in the spinal cord and of cell cycle markers in the regenerative and non-regenerative stage of Xenopus spinal cord with comparison of those observed in the embryonic mouse spinal cord.

(COI: No)

P2-026

Role of neuropeptide PACAP in hematopoiesis via its specific receptor PAC1R

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Pituitary adenylate cyclase-activating polypeptide (PACAP) is a multifunctional neuropeptide and contributes to anti-apoptosis, anti-inflammation, cell proliferation and differentiation. However, the role of PACAP in hematopoiesis is still unclear. The purpose of this study is to investigate the localization of PACAP and specific PAC1 receptor (PAC1R), and regulatory action of PACAP in mouse bone marrow (BM). The mRNA and protein expressions of PAC1R were detected in BM aspiration and tissues by RT-PCR and immunohistochemical staining. In particular, PAC1R strong immunoreaction was co-localized with $CD34^+$ hematopoietic stem/progenitor cells (HSPCs). By using flow cytometry (FCM) analysis, % PA1CR was found rich in HSPCs population ($CD34^+/SCA1^+$), seemed to decrease with lineage maturation (low % in $Gr\text{-}1^+/CD34^-$ and $CD45R^+/CD34^-$populations). Meanwhile, the colonies-forming unit counts of HSPCs were increased by PACAP. Few PACAP mRNA was detected in the BM aspiration. However, lumbar 1-4 paravertebral ganglions were retrograde traced by flouro-gold from tibial BM. In these ganglions, neuron cell bodies were strongly expressed with tyrosine hydroxylase and PACAP. Therefore, PACAP from nervous system may promote the proliferation of HSPCs via PAC1R signaling pathway.

(COI: No)

P2-027

Transcription factor FoxP1 is involved in the induction of apoptosis specific to the cervical spinal cord of chick embryo

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During the early development of chick embryo, a certain population of motoneuron within the cervical spinal cord undergoes apoptosis between embryonic day 4 and 5. However, the identity of this specific population remains largely unknown. Recently, it has been reported that transcription factor FoxP1 plays a critical role in defining the motoneuron identity in brachial, thoracic, and lumbar spinal cord. However, role of FoxP1 in the cervical region remain unclear. To elucidate the function of FoxP1 regarding apoptosis, we previously examined the effect of overexpression of micro RNA-9 (miR-9), which is known to suppress *FoxP1*, and demonstrated that apoptosis in the cervical spinal cord is reduced by miR-9 overexpression. However, miR-9 also suppresses *Onecut1* (*Oc1*), the expression of which initiates earlier than and overlaps with *FoxP1* expression in the developing spinal cord, and is likely to suppress some other unknown target genes. Thus, the overexpression of miR-9 is an ambiguous condition to examine the precise function of FoxP1. In the present study, we have designed shRNA expression vector that specifically repress *FoxP1*. When the shRNA against *FoxP1* was induced in the cervical spinal cord by electroporation, the number of $FoxP1^+$ cells was reduced to approximately 60% compared to the contralateral side. In contrast, the numbers of $Oc1^+$ or $Lhx3^+$ motoneurons were unchanged. In this condition, signals of TUNEL or active caspase3 were reduced, indicating that FoxP1 is required for the induction of apoptosis in the developing chick cervical spinal cord.

(COI: No)

P2-028

The evolutionary origin of cerebellar neural circuit in vertebrates

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In agnathans including lamprey and hagfish, the cerebellum consists of immature corpus cerebelli and few commissural tracts. By contrast, chondrichthyans, a group of gnathostome, possess the well-organized corpus cerebelli and the elaborated neural connection with the precerebellar nuclei. These observations indicate that the cerebellar neural circuits have been improved after the sprit between agnathans and gnathosotmes. To clarify the origin and evolution of the cerebellum, we investigated the developmental mechanism to form the spinocerebellar tract, which is conserved in every vertebrate.

We injected the neural tracer into the spinal cord to visualize the spinocerebellar tract in various vertebrate embryos, and compared its trajectory with the expression pattern of *Slit2*, a repulsive axon guidance molecule. In both lamprey (*Lethenteron japonicum*) and gnathostomes including mouse and *Xenopus laevis*, the spinal ascending axons crossed the dorsal midline at the posterior side of midbrain-hindbrain boundary, corresponding to the *Slit2*-negative region. Moreover, treatment with *Slit2* antisense morpholino disrupted the spinocerebellar tract in *Xenopus* embryo. These data suggest that *Slit2*-dependent axon guidance mechanism, which is conserved through the vertebrate evolution, plays an important role for the formation of spinocerebellar tract.

(COI: No)

P2-029

Specification of select hypothalamic circuits and innate behaviors by the embryonic patterning gene, Dbx

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The hypothalamus integrates information required for the output of a variety of innate behaviors such as feeding, mating, aggression and predator avoidance. Despite an extensive knowledge of hypothalamic function, how embryonic developmental programs specify circuits that regulate innate behaviors remains unknown. Here, we find that in the hypothalamus the developmentally regulated homeodomain-containing transcription factor, Dbx1, is selectively required for the generation of subclasses of neurons within the feeding-associated lateral hypothalamic area/zona incerta and arcuate nucleus. Consistent with this specific developmental role, Dbx1 hypothalamic-specific conditional knockout mice display alterations in energy homeostasis and innate stress responses to predator odor, but not other innate behaviors such as mating or conspecific aggression. Thus, Dbx1 is a common developmental genetic mechanism for specification of neurons in two distinct but functionally related hypothalamic nuclei, and links energy homeostasis and innate stress.

(COI: No)

P2-030

The mechanism for induction of ascidian peripheral neurons

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The vertebrate peripheral nervous system (PNS) originates from the neural crest and neurogenic placodes, which arise at the boundary between the neural plate and non-neural ectoderm. The neural crest and neurogenic placodes are considered to be vertebrate innovations, but their evolutionary origins remain unresolved. To gain more insight into the evolution of the PNS, we investigated the development of ascidian PNS, because ascidians are protochordates closely related to vertebrates and expected to provide information about the transition between invertebrates and vertebrates. We found that epidermal sensory neurons (ESNs), which mainly constitute the entire peripheral nervous system of the ascidian young tadpoles, are derived from the neural plate border, as is the case in the vertebrate PNS, and demonstrated that FGF, Nodal and BMP signaling are required for ESN specification. Gene knockdown experiments showed that moderate levels of BMP activity induce ESNs at the tailbud stage, suggesting that the role of BMP signaling in PNS formation is conserved among chordates. We also found that Nodal signaling regulates expression of BMP signaling molecules in the lateral neural plate, and consequently specifies ESNs, which clearly differs from the BMP gradient model proposed for vertebrate neural induction.

(COI: No)

P2-031

The role of BMP in the process of the developing dentate gyrus

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In the process of dentate gyrus (DG) formation, neural stem cells (NSCs) and progenitors in near the cortical hem proliferate and migrate to form granule cell (GC) layer. Our previous analysis using transgenic mice expressing GFP under the control of glial fibrillary acidic protein (Gfap) promoter (Gfap-GFP mice) reveals that during embryonic stages GFP-expressing cells confined to the dentate primordium produce granule cells (GCs). Since DG formation is reported to be impaired in the BMP receptor (BMPR) deficient mice, we thought that the malformation is due to the reduction of Gfap-expressing cells by deficient of BMP signaling. Here we investigated the role of BMP signaling in the production of GCs by Gfap-expressing cells. When neurosphere assay was performed using embryonic hippocampal cells from Gfap-GFP mice, Gfap-expressing neurospheres are detected, suggesting that they contain NSCs. RT-PCR analysis revealed that bone morphogenetic proteins (BMP2 and BMP4) are strongly expressed by the developing hippocampus. Thus, to examine the effect of BMP signaling in vivo, Noggin, BMP signaling inhibitor or dominant negative form of BMPR was introduced in the embryonic brain. The suppression of BMP signaling reduced Gfap-expressing cells in the developing hippocampus as well as Prox1$^+$ GCs. Taken together, these results indicate that Gfap-expressing NSCs that contribute to the formation of the DG are induced by BMPs that are abundantly contained in the developing hippocampus.

(COI: No)

P2-032

Cell-tracing Analysis for Progenitor Cell Migration in the Embryonic Dentate Gyrus

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In general, neurogenesis occurs during embryonic and early postnatal stages, and ceases at adult stage. However, the dentate gyrus (DG) continues neurogenesis from embryonic to adult stages. In the adult DG, granule neurons are generated in the subgranular zone, while during embryonic period, dentate neural progenitors are initially produced in the ventricular zone (VZ), and then migrate through the suprafimbrial region to the subpial region (SP) where a new proliferative zone is formed to develop the presumptive dentate gyrus. During the migration, the progenitors differentiate into granule neurons or maintain property of neural progenitors that further contribute to perinatal and postnatal neurogenesis. Although the migration of the neural precursors and relocation of the region of neurogenesis are key processes for the formation of the DG, the exact temporal and spatial patterns are still unknown. To address the problem, we performed cell-tracing analysis of the DG by in utero electroporation. RFP-positive cells originated from the VZ migrated to the DG. Immunohistochemical studies revealed that the RFP+/Tbr2+ cells were present in the SP, whereas the RFP+/Sox2+ cells were localized in both the SP and the hilus. Moreover, we performed time-lapse imaging in cultured hippocampal slices and found some types of cell migration in the DG: pia-touching cells, presumptive hippocampal fissure-touching and somal translocation-like cells. We will discuss possibility that the correlation between cell-type specification and modes of cell migration.

(COI: No)

P2-033

Tangential cell migration in the superficial layers of the developing optic tectum

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The optic tectum is composed of multiple layers, which are formed by the radial and tangential migration during development. We have previously reported a population of tangentially migrating cells in the deep layers of the developing chick optic tectum. Here, we report another tangential cell migration in the superficial layers. When the ventricular cells of chick tectum were labeled by the electroporation of GFP-expression vector at E4.5-E5.5, GFP-labeled cells migrated radially and some of them turned horizontally in the superficial layers after E7.0 and began to spread throughout the tectum. In contrast to the tangential migration in the deep layers, which is strictly guided by the tectal efferent axons in dorso-ventral and ventro-dorsal directions, these tangential migrants in the superficial layers moved freely in multiple directions with bifurcated leading processes. We are currently trying to identify their cell fates after the migration to characterize this novel superficial migration in the developing optic tectum.

(COI: No)

P2-034

Expression and function of ADP-ribosylation factor 6 (Arf6) in the neuronal migration during cortical layer formation

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Cortical layer formation in the cerebral cortex is one of the typical events in the mammalian brain. Neurons that are born in the ventricular zone migrate to the pial surface with an inside-out manner along the fiber of radial glia. Recent studies revealed that transmembrane proteins such as connexins, integrins, and N-cadherin, regulate neuronal migration through cell-cell and/or cell-matrix interactions, and their expression on the plasma membrane is tightly regulated by vesicle trafficking factors that are involved in the process of secretion, endocytosis and recycling. However, it largely remains unclear the mechanistic details of how vesicle trafficking factors regulate neuronal migration. In this study, we examined the functional role of ADP-ribosylation factor 6 (Arf6), a critical regulator of endosomal trafficking, in the cortical layer formation. In situ hybridization analysis revealed that Arf6 mRNA was expressed in all layers including ventricular zone, intermediate zone, and cortical plate in the dorsal pallium of embryonic cerebral cortex. Knockdown of Arf6 by in utero electroporation resulted in the decrease in the cell population invading to layer II-IV. Furthermore, time-lapse observation demonstrated that neuronal migration was delayed in the intermediate zone by knockdown of Arf6. These results suggest that Arf6 regulates the neuronal migration in multipolar mode through vesicle trafficking.

(COI: No)

P2-035

Cell divisions of neural progenitor cells are regulated by NRG1-ErbB signaling in the developing zebrafish optic tectum

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Post-mitotic neurons are generated from neural progenitor cells (NPCs) at the expense of their proliferation. Molecular and cellular mechanisms that regulate neuron production should impact on the size and shape of the brain. While transcription factors govern progression of neurogenesis as cell-intrinsic mechanisms, recent studies show regulatory roles of several cell-extrinsic signaling molecules in production of NPCs from neural stem/radial glial cells. However, it remains elusive what regulates production of post-mitotic neurons from NPCs. In the optic tectum (OT) of zebrafish embryos, newborn neurons accumulate in the basal-to-apical direction. Here, we show that this neurogenesis depends on Neuregulin 1 (NRG1)-ErbB signaling. Transient treatment with an ErbB inhibitor, AG1478 impairs mitoses in the sub-basal region of the OT prominently. Removal of AG1478 resumes sub-basal mitoses and basal-to-apical accumulation of neurons without affecting mitoses in the apical ventricular (V) region, suggesting critical roles of ErbB signaling in mitoses of post-mitotic neuron production. Depletion of NRG1 type II isoform (NRG1-II) impairs both mitoses in the sub-basal and apical regions. Injection of soluble human NRG1 into the developing brain ameliorates neurogenesis of NRG1-II-depleted embryos. These results imply that NRG1-ErbB signaling promotes neurogenic competence of NPCs in the developing vertebrate brain.

(COI: No)

P2-036

Newborn mice exposed prenatally to bisphenol A show hyperactivity and defective neocortical development

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The central nervous system is especially sensitive to toxic insults during development. Prenatal administration of bisphenol A (BPA) induces histologic changes in the dorsal telencephalon of the embryo. Whether these changes affect the morphogenesis and maturation of neuronal function of the newborn neocortex, however, is unknown. To evaluate the neurodevelopmental and behavioral effects of prenatal BPA exposure at 20 and 200 μg/kg/day in newborn mice, we performed a detailed histologic analysis of the neocortex and tested for the presence of behavioral abnormalities in newborn mice prenatally exposed to BPA using the behavioral test. Observations of newborn mice prenatally exposed to BPA revealed abnormal neuronal distribution and layer formation, hypoplasia of layer 6b, and abnormal dopaminergic neuronal projections in the neocortex. Further, the newborn mice exhibited hyperactivity. These findings suggest that prenatal BPA exposure induces neurobehavioral toxicity associated with abnormal dopaminergic neuronal projections, and abnormal corticogenesis. Histologic and behavioral analyses of newborn mice are considered useful for assessing the neurodevelopmental and behavioral toxicity of chemicals.

(COI: No)

P2-037

Live-cell imaging of nephrogenesis

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The mammalian kidney arises via reciprocal interactions between ureteric bud (UB) and the surrounding metanephric mesencyme (MM). UB grows and branches repeatedly in the MM. Around the UB tip, MM cells become densely packed to form a cap condensation (CC). A subset of CC cells then forms a pretubular aggregate (PA). As a result of mesenchymal epithelial transition, PA forms a single epithelial renal vesicle, which is a progenitor cell population for the all nephron epithelia. These processes of kidney development have been mostly studied by histology of the fixed kidney rudiments of various developmental stages. Thus, there are considerable limitations in understanding the dynamics of cellular events during nephrogenesis. Here, we cultured the rudimental metanephros of embryonic day-11 mice in a medium containing a non cell-permeable fluorescent tracer, and observed cell behavior by the time-lapse confocal microscopy. This method allowed us to trace the migration and proliferation of individual UB or MM cells, as a shadowgraph movie. We found extensive cell divisions in the CC and PA, and some in the UB. These were confirmed by the EdU (a thymidine analogue) incorporation assay. Roles and fates of these highly proliferative cells on the nephrogenesis will be discussed.

(COI: No)

P2-038

FGF9 and BMP4 regulate the competence of Wolffian duct

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The metanephros starts to develop by ureteric bud formation from Wolffian duct (WD) in response to GDNF secreted from metanephric mesenchyme. BMP4 secreted by surrounding mesenchyme has been reported to suppress ureteric bud formation, thereby contribute to the orthotopic formation of ureteric bud. However, the mechanisms of this suppression have not been fully elucidated. We have reported that FGF9 expressed in both WD and its mesenchyme supports the survival and responsiveness of WD, maintaining the gene expression of GDNF receptors Ret and Gfra1, Fgf9 and Sox9. In the present study, we assessed the effect of FGF9 on the expression of growth factors including BMP4 in the mesenchyme, and also examined the effect of BMP4 on WD in vitro. In rat embryos at E12 and 13, expression of several growth factors such as Bmp4, Wnt4 and Wnt2b was verified in the WD mesenchyme. Addition of FGF9 to the culture of mesenchyme significantly increased the expression of CyclinD1, while decreased that of Bmp4, Wnt4 and Wnt2b. Addition of BMP4 to the FGF9-maintained WD culture significantly decreased the expression of Ret, CyclinD1 and especially Fgf9, whereas WNT4 showed no apparent effect on these gene expressions. From these findings, FGF9 of WD and mesenchymal BMP4 appeared to reciprocally inhibit at the gene expression level. FGF9 may enhance competence of WD by suppressing mesenchymal Bmp4 expression. In turn, mesenchymal BMP4 may suppress ureteric bud formation, at least in part, through downregulation of Ret and FGF9 in WD.

(COI: No)

P2-039

Interkinetic nuclear migration in the mouse embryonic ureteric epithelium

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Purpose: Interkinetic nuclear migration (INM) is the phenomenon that progenitor cell nuclei migrate along the apicobasal axis of the pseudostratified epithelial layer synchronously with the progression of the cell cycle, and is suggested as a regulatory mechanism of stem cell proliferation. INM has been reported in epithelia of ectodermal origin. We previously reported INM in the endoderm-derived midgut epithelium in mice. We here examined whether INM exists in the mesoderm-derived ureteric epithelium.

Methods: At E11.5, E12.5 and E13.5, C57BL/6J mouse dams were injected with bromodeoxyuridine (BrdU) and sacrificed 1, 2, 4, 6, 8, 10 and 12 hours later to collect embryos. Transverse sections were BrdU-immunostained. We measured the position of BrdU-positive nuclei in ureteric epithelia along the apicobasal axis at each time point. We analyzed the distribution patterns of BrdU-positive nuclei in histograms at each point using the multidimensional scaling method.

Results: Changes in nucleus distribution patterns that suggest nucleus movement characteristic of INM was found in ureteric epithelia. Nucleus distribution patterns varied depending on the date.

Conclusions: INM exists in the ureteric epithelium of mesoderm origin.

(COI: No)

P2-040

Cathepsin D-deficient mice exhibit impairment of postnatal growth of kidney and liver cells

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Cathepsin D (CTSD) is the principal lysosomal aspartate protease and expressed in most of tissues, but the level of expression varies considerably. Mice deficient in CTSD, generated by gene targeting, develop normally during the first 2 weeks, stop thriving in the third week and die in a state of anorexia at day 26 ± 1. An atrophy of the ileal mucosa first observed in the third week progresses towards widespread intestinal necroses accompanied by thromboemboli. From these results, Saftig et al. (1995) suggested that vital functions of cathepsin D are exerted by limited proteolysis of proteins regulating cell growth and/or tissue homeostasis. The mechanism of growth disorder caused by CTSD deficiency is still unknown. To study the relationship between CTSD and cell proliferation, immunostaining for ki67, a cellular marker for proliferation, was performed in the kidney and liver of CTSD-KO mice. Positive staining for ki67 was significantly less in number in proximal renal tubular cells and hepatocytes of CTSD-KO mice at the age of 23-25 days (p23-p25) after birth than in those of wild-type littermates. These results suggest that proliferation and/or cell cycle were impaired in comparison with age. As weight difference between the CTSD-deficient and wild-type mice became remarkable after p14, CTSD may be involved in the activation of some kinds of growth factors for the small intestine, liver and kidney.

(COI: No)

P2-041

Prosaposin mRNA and protein expression in rat testis

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Prosaposin is the precursor of sphingolipid hydrolase activator proteins called saposins (saposin A-D). However, prosaposin is not merely a precursor of saposins, it also functions as a trophic factor. Prosaposin is found in cerebrospinal fluid, bile, pancreatic juice, milk, and semen. In this study, we performed immunohistochemical and in situ hybridization analyses to clarify the role of prosaposin during spermatogenesis in rat testis. We performed triple immunostaining using specific anti-prosaposin antibodies. Intense prosaposin immunoreactivity was observed mainly in spermatogonia, spermatocytes, spermatids, and Sertoli cells. We also examined the expression patterns of alternatively spliced forms of PSAP mRNA in rat testis. In rats, alternative splicing of the PSAP gene generates two forms of mRNA: Pro+9, containing a nine-base insertion, and Pro+0, which lacks the insertion. According to Madar-Shapiro et al. (1999), the Pro+9 form is preferentially secreted from cells, whereas Pro+0 is mainly found in lysosomes. In the present study, prosaposin mRNA was expressed in the basal half of the testicular tubules, while the secreted form was mainly expressed in testis.

(COI: No)

P2-042

Roles of Bmp signaling in the L-R asymmetric development of female chicken gonads

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The primordial gonads are formed on the left and right ventromedial surfaces of the mesonephros. In the chicken embryos, as in many other avian species, only the left ovary develops while the right one eventually degenerates after sexual differentiation. To understand molecular mechanisms underlying this remarkable phenomenon, we investigated the roles of *Bmp* in the L-R asymmetric development of female chicken gonads. We observed expression pattern of *Bmp7* and *Smads* and overexpressed *Follistatin* (*Fst*), an antagonist of *Bmp*, in the presumptive gonadal region and analyzed gene expression patterns in both ovaries. The overexpression of *Fst* caused the right ovary to form cortex including germ cells. In addition overexpression of *Fst* induced *Pitx2* expression in the right gonad and stimulated proliferation of somatic cells and germ cells. These results strongly suggest that *Bmp* signaling may play an important role in the L-R asymmetric gonadogenesis in female chicken embryo.

(COI: No)

P2-043

Decidual natural killer cells uptake placenta-associated miRNAs during early pregnancy

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Objective: Decidual natural killer (dNK) cells play important roles in the maintenance of early pregnancy. We hypothesized that circulating placenta-associated miRNAs might be transferred via exosomes from placental trophoblasts into maternal immune cells. We investigated whether dNK cells contain the chromosome 19 miRNA cluster (C19MC) miRNAs, which are expressed exclusively in the placenta.
Methods: Decidual tissue and peripheral blood samples from patients who gave informed consent were aseptically obtained after legal abortions (at 6-7 weeks of gestation, n = 3). The expression levels of miRNAs were examined by real-time PCR using a TaqMan microRNA Assay, and gene expression profiling was conducted using Agilent microarrays. Integrated miRNA-mRNA expression profiling and pathway analysis were performed using Ingenuity Pathway Analysis software.
Results & Conclusion: The miRNA array analysis showed that C19MC miRNAs were detected in dNK cells. By in silico analysis, twenty-one C19MC miRNAs targeted many genes that were downregulated in dNK cells compared to peripheral blood NK cells. The miRNA-mRNA network analysis indicates the inhibition of NK cell cytotoxicity by C19MC miRNAs in dNK cells. C19MC miRNAs in dNK cells may contribute to the maintenance of early pregnancy.
(COI: No)

P2-044

The expression of H19 non-coding RNA in developmental stages of the mouse placenta

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Objective: The maternally imprinted gene H19 encodes a non-coding RNA (ncRNA). H19 is strongly expressed during embryogenesis. However, there is little information available on the expression pattern of this gene in placenta development. We examined the expression level of H19 ncRNA in the mouse placenta by real-time quantitative reverse transcription PCR (real-time PCR) and in situ hybridization.
Methods: We studied the expression level of H19 ncRNA in B6D2F1 mouse placentas at four different developmental stages; E7.5, E10.5, E13.5, and E16.5. We extracted total RNA from these different stages of mouse placentas and adult mouse organs (i.e., brain, heart, lung, liver, kidney, intestine, spleen, uterus, ovary, and testis,) and performed real-time PCR for detection of expression of H19 ncRNA. In addition, we investigated expression level of H19 using in situ hybridization using DIG-labelled RNA probe.
Results: We found that H19 ncRNA was exclusively expressed in the mouse placenta by real-time PCR. The expression levels of H19 were higher than those of adult organs examined in this study; the placenta had 80, 800, 1000, and 700 fold higher expression of H19 in E7.5, E10.5, E13.5, and E16.5, respectively when compared to the adult organs. H19 ncRNA was detectable in the placenta using in situ hybridization.
Conclusion: Our findings showed that the placenta expressed H19 ncRNA in a development-dependent manner, especially in the middle and late stages.
(COI: No)

P2-045

Comparative developmental analysis of middle ear formation in mouse and chicken embryos

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How the amniote middle ear evolved remains an intriguing question. Although paleontological studies suggest that the middle ear evolved independently in mammals and diapsids (modern reptiles and birds), little is known about the developmental basis for independent evolution. We have previously found that the relative positions of the primary jaw joint (PJJ: the articulation between the quadrate- and articular-homologue) and first pharyngeal pouch (PP1) led to the coupling of tympanic membrane formation with the lower jaw in mammals, but with the upper jaw in diapsids. In this study, we further compared middle ear formation in mouse and chicken embryos. We found no difference in the expression pattern of genes central to lower jaw specification at comparable stages (E9.5 in the mouse and HH18 in the chicken). However, within a day, these genes were detected more dorsally in the mouse compared to the chicken, resulting in different positioning of the PJJ relative to PP1 in these animals. We also found that the chicken external auditory meatus originates from the ectoderm in the second pharyngeal arch (PA2), not in the first pharyngeal arch (PA1) as in the mouse. These results suggest that although early patterning of the pharyngeal arches is comparable, middle ear formation basically associates with PA1 in the mouse, but with PA2 in the chicken, supporting the idea of independent origin of the middle ear in mammals and diapsids.
(COI: No)

P2-046

Tlx3 promotes glutamatergic neuronal differentiation through the interaction with epigenetic co-factor CBP

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The homeodomain transcription factor T cell leukemia 3 (Tlx3) functions as a selector gene determining glutamatergic cell fate. However, how Tlx3 promotes glutamatergic neuronal specification is unknown. In this study, we show that Tlx3 directly interacts with the epigenetic co-regulator CREB-binding protein (CBP) via its homeodomain. In addition, the interaction between Tlx3 and CBP is enhanced by Pbx3, a member of the TALE family of transcription factors. Using mouse embryonic stem (ES) cells stably expressing Tlx3 to evaluate glutamatergic lineage commitment, we further demonstrate that Tlx3 binds CBP only after neural induction. The expression of Pbx3 was increased upon neural differentiation of ES cells. A deletion mutation in the homeodomain of Tlx3 abolishes glutamatergic neuronal specification of ES cells but has no effect on neural differentiation. Taken together, these data suggest that functional interplay between Tlx3 and CBP plays an essential role in glutamatergic neuronal subtype specification.
(COI: No)

P2-047

Programmed cell death in the developing optic cup in anophthalmia mutant (kAP)-rat

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Introduction: The kAP-rat has anophthalmia, but not macroscopic complications in other organs, unlike other anophthalmia model animals. In our previous study, we showed that the optic cup disappeared between embryonic day (E) 13.5 and E14.5. In this study, we examined programming cell death in the optic cup to analyze the cause of anophthalmia.
Methods: The number of the TUNEL-positive nuclei was counted in the inner and outer layersof the optic cup in female kAP- and control Wistar rat embryos (E12.5). The number of them per unit length was compared between kAP and control embryos.
Result: TUNEL-positive nuclei per unit length were significantly larger in the outer layer of the optic cup in kAP-rat embryos than that in controls ($P = 0.019$ and $P = 0.021$ in the left and right eyes, respectively), whereas there was no significant difference in the number of the TUNEL positive nuclei in the inner layer between kAP and control embryos.
Conclusion: This result suggests that degeneration of the outer layer triggers disappearance of the eye in the kAP-rat.
(COI: No)

P2-048

Morphological analysis of prosensory epithelium in the extension of organ of Corti

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In the development of the mouse cochlea, prosensory epithelium extends along the cochlea spiral. Since the extension requires PCP signal components and non-muscle myosin, and exhibits rosette formation of the epithelial cells, it is thought to be convergent extension. However, detailed mechanisms for the extension remain obscure, since myosin has been reported to accumulate at the cell-cell boundaries parallel to the tissue elongation in the cochlea, which is orthogonal to known accumulating pattern of myosin in a typical convergent extension. We thoroughly analyzed the cellular morphology and distribution of non-muscle myosin proteins in the prosensory epithelium to dissect the processes of cochlear extension. The analyses showed that (1) non-muscle myosin accumulated in punctate dots along the apical cell-cell boundary of each epithelial cell and (2) expression level of non-muscle myosin was dependent on cell types and stages of differentiation. In addition, non-muscle myosin seemed not yet to have accumulated in parallel to the tissue elongation when the rosette formation occurs. The present survey items could be used as indices to evaluate the achievement of each process during the extension and we are intending to evaluate a defect in cochlear extension in Dlg1 gene-targeted mice, which exhibit multiple developmental abnormalities similar to PCP phenotypes. The evaluation is currently in progress and results will be presented on posters.
(COI: No)

P2-049

Possible involvement of olfactory placode-derived neurons in development of the telencephalon

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Olfactory placode (OP), anlage of the olfactory epithelium, is known to produce various kinds of cells in addition to olfactory receptor neurons. OP-epithelial cell migration of chick embryos starts at embryonic day 2.5 (E2.5). The migratory cells are immunoreactive (-ir) for polysialylated NCAM (PSA-NCAM, a marker of immature neurons) and HuC/D (a neuronal marker). The neurons then form a cellular cord toward rostral telencephalon (TEL) and the olfactory nerve axons grow along the cord from E3.5. In the early stage of migration, many neurons migrate towards various directions from the OP or the cellular cord. To know the fate of such migratory neurons, OP-epithelial cells were labeled with GFP or Tol2-GFP vector by electroporation at E2.5 (HH stage 14-18). Embryos were fixed 38 to 72 hours after the treatment and whole-mount specimens were immunostained for PSA-NCAM, and HuC/D or laminin. GFP-labeled cells with HuC/D-ir cell bodies and PSA-NCAM-ir long horizontal processes were detected in the olfactory nerve and in the lateral and medial wall of dorsal TEL of HH stage 21 to 27 embryos. At TEL most GFP-labeled cells were detected on or just beneath the pia mater. In the subpial layer of the dorsal TEL PSA-NCAM-ir neuronal network, which may correspond to Cajar-Retzius cells in mammals were present in E4 to E5 embryos. OP-derived PSA-NCAM-expressing neurons appeared to join the network at later stages. These results indicate involvement of the OP-derived neurons in development of the telencephalon.

(COI: No)

P2-050

Expression of epigenetic factors in the developing mouse retina

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Many studies suggest that epigenetic regulation affects proliferation and cell differentiation during development. Epigenetic modification, such as histone methylation, induce gene activation or silencing by regulating the accessibility of regulatory molecules to chromatin. To understand the role of epigenetic factors in retinal development, we investigated temporal gene expression patterns of histone demethylases and methylases using in situ hybridization. We found that histone demethylases KDM1A, B, KDM2A, B, KDM3A, B, KDM4A, B, C, D, KDM5A, B, C, D, KDM6B were expressed in retinal progenitor cells in embryonic stages. Histone methylases G9a, SETDB1, SUV39H1 also expressed in retinal progenitor cells. These results indicate that many epigenetic factors may regulate the function of retinal progenitor cells during the early retinal development.

(COI: No)

P2-051

Identification of the causal gene of gastrointestinal atresia using medaka mutant

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Gastrointestinal Atresia (GA) is a common feature of congenital malformations and occurs in approximately 1:500 to 1:4000 newborns. GAs can occur as sporadic and are generally thought to originate from mechanical or vascular incidents. However, recent data have showed that genetic components might be also present. To understand the genetic basis of GA, we analyzed medaka mutant which revealed a GA during the embryonic development.

The medaka *g1-4* mutant was isolated in our ENU-driven screen for mutants with defects in embryonic development and organogenesis. *g1-4* revealed GA at 4 to 5 days after fertilization. The carrier pairs had produced around 10% mutant siblings, suggesting that g1-4 was likely a recessive mutant although its penetrance was lower than expected Mendelian frequency.

To determine the molecular structure of the *g1-4* locus, we first mapped it to linkage group 23 (LG 23) using M-marker analysis (Kimura et al., Mech Dev, vol 121 pp915-32, 2004). Subsequently, high-resolution linkage analysis was performed using an F2 mapping panel. This confined the mutation between two markers, *g1-4*_LG23-4.4 and *g1-4*_LG23-4.6, spanning a region of 24 kb. This region contained only one gene. Comparison of genomic sequences between the wild-type and *g1-4* mutant revealed a C to A transversion that led to a premature stop codon in one exon in the mutant.

(COI: No)

P2-052

Evolution of the pectoral fin/limb and the vertebrate neck

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Fin/limb in vertebrates is one of the evolutionary novelties, and its evolutionary origin remains as an enigma. To gain some insight into the question, cell lineage of the lateral plate mesoderm (LPM) was analyzed by using chicken-quail chimera. The most rostral LPM formed pharyngeal mesoderm during the middle stage of the development, and developed cucullaris muscle in the later stage, implying that the region would be head mesoderm. The LPM just caudal to the source of the cucullaris muscle developed the clavicle. Thus, the clavicle adjoins the cucullaris from the early stage in chick. Because the shoulder girdle of the teleosts marks the caudal rim of gill (pharyngeal) arches, the results suggest that, the shoulder girdle is always attached to the head independently of the number of the cervical vertebrae. The infrahyoid muscle in medaka developed adjacent to the fin muscle. These muscles developed from the same somite, and shared the same developmental mechanisms. The mechanism is also used for the development of the infrahyoid muscle in agnathans, lamprey, which do not possess paired fin. Thus, pectoral fin/limb inevitably attaches to the head, implying that it appears to have evolved adjacent to the gill arches, and pectoral fin/limb muscle would have established through co-option of the developmental program of the infrahyoid muscle.

(COI: No)

P2-053

Morphogenesis of the rat glenohumeral joint

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The mechanism of development of the shoulder joint was shown in some studies. The shoulder joint has an interesting structure, for example, SLAP (superior labrum anterior and posterior) and enthesis of rotator cuff. To observe in detail the development of the structure of the shoulder in rat, white Wistar rat embryos of E18 to P1 (postnatal day one) were employed. After mating, the morning when sperm was observed in a vaginal smear was designated as gestational day 0. All the mother rats were anesthetized with ether gas for sacrifice. For paraffin histology, samples of the whole shoulder joint were fixed in 4% paraformaldehyde at 4 oC overnight. Then they were dehydrated in graded ethanol and embedded in paraffin wax. Sagittal and axillar sections were serially cut at 4-6-μm thickness, and were stained with hematoxylin eosin staining. Immunochemical stain of the collagen type I and III was also performed in this study. The shape of rat scapula was more rectangular shape than human that. And acromion is placed on the scapula. In the stage of E18.5, cavitation was clearly recognized in the lateral side of the joint although that was not shown around the articular surface. SLAP and enthesis of the rotator cuff was detected in this stage. In E19.5, vascular formation was recognized in the medial side of the enthesis of the rotator cuff. We showed re-evaluation of the development in the rat shoulder joint.

(COI: No)

P2-054

Generation of Rat-Induced Pluripotent Stem Cells Using Mesenchymal Stromal Cells from a New Model of Metabolic Syndrome

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We recently characterized DahlS. Z-*Lepr^{fa}/Lepr^{fa}* (DS/obese) rats, derived from a cross between Dahl salt-sensitive rats and Zucker rats, as a new animal model of metabolic syndrome (MetS). Although the phenotype of DS/obese rats is similar to that of humans with MetS, the pathophysiological and metabolic characteristics in each cell type remain to be clarified. Hence, the establishment of induced pluripotent stem cells (iPSCs) derived from MetS rats is essential for investigations of MetS *in vitro*. Reports of rat iPSCs (riPSCs), however, are few because of the difficulty. Recently, the advantage of using mesenchymal stromal cells (MSCs) as a cell source for generating iPSCs was described. We aimed to establish riPSCs from MSCs of both DS/obese rats and their lean littermates, DahlS. Z-*Lepr^{+}/Lepr^{+}* (DS/lean) rats. The established colonies showed ES cell (ESCs)-like properties, and the differentiation potential into cells from all three germ layers both *in vitro* and *in vivo*(teratomas). Both riPSCs became adipocytes after induction of adipogenesis. Real-time PCR analysis also revealed that both riPSCs and the adipose tissue from DS/obese and DS/lean rats possess similar expression patterns of adipocyte differentiation-related genes. We succeeded in generating riPSCs effectively from MSCs of both DS/obese and DS/lean rats. These riPSCs may well serve as highly effective tools for the investigation of MetS pathophysiology *in vitro*.

(COI: No)

P2-055

Lumbar lateral plate cells stop migration of thoracic somite cells: an in vitro model of region specific morphogenesis of the chick axial skeleton

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The rib is restricted to the thoracic region of birds and mammals. Although the thoracic somites have potency to form ribs, we have shown that morphogenesis of the rib is depending on circumstance of the somite. When somatic mesoderm of the limb forming region was transplanted into the thoracic region (Liem and Aoyama, 2009) or thoracic segmental plate to lumbosacral region, the grafted mesoderm gave rise to ectopic ribs, which were much shorter than usual (Matsutani et al., JAA cong., 2014). In the latter case, the lateral somite lips derived from the graft did not penetrate the lumbar lateral plate. The lateral plate appears to stop the migration of the somite cells. To examine the interaction between these embryonic tissues, we co-culture the thoracic somites with lateral plate, and found that when the somite cells collided with lumbar somatic mesoderm cells, they rapidly moved away from lumbar somatic mesoderm cells (Matsutani et al., JAA cong., 2014). For quantitative analysis we cultivated the somite and the lateral plate on the 10 μm wide hydrophilic straight paths. When they collided, while thoracic somatic mesodermal cells generated a new protrusion and began to migrate in the opposite direction to the somite cells and, lumbar somatic mesodermal cells remained at the encounter point and inhibited somitic cells migration. The authors declare no conflict interests.

(COI: No)

P2-056

Effect of transplantation of choroid plexus epithelial cells for spinal cord injury in rats

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Although spinal cord injuries have been extensively studied, no effective treatment for spinal cord injury (SCI) is currently available. The development of effective treatments is urgently needed. Recently, transplantation of several kinds of cells for regenerative medicine has attracted a great deal of attention. We previously reported the effect of bone marrow stromal cells (BMSCs) transplantation for SCI, and choroid plexus epithelial cells (CPECs) transplantation for acute ischemic brain injury. CPECs, producing the cerebrospinal fluid, are known to express various neurotrophic factors such as IGFs, FGFs, and EGF. We have detected that cultured CPECs express several neurotrophic factors such as NGF, VEGF, HGF, BDNF, and FGFs. In the present study, we examined the therapeutic effect of CPEC transplantation for SCI in rat. Locomotory behaviors assessed by the BBB score were significantly improved in the cell transplantation group. The transplanted CPECs survived in the spinal cord at least 2 weeks after transplantation, in our previous studies, BMSCs disappeared at 1-2 weeks after transplantation. The differentiation of transplanted CPECs into other neuronal cells has not been identified. Therefore, it is probable that neurotrophic factors secreted from transplanted CPECs might contribute to neuronal regeneration in injured spinal cord. Further studies are needed to explore the therapeutic mechanisms of CPEC transplantation.

(COI: No)

P2-057

Effect of treadmill exercise on the motor recovery and neurogenesis after photochemically induced infarction of unilateral motor cortex in rats

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It is well known that the exercise therapy in rehabilitation promotes the recovery from impaired motor function after cerebral infarction. However, it still remained largely unknown whether the exercise can induce neurogenesis to reconstruct the neuronal circuit damaged by cerebral infarction. To address this issue, we have examined the role of physical exercise on neurogenesis using photochemically-induced thrombosis (PIT) model in rats. Here, we established motor cortex infarction model and also examined whether the exercise also affects the motor recovery and neurogenesis in this model. One day after operation, exercise group was forced to running exercise using a treadmill for 30 min every day for four weeks. Rota-rod tests revealed that exercise seemed to promote to recovery of motor function during late phase. We performed BrdU labeling experiments which can follow the progeny of newly dividing cells. BrdU immuno-positive cells were detected only around the damaged area in PIT-operated rats. Interestingly, there were more robust BrdU-positive cells in exercise group compared to non-exercise group. These results suggest that physical exercise can promote the motor recovery and the neurogenesis around the damaged areas in rats with unilateral motor cortex infarct.

(COI: No)

P2-058

3D-ultrastructural analysis of the development at the supraspinatus tendon insertion with FIB/SEM tomography

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Introduction: After rotator cuff repair, the repaired tendon-bone insertion is totally different from that of the normal insertion. To regenerate tendon-bone insertion, the morphological development of this region would contribute to the clarification of the pathophysiology in rotator cuff tear. In this study, we analyzed development of the normal tendon-bone insertion in terms of the expression of SOX9/SCX and the 3D ultrastructure.
Materials and methods: 1-, 2-, 3-, 4-week-old SD rats were used. 8 supraspinatus tendon insertion were isolated per each time point. 4 specimens were observed with the fluorescent immunostaining of the SOX9/Scleraxis antibody, and remaining 4 specimens were observed with FIB/SEM tomography.
Results: At 1-week-old insertion, the cells in the insertion region expressed SCX(+)/SOX9(+), the chondroid cells localized between immature collagen bundles. They had many cell processes and connected with each other. As postnatal week passes, the morphology of the cells changed from spherical to the ellipsoidal formation. The number and length of the cell processes were decreased, however, the direction of the cell processes seemed to be extended regularly.
Discussion: The pattern of the expression SOX9/Scrolaxis and the 3D ultrastructural changes in this study would clarify the postnatal development of the normal tendon-bone insertion, may help better understand the pathophysiology of the tendon-bone insertion, especially rotator cuff tears.

(COI: No)

P2-059

Study on structural changes of bone matrix and periostum of femur in growing rats

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Purpose: It is known that structures of bone matrix and periosteum change with growth. Purpose of this study was to investigate relationships between the bone matrix, the periosteum and the bone strength, by observing those structures of the bone matrix and the periosteum, and measuring the bone strength, in growing rats.
Materials and methods: In this study, thirty two male rats (wistar strain, 3, 7 and 13 weeks old) were used as materials, and their femurs were excised after euthanasia. Their right and left femurs were used for measurement of the bone strength and histological analyses, respectively. Both the measurements and the analyses were performed at middle and distal 1/3 portions of diaphysis. Each parameter of the bone strength were measured by bone strength tester, using non-fixation samples, and the structures of bone matrix and periosteum were observed histologically, using decalcified and undecalcified specimens.
Results: Thickness of femoral cortical bone increase wholly, but lamellar structures and thickness of periosteum increased at posterior medial face of femur, with growth. Bone strength indicated higher value at middle portion in the immature stage, but difference of that values between middle and distal portions became lesser with growth.
Conclusion: It was understood that the bone strength increased from middle portion toward distal portion with growth, and the changes were related to thickening of the lamellar bone and the periosteum.

(COI: No)

P2-060

Functional morphology of lumbar spine using X-ray in vivo

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Introduction: Spinal anterior and middle column consists of vertebra and disc. They have a role of weight bearing through vertical direction. Also flexibility and stability are needed especially for bipedal animal. These complex load cause spinal deformity and lifetime morbidity of low back pain is over 80% in humans.
Method: Ninety-five patients with low back pain underwent X-ray photos. Angle of upper and lower edges of vertebra and disc were calculated respectively and analyzed in level, sex and age.
Result: The vertebra-angles at L1, L2, L3, L4 and L5 were 4.2° ± 0.4, 2.1° ± 0.3, 0.8° ± 0.3, -1.3° ± 0.3 and -5.5° ± 0.4, respectively. The disc-angles at L1/2, L2/3, L3/4, L4/5 and L5/S were -2.9° ± 0.3, -4.0° ± 0.3, -6.2° ± 0.3, -7.6° ± 0.4 and -10.9° ± 0.4, respectively. The vertebra-angles were -0.6° ± 0.3 (female) and 0.4° ± 0.3 (male). In terms of disc-angles, there were no statistical differences between female and male. A significant difference in disc and vertebrae was not detected among age groups (20's, 30's, 40's and 50's).
Discussion: Significant level dependence was detected in vivo. The vertebra-angles and disc-angles both showed backward tilting with caudal level and disc showed more retroversion. Female has more lordotic vertebra. It will relate to the difference of pelvic organ. Bone density and muscle volume changes especially in youngers and elderlies. Spinal shape may also be affected with these findings. Although the current study did not show age-dependency, further studies are needed to include larger numbers include other generation.

(COI: No)

P2-061

Age-related changes of elements in the thyroid cartilage of monkey

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The purpose of the present study is to elucidate age-related changes of elements in the thyroid cartilages. The authors determined the elemental contents of the monkey thyroid cartilages by inductively coupled plasma-atomic emission spectrometry (ICP-AES). The monkey subjects consisted of nine rhesus monkeys, one Japanese monkey and three crab eating monkeys, ranging in age from 0.1 to 27 years. It was found that the average content of calcium was 30.9 mg/g in the monkey thyroid cartilages and it is easy to calcification when calcium content of tissue is higher than 10 mg/g. This finding indicates that calcification occurs easily in the monkey thyroid cartilages. Regarding age-related changes of element contents, it was found that the accumulation of calcium increased progressively with aging and showed a sudden rise at 7 years old in the monkey thyroid cartilages. In addition, all the monkeys that calcium content exceeded 20 mg/g were over 7 years old. Likewise, the trend of change of phosphorus was parallel with that of calcium. Therefore, it is likely that the accumulation of calcium and phosphorus may mainly occur after reaching a certain age in the monkey thyroid cartilages. In regard to relationships among the average contents of elements, there were very significant direct correlations among the average contents of calcium, phosphorus, and magnesium in the monkey thyroid cartilages.

(COI: No)

P2-062

Analysis of biological apatite crystal orientation in posterior cortical bone of human maxilla using microbeam X-ray diffractometry

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The purpose of this study was to quantitatively evaluate BMD and BAp crystal orientation using micro-computed tomography(micro-CT) and microbeam X-ray diffractometry in the posterior cortical bone of human maxilla. The intensity and direction of mechanical stresses in both the buccal and lingual area were compared. The maxillary first molar region in Japanese bone samples was designated as the region of interest and BMD and BAp crystal orientation in the buccal and lingual area measured. The results showed no difference in BMD values among regions. BAp crystals were oriented predominantly in the mesiodistal direction in the lingual area and along the direction of masticatory force in the buccal area. These findings suggest that the lingual area exhibits form maintenance such as the dentition maintenance, while in the buccal area alignment takes place in the direction of masticatory force resulting from mechanical stress exerted via the teeth. Qualitative evaluation revealed clear differences between the buccal and lingual area, suggesting that BAp crystal orientation offers a more precise indicator of bone quality than BMD.

(COI: No)

P2-063

Cortical bone water changes in ovariectomized rats during the early postoperative period: objective evaluation using sweep imaging with Fourier transform

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It is important to evaluate bone quality in osteoporosis for the early diagnosis. The purpose of this study was to evaluate the cortical bone signal-to-noise ratio (SNR) in ovariectomized (OVX) rats during the early postoperative period as a method to measure bone quality using the sweep imaging with Fourier transform (SWIFT) technique. Twelve-week-old female Sprague-Dawley rats (n=64) were divided into sham and OVX groups. Preoperative tetracycline was immediately administered subcutaneously to distinguish new cortical bone area, and tibial samples were collected at 2, 4, 8, and 12 weeks postoperatively. Magnetic resonance imaging (MRI) was performed using SWIFT to obtain cross-sectional images of the tibial diaphysis. The cortical bone SNR was calculated. Bone histomorphometry was performed. Histomorphometry findings showed that the new bone area was significantly greater at 8 and 12 weeks postoperatively in the OVX group ($P<0.05$). The SWIFT technique showed that the SNR was significantly higher at 8 and 12 weeks postoperatively in the OVX group ($P<0.05$) and was correlated with the new bone area ($R^2=0.430$). The SWIFT findings suggest that the SWIFT technique may depict early changes in cortical bone quality.

(COI: No)

P2-064

Effects of chewing during prenatal stress on bone microstructure in mice

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Objective: Chronic stress is a risk factor for osteoporosis. Chewing inhibits the stress-induced response. In the present study, we examined the effects of maternal chewing during prenatal stress on bone microstructure of the adult offspring.
Methods: We used three-month-old ddY mice. Animals were divided into control, stress, and stress/chewing groups. Mice in the stress and stress/chewing groups were placed in a restraint tube for 30 minutes, thrice a day from day 12 of pregnancy. Mice in the stress/chewing group allowed to chew on a wooden stick during the same period. The blood corticosterone levels in dams were measured. The male offspring were raised until 5 months old, at which point the trabecular bone in the femur and vertebra was evaluated using micro-CT. The bone formation rate was analyzed and osteoclast number was calculated.
Results: Blood corticosterone levels were significantly higher in the stress group. Chewing under chronic stress prevented the increase of the corticosterone level. Prenatal stress caused a significant reduction of trabecular bone of the offspring. Bone formation rate was decreased and osteoclast number was increased in the stress mice. Chewing under prenatal stress attenuated reduced bone formation and stimulated bone resorption, and improved the trabecular bone loss.
Conclusions: These findings indicate that prenatal stress induced excess secretion of corticosterone, triggered the bone loss. Chewing prevented the increase of corticosterone level, improved the balance of bone formation and bone resorption, ameliorated bone loss induced by prenatal stress.

(COI: No)

P2-065

Histochemical assessment on bone tissue in transgenic mice overexpressing parathyroid hormone-related peptide (PTHrP) driven by type1 collagen promoter

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Purpose: This study aims to elucidate the biological function of parathyroid hormone-related peptide (PTHrP) in bone cells, by examining long bones in PTHrP overexpressing transgenic (Tg) mice.
Materials and Methods: Tg mice overexpressing PTHrP were generated by inserting the PTHrP cDNA downstream type1 collagen promoter specific to osteoblasts. Fetuses at E18 were harvested and immersed in 4% paraformaldehyde solution. The paraffin sections of femora and tibiae were histochemically examined for tissue nonospecific alkaline phosphatase (ALP), tartrate-resistant acid phosphatase (TRAP), and ecto-nucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1).
Results and discussion: PTHrP Tg mice showed enlarged epiphyseal cartilage with no hypertrophic zone, and therefore, vascular invasion and mixed spicules of cartilage cores and surrounding bone matrix were not observed in the chondro-osseous junction; The resultant bone showed no distinct metaphysis and diaphysis. There were many ALP-positive preosteoblastic cells throughout the Tg bone, but a less number of ENPP1-reactive cells were observed on trabeculae accompanied with a few TRAP-reactive osteoclasts. Taken together, the overexpressed PTHrP appears to affect chondrocyte proliferation and the entry into the hypertrophic phenotype, and also stimulate preosteoblastic proliferation rather than differentiation into osteoblasts.

(COI: No)

P2-066

Effects of mechanical loading on structures of tibial growth plate and primary cancellous bone in rats

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Purpose: Bone trabecula formation is related not only bone addition and resorption at there but also structures of calcified cartilage trabeculas derived from growth plate. We had already recognized that acute bone resorption caused in the early stage of exercise period. Purpose of this study was to investigate structural changes of tibial primary cancellous bone and growth plate accompanied with short-term exercise in growing rats.
Materials and methods: Thirty two male rats (wistar strain, seven weeks old) were used as material, and they were divided into exercise group (EX) and control (CO), randomly. Furthermore, EX was divided into EX2, EX4, EX7 and EX14, according to difference of experimental periods, and CO was also divided into CO2, CO4, CO7 and CO14 similarly. EX2, EX4, EX7 and EX14 performed jumping exercise (45cm height, 100 times per day, every day) for 2, 4, 7 or 14 days. Tibiae were excised from rats after each experimental periods. Bone structures were observed histologically and immunohisotologicaly.
Results: No differences were found in thickness of calcified cartilage trabeculas between every groups. Many osteoclasts were recognized in the early stage of experimental period, but starting portion of bone formation on the bone trabecula got closer to growth plate in EX.
Conclusion: It was suggested that mechanical loading promoted both resorption in the early stage of exercise, but little effects were given to the growth plate.

(COI: No)

P2-067

Comprehensive analysis of osteons in human femoral cortical bones with circularly-polarized microscope

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Background: The increase of the fragile fractures and atypical fractures with osteoporosis in elderly people becomes the problem clinically. The main locus of the bone modification in the cortical bone is osteon. However, there are much unsolved problems on these osteons. We will report a manufactural technique to make polishing specimens from large cortical bones and image analysis measurement.
Method: Subjects were the human femoral bones from the anonymous individuals. We made a 5mm width specimen from the shaft of femur and performed resin embedding. They were polished to make a specimen of 100 μm width manually. Then, the images were taken under the circularly-polarized microscope (CPM). Finally we could analyze the whole section as one large image using a synthetic software.
Results: It was possible to evaluate the distribution patterns of primary and secondary osteons in the whole section. Moreover, all of the osteons could be classified from the aspect of polarization pattern under the CPM.
Discussion: Ascenzi reported that the difference in features of osteon with CPM images depended on the difference in collagen alignment patterns. Qualitative or quantitative analysis of the osteons was possible with CPM in the human femur polishing specimen. All of the osteons in a large axial section could be evaluated with our technique. Hereafter, it will be possible to analyze osteonal structures in cortical bones comprehensively.

(COI: No)

P2-068

Histological examination on bone matrix surrounding osteocytic lacunae after PTH-administration or during lactation of mice fed with low calcium diet

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Introduction: In this study, we have attempted to histologically verify "osteocytic osteolysis" proposed by Bélanger in 1960's., using two mouse models - PTH administer mice and lactating mice with low calcium (Ca) diet.
Materials and Methods: Wild-type (*wt*) ICR and *Rankl*$^{-/-}$ male mice were injected with hPTH (1-34), and then, bone matrix surrounding the osteocytic lacunae was examined under TEM, fluorescence microscopy and confocal laser microscopy. Furthermore, we additionally investigated osteocytic lacunae in lactating mice fed with low Ca diet.
Results and Discussion: Serum Ca concentration was increased at 1 hour after PTH administration in *wt* and *Rankl*$^{-/-}$ mice. At six hours after PTH administration, enlarged osteocytic lacunae were observed mainly in the cortical bone, and von Kossa staining demonstrated broadly demineralized bone matrix surrounding the osteocytes. Under TEM, fragmented collagen fibrils and pieces of mineralized matrices were observed in the enlarged osteocytic lacunae with irregularly-shaped walls. In addition, calcein labeling was seen on the walls of some osteocytic lacunae. In lactating mice with low Ca diet, consistently, the osteocytic lacunae were enlarged, and sometimes labeled with calcein. It seems likely that osteocytes erode the surrounding bone matrix i.e., osteocytic osteolysis, and deposit minerals on their lacunae.

(COI: No)

P2-069

Three-dimensional reconstruction of osteocytic lacunar-canalicular system in murine bone by using FIB-SEM

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Osteocytes extend their thin cytoplasmic processes, by which they communicate with not only neighboring osteocytes but also osteoblasts on bone surface. Thereby, osteocytes establish the cellular network referred to as osteocytic lacunar-canalicular system (OLCS). Focused ion beam-scanning electron microscope (FIB-SEM) is one of the most powerful microscopy for reconstructing the ultrastructural 3D of the objects. In order to clarify the 3D of cellular network of OLCS, we have investigated it using FIB-SEM.
Eight-weeks old ICR mice were fixed with 1/2 Karnovsky solution, and their tibiae were decalcified with 5% EDTA solution. The specimens were post-fixed with OsO4, immersed into an aqueous solution of uranyl acetate and embedded in epoxy resin prior to FIB-SEM observation.
Under FIB-SEM, osteocytes were shown to expand their stout protrusions of the osteocytes' cell bodies, and then, many fine cytoplasmic processes branched off. Some fine processes from the stout cellular protrusion extended horizontally, some turned immediately at a right angle to reach the bone surface, and others arose directly from the osteocytes' bodies and ran perpendicularly to the bone surfaces. Thus, there seemed to be several pathways for cytoplasmic processes of osteocytes to reach the bone surfaces. In summary, FIB-SEM is able to clearly demonstrate the fine ultratrsuctures of 3D reconstruction of osteocytes and their cytoplasmic processes.

(COI: No)

P2-070

PP2A Calpha in osteoblasts controls osteoblast and adipocyte differentiation

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The serine/threonine protein phosphatase 2A (PP2A) participates in regulating many important physiological processes. We examined the role of alpha-isoform of PP2A catalytic subunit (PP2A C α) in osteoblast and adipocyte differentiation. Transgenic mice that specifically express dominant negative PP2A C α in osteoblastic cells showed higher cortical bone mineral density and increase in body weight and adipose tissue of tibia bone marrow. The expression and phosphatase activity of PP2A C α decreased during osteoblast differentiation in osteoblasts. PP2A knockdown cells (shPP2A) were established by infecting lentivirus particles expressing shRNA specific for PP2A C α. shPP2A cells showed accelerated osteoblast differentiation with the upregulation of bone-related genes such as Osterix, Bone sialoprotein, and Osteocalcin. Transcriptional activity of Osterix promoter region was higher in shPP2A cells than that of the control cells, which was controlled by transcription factors Dlx5 and Runx2. To examine the effect of PP2A C α in osteoblasts on adipocyte differentiation, mesenchymal stem C3H10T1/2 cells were co-cultured with shPP2A cells. shPP2A cells showed higher ability to induce adipocyte differentiation and the expression of adipocyte marker genes in C3H10T1/2 cells. Our results indicate that PP2A C α plays an important role in the regulation of bone formation and osteoblast differentiation through the bone-related transcription factors. PP2A C α in osteoblasts is also thought to be involved in controlling adipocyte differentiation.

(COI: No)

P2-071

Histone demethylase Jmjd3 regulates osteoblast differentiation and apoptosis

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Posttranslational histone modifications including methylation are closely linked to regulation of eukaryotic gene expression. Jumonji domain-containing 3 (Jmjd3) is a histone demethylase, which specifically catalyzes the removal of trimethylation of histone H3 at lysine 27 (H3K27me3). In this study, we examined the role of Jmjd3 in osteoblast differentiation and apoptosis. Jmjd3 expression was induced in response to the stimulation of osteoblast differentiation. Silencing of Jmjd3 expression suppressed osteoblast differentiation in vitro and in vivo. Silencing of Jmjd3 decreased the promoter activities of osteoblast-specific transcription factors Runx2 and Osterix and increased the level of H3K27me3 on the promoter regions of these genes. Introduction of the exogenous Runx2 and Osterix partly rescued osteoblast differentiation in the Jmjd3 knockdown cells. On the other hand, knockdown of Jmjd3 in osteoblasts promoted apoptosis in response to serum deprivation. Cleavage of Caspase-3 and PARP induced by serum deprivation, which are mediators of apoptosis, were increased in Jmjd3 knockdown cells. The expression of anti-apoptotic molecule B-cell lymphoma-2, was inhibited in Jmjd3 knockdown cells. The present results indicate that Jmjd3 plays important roles in regulating osteoblast differentiation and apoptosis.

(COI: No)

P2-072

Effect of Nitrogen-containing bisphosphonates on collagen-induced arthritis model mice

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Nitrogen-containing bisphosphonates (NBP) is the strong inhibitor of osteoclastic bone resorption. It has been indicated that one of NBP, alendronate, exacerbated collagen-induced arthritis (CIA) in mice. Minodronate (MIN) is one of another NBP and more strongly inhibit osteoclastic bone resorption than alendronate. However, the effect of MIN on CIA has not been revealed yet. In this study, we examined whether the bone destruction and inflammation induced by CIA was exacerbated by MIN or not. CIA was induced in male DBA/1 mice (8 weeks old) by the sensitization with type II collagen. MIN (4 μmol/kg) was injected once a week from one week before the onset of the first sensitization. At indicated periods, mice were killed and processed for the experiments. MIN-treated group showed a higher clinical arthritic score at every time point than non-treated group. Flow cytometric analysis indicated the enhancement of granulopoiesis in bone marrow in both groups. Granulopoiesis in MIN-treated group was more augmented than non-treated group. Histological analysis indicated the thickening of growth plate and the severe and sustained invasion of granulocytes into the joint cavity in MIN-treated group. Furthermore, Gr-1+ granulocytes directly attached to bone surface. These results indicated that MIN strongly inhibited the physiological bone resorption mediated by osteoclasts and exacerbate inflammation in CIA mice. Further study is necessary to clarify the mechanism of bone destruction in this model.

(COI: No)

P2-073

Critical role of PKR in TNF-α-induced osteoclastogenesis

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Double-stranded RNA-dependent protein kinase (PKR) is also to signal transduction pathways, such as MAPK, NF-κB and Smad. TNF-α, one of the inflammatory cytokines, induces osteoclast differentiation and plays a role in progression of inflammatory bone destruction. However, it is unknown about the roles of PKR in TNF-α-induced osteoclast differentiation. Therefore, the present study was undertaken to clarify the role of PKR in TNF-α-induced osteoclastogenesis. The expression of PKR in RAW264.7 cells increased by TNF-α. The TNF-α-induced osteoclast differentiation was markedly suppressed by the pre-treatment of 2-aminopurine (2AP) and PKR inhibitor, a specific inhibitor of PKR as well as PKR siRNA. PKR inhibition also suppressed bone resorption activity. PKR siRNA or 2AP suppressed the TNF-α-induced activation of NF-κB and MAPK in osteoclast precursor. Translocation of NF-κB to nucleus was also suppressed by 2AP. 2AP inhibited the TNF-α-induced expression of NFATc1 and c-fos, master transcription factors in osteoclastogenesis. TNF-α-induced nuclear translocation of NFATc1 in mature osteoclasts was clearly inhibited by the 2AP treatment. The PKR inhibition decreased the TNF-α-induced osteoclast formation and bone resorption in mouse calvaria. Collectively, these results demonstrated that PKR regulates TNF-α-induced osteoclast differentiation and suggested to be a pivotal therapeutic target for TNF-α-induced bone destruction.

(COI: No)

P2-074

Chondroitin sulfate inhibits osteoclast differentiation and bone resorption activity, and improve bone metabolism

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Chondroitin sulfate (CS) is a kind of glycosaminoglycan, which existed in the bone extracellular matrix. In present study, we investigated the osteoclast inhibition ability compared to with or without sulfation groups in CS and the mechanism how CS inhibit osteoclast differentiation in vitro. Both sulfated-CS (s-CS) and non-sulfated-CS (ns-CS) were significantly inhibited the increase of tartrate-resistance acid phosphatase (TRAP)-positive multinucleated cells and had negative effect on increasing osteoclast size. Pit formation assay revealed that CSs also suppressed bone resorption activity. Whereas chondroitinase ABC-digested CS did not show the inhibition activity of osteoclast differentiation and function. Quartz-crystal microbalance analysis clarified that CS possessed the binding ability to receptor activator of NF-κB ligand (RANKL) and interrupted binding of RANKL to RANK. Furthermore, CS reduced the phosphorylation of extracellular signal-regulated kinase in pre-osteoclast cells shown by flow cytometrical analysis. These in vitro results indicate that CS suppresses both osteoclastogenesis by binging to RANKL and osteoclast size increase. Moreover, CS inhibits RANKL-induced signal pathway, which results in decrease of the osteoclast bone resorption area. Moreover, we treated CS to osteoporosis model mouse for 8 week by intraperitoneal administration. The osteoclast activity of the animal was downregulated and osteogenesis in bone was promoted. These results suggest that CS have a potential to improve bone metabolism in vivo.

(COI: No)

P2-075

Three-dimensional morphology of Golgi apparatus of osteoclasts by scanning electron microscopy using OsO_4 maceration method

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Introduction: Osteoclasts have highly-developed Golgi apparatus around their nuclei. Although two-dimensional structure of Golgi apparatus in osteoclasts have been reported, its three-dimensional structure is still veiled. This study was designed to elucidate the three-dimensional structure of the Golgi apparatus in osteoclasts by scanning electron microscopy using OsO_4maceration method.

Materials and methods: Eight-week-old Wistar rats were perfused with a mixture of 0.5% glutaraldehyde and 0.5% paraformaldehyde. The femora were dissected out, sagittally freeze-cracked, and post-fixed with 1% OsO_4. The specimens were then immersed in 0.1% OsO_4 for 10-12 days at 20℃ according to Tanaka et al (1984). They were dehydrated, critical point-dried, and coated with OsO_4 or platinum-palladium.

Results and discussion: Actively bone-resorbing osteoclasts on femoral trabeculae were clearly identified by multi-nuclei, a lot of vacuoles and mitochondria, and well-developed ruffled border. The Golgi apparatus in the vicinity of the nuclei consisted of 4-5 layers of cisterns and small vesicles. The cis-most cistern facing the nucleus revealed the meshwork with regularly-arranged small pores. In contrast, the trans-most cistern demonstrated a plate-like structure with a few pores. The network of such Golgi apparatus widely covered the nuclear surface, with leaving focal fenestrations. These findings suggest that the Golgi apparatus in osteoclasts almost encompasses the entire surfaces of nuclei like a basket.

(COI: No)

P2-076

Expression of MicroRNAs in the Extracellular Vesicles during Osteoclastogenesis

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MicroRNAs (miRNAs) are small, non-coding RNAs that are involved in various biological processes, including cellular differentiation, proliferation, apoptosis, and organ development. We previously profiled miRNA expression during osteoclastogenesis using microarrays. Recently, the presence of miRNAs in extracellular vesicles was reported. It is not known whether osteoclasts secrete extracellular vesicles containing miRNAs. We investigated miRNA expression in the extracellular vesicles in conditioned medium of cultured osteoclasts using RT-PCR. Specifically, we investigated eight miRNAs deemed important for osteoclastogenesis in our previous study: let-7e, miR-21, miR-33, miR-155, miR-210, miR-223, miR-378, and miR-1224. Of these, the expression levels of miR-378, miR-210, and miR-21 were very high, while no significant miR-33 or miR-1224 expression was detected. These results suggest that osteoclasts secrete extracellular vesicles containing specific miRNAs, but that they do not contain the entire set of intracellular miRNAs.

(COI: No)

P2-077

Lidocaine induces ROCK-dependent membrane blebbing associated with subsequent cell death in rabbit articular chondrocytes

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Local anesthetics are administered intraarticularly for pain control in orthopedic clinics and surgeries. Previous studies have shown that local anesthetics can be toxic to chondrocytes, although the underlying mechanism remains unclear. The present study was undertaken to investigate the cellular mechanisms associated with lidocaine-induced toxicity to articular chondrocytes. Isolated rabbit articular chondrocytes were exposed to lidocaine and monitored under a light microscope. Clinical concentrations of lidocaine caused membrane blebbing. ROCK inhibitors Y-27632 and fasudil completely prevented the lidocaine-induced blebbing, suggesting that ROCK activation is required for the bleb formation. The GTP-bound RhoA level was significantly increased (3.01 ± 0.76 folds, $P < 0.0001$) by 20-min treatment with 10 mM lidocaine, suggesting that RhoA activation is involved in ROCK activation. Chondrocyte viability significantly decreased to 17.6 ± 5.7% after 1-hour exposure to 30 mM lidocaine, compared with the control viability of 94.8 ± 2.4% ($P < 0.0001$). Pretreatment with 10 μM Y-27632 or 100 μM fasudil attenuated the lidocaine induced-cytotoxicity (49.4 ± 12.5% and 47.2 ± 9.1% viability respectively, $P < 0.0001$). These findings show that lidocaine induces a cytotoxic effect on chondrocytes through a mechanism involving membrane bleb formation and ROCK activation and that caution should be taken when administering lidocaine intraarticularly.

(COI: No)

P2-078

Effect of glutathione on TNFα-induced osteoclast differentiation in murine bone marrow-derived macrophages

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Osteoclast differentiation is regulated by TNF α and RANKL in inflammatory bone destruction in rheumatoid arthritis; and reactive oxygen species (ROS) have been shown to act as a signaling molecule in TNF α signaling suggesting that ROS play a role in TNF α-mediated osteoclast differentiation. Glutathione is an intra- and extra-cellular antioxidant against oxidative stress in inflammation. Therefore, we have investigated the effect of glutathione on TNF α-induced osteoclast formation using murine bone marrow-derived macrophages. Glutathione significantly stimulated the TNF α-induced osteoclast formation and buthionine sulfoximine, an inhibitor of glutathione synthesis, suppressed the TNF α-induced osteoclast formation. Glutathione facilitated the protein expression and nuclear translocation of NFATc1, a master regulator of osteoclastogenesis as well. In time-lapse analysis, glutathione increased the incidence of TNF α-induced cell fusion of osteoclasts. Furthermore, N-acetylcysteine, a substrate of glutathione synthesis, also stimulated osteoclast formation and NFATc1 nuclear translocation. Thus, these results suggest that glutathione is positive regulator of TNF α-stimulated osteoclast differentiation.

(COI: No)

P2-079

Ultrastructural assessment for biological function of vascular endothelial cells at chondro-osseous junction

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Purpose: Chondro-osseous junction is the site of vascular invasion in the process of endochondral ossification, which replaces epiphyseal cartilage with bone. Osteoclasts, bone-resorbing cells, are not intrinsic for endochondral ossification, because the long bones of osteoclast-less mice can grow longitudinally. In this study, we have histologically examined vascular invasion of endothelial cells at the chondro-osseous junction.
Materials and Methods: ICR mice at eight weeks of age were perfused with 1/2 Karnovsky solution from the left ventricle, and then, tibiae were extracted and immersed in the same fixatives. The specimens were decalcified with 5% EDTA and embedded into epoxy resin. Ultrathin sections were stained with uranyl acetate and lead citrate prior to TEM observation.
Results and discussion: At the tibial chondro-osseous junction, the cytoplasmic processes of vascular endothelial cells were shown to extend into the transverse partitions of columns of hypertrophic chondrocytes. Hypertrophic chondrocytes adjacent to such vascular invasion seemed to be intact featuring normal cell organelles and enlarged cell bodies. But, some cell debris was observed in blood vessels close to the junction, and cells neighboring the endothelial cells possessed large secondary lysosomes. Thus, the cellular interplay between the endothelial cells and the surrounding cells seems to be essential for vascular invasion during endochondral ossification.

(COI: No)

P2-080

Detection of early changes after growth plate injury

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Growth plate injuries can cause a premature closure of the growth plate, and may lead to limb shortening or deformity. The purpose of this study was to elucidate the relationship between the size of the growth plate injury and the timing of the beginning of physeal growth disturbance in non-injured regions. Thirty-two 5-week-old male Japanese white rabbits were used. Injuries were made to the central region of the proximal growth plate of the right tibia, by using a 3.0-mm drill (3.0 mm group) and a 1.2-mm drill (1.2 mm group). Left tibia was used as the control. Imaging of the growth plates was performed at 1, 4, 8, 10 and 12 weeks after the injury, by using MRI. Findings at 10 and 12 weeks after injury in the 3.0mm group showed that the growth plates in non-injured regions were significantly reduced on both the medial and lateral sides, compared to those found in the control. Findings in the 1.2mm group showed that at 12 weeks after injury, growth plates in non-injured regions were significantly reduced on both the medial and lateral sides. Tibia length in both groups was significantly shorter than that found in controls at 12 weeks after injury. These findings showed that the size of the growth plate injury was associated with the timing of the beginning of physeal growth disturbance in non-injured regions.

(COI: No)

P2-081

The roles of promyelocytic leukaemia zinc finger (PLZF) in Glucocorticoid-induced cell cycle arrest in a chondrogenic progenitor cells, ATDC5

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Glucocorticoids inhibit long-bone growth by suppressing the proliferation of chondrocytes in growth-plate. However, the mechanisms by which glucocorticoids induce cell cycle arrest or apoptosis in chondrocytes are not well understood. In this study, we investigated the expression of the glucocorticoid-induced transcription factor promyelocytic leukemia zinc-finger (PLZF) during chondrocyte differentiation using a chondrogenic progenitor cell line, ATDC5. PLZF expression was up-regulated during chondrocyte differentiation. Furthermore, treatment with a GR antagonist showed that glucocorticoid-induced up-regulation of PLZF is mediated by the GR. To elucidate the roles of PLZF in chondrocyte proliferation, we transfected ATDC5 cells with the PLZF gene and found that PLZF overexpression suppressed proliferation by up-regulation of a cyclin-dependent kinase inhibitor, p21WAF1/CIP1 (p21) expression. In contrast, PLZF short hairpin RNA (shRNA) suppressed differentiation into hypertrophic chondrocyte, and promoted cell cycle progression by down-regulation of type X collagen and p21 mRNA expression. Furthermore, PLZF shRNA attenuated glucocorticoid-induced cell cycle arrest by down-regulation of p21 mRNA expression. These results clearly indicate that physiological levels of PLZF promote hypertrophic phenotypes, whereas excess levels of PLZF are involved in glucocorticoid-induced cell cycle arrest by regulation of CDK inhibitor in chondrocytes.

(COI: No)

P2-082

Infruence by excess and deficiency of retinoic acids on septoclasts in the epiphyseal plate of mice

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Objective: We found that the septoclast, a mononuclear cartilage-resobing cell, express exclusively epidermal type-fatty acid-binding protein (E-FABP) in the epiphyseal plate of ddY mice. E-FABP has high affinity for retinoic acids (RAs) as well as fatty acids. We investigated the effects of both excess and deficiency of RAs on septoclasts.
Methods: RA-excess ddY mice were given a single oral dose of 300 mg/kg RAs in soybean oil at 4-weeks old, and RA-deficient mice received the vitamin A-free diet from weaning to 9-weeks old. Frozen sections of the epiphyseal plate were obtained from the proximal tibia of these mice. Immunohistochemistry, immunoelectron microscopicopy and 3-D analysis were performed to detect E-FABP-positive septoclasts. Double immunohistochemistry of E-FABP and peroxisome proliferator-activated receptors (PPARs) were performed.
Results: In both RA-excess and -deficient mice, number of septoclasts decreased compared with control ones. E-FABP-positive cells posessed reduced number and shortened cell processes projecting to the transverse septa lining the chondro-osseous junction. E-FABP-positive septoclasts were simultaneously immunostained with PPARβ/δ.
Discussion: These results suggest that RAs are incorporated by septoclasts and used with PPARβ/δ to regulate cartilage-resorption activity of septoclasts. RAs are important for morphological maintenance of septoclasts.

(COI: No)

P2-083

Suppressive activity of glucosamine on osteopontin-induced nitric oxide (NO) production from human synoviocytes in vitro

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Objective: The present study was designed to examine the influence of glucosamine hydrochloride (GH) on the ability of synoviocytes to produce nitric oxide (NO), which is the important effector molecule in the development of osteoarthritis, in response to osteopontin (OPN) stimulation in vitro.
Methods: Synoviocytes (5 x 10^5 cells/ml) derived from osteoarthritis patients were stimulated with 330 ng/ml OPN in the presence of various concentrations of GH for 24 hours. The levels of NO in culture supernatants was examined by NO_2/NO_3 assay kits. To examine the influence of GH on transcription factor, NF-κB, activation and iNOS mRNA expression, synoviocytes (5 x 10^5 cells/ml) were also cultured in a similar manner for 4 and 12 hours, respectively. The levels of both mRNA expression and transcription factor activation were measured by ELISA.
Results: Addition of GH into cell cultures caused the suppression of OPN-induced NO production from synoviocytes. The minimum concentration that caused significant suppression of NO production was 1.0 mg/ml. GH at more than 1.0 mg/ml also inhibited iNOS mRNA expression and NF-κB activation, which were increased by OPN stimulation in synoviocytes.
Conclusion: These results strongly suggest that GH favorably modify the clinical condition of osteoarthritis patients through the suppression of NO production from synoviocytes.

(COI: No)

P2-084

Fourier transform infrared spectroscopy and atomic force microscope observations of regenerative tendon biomaterial

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In this study, we used Fourier transform infrared (FT-IR) spectroscopy and atomic force microscopy (AFM) to analyze tendon gel, a biomaterial produced from injured tendons in mice, before and after applying tensile stress to obtain quantitative information regarding the mechanism of regeneration in injured Achilles' tendons. AFM revealed periodic striations of collagen fibers aligned along the tensile direction in the tendon gel. The FT-IR spectrum showed that cross-linking of collagen molecules began in the tendon gel after applying tensile stress. Thus, FT-IR and AFM are effective techniques for quantitative evaluation of the regenerative process in tendon biomaterials.

(COI: No)

P2-085

Three-dimensional construction of the twisted structure of the Achilles tendon: focusing on twisted angle

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Many studies have investigated the twisted structure of the Achilles tendon (AT) for many years. However, no consensus for twisted angle has been reached by the differences in measurement methods or reference axes. The purpose of this study was to three-dimensionally construct the structure of each tendon fiber bundle constituting the AT and to clarify twisted angles in three dimensions (3D). Three types of AT twists categorized by our previous study were used; one each of Type I (least), Type II (moderate) and Type III (extreme) (3 legs). Using a Microscribe device, fascicles that originate from the medial head of the gastrocnemius (MG), lateral head of the gastrocnemius (LG), soleus muscle (Sol), and the calcaneal tuberosity (4 points) were digitized to construct 3-dimensional models. An absolute coordinate system was created on the basis of an arbitrarily determined rotation center of the calcaneal tuber, and the angles of each fiber bundle to the three axes of the absolute coordinate system were calculated. SCILAB-5.5.0 was used for the analysis. Each type of AT twists was as follows; for Type I, lateral axis: 86.1 ± 3.3°, vertical axis: 173 ± 2.4° and longitudinal axis: 92.6 ± 1.7°; for Type II, 93 ± 3°, 175 ± 2° and 91 ± 1°; for Type III, 70 ± 4°, 60 ± 4° and 90 ± 1°. This study demonstrated that the AT has a three-dimensional twisted structure. Further studies should be needed by calculating twisted angles to the joint axes such as the talocrural or the talocalcaneal joints.

(COI: No)

P2-086

Immunolocalization of E-FABP in Meckel's Cartilage of Mice

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Objective: Septoclasts, mononuclear cartilage-resorbing cells in the epiphyseal growth plate, express epidermal-type fatty acid binding protein (E-FABP or FABP5). They locate at the chondro-osseous junction and project several long processes onto the uncalcified cartilage matrix. Meckel's cartilage (MC) develops earlier than mandibular bone and degenerates before birth except for the portion forming the ear ossicles. The present study aims to clarify the localization of E-FABP-positive septoclasts during the degeneration of MC.
Methods: Embryos at 15th day (E15), E16, E17 and E18 of ddY mice were fixed by 4% paraformaldehyde and prepared for frozen serial sections. Immunohistochemical procedures were performed using a specific polyclonal antibody against mouse E-FABP.
Results: E-FABP-positive small, spindle-shaped cells were initially detected at the junction of hypertrophied MC and fibrous connective tissues in E16, and increased in number to E17 and E18. E-FABP-positive cells with or without cell processes were located interior of opened hypertrophic cartilage lacunae. No cells expressing E-FABP were found at any healthy portions of MC comprising resting or proliferating chondorocytes in mice of E15 to18.
Discussion: These results suggest that septoclasts participate in the resorption of MC during its degeneration. Fatty acids and relative lipids affinity to E-FABP may regulate MC degeneration.

(COI: No)

P2-087

Cocktails of certain growth factors that induce differentiation of periodontal ligament cells

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Object: To establish a model *in vitro* that demonstrates the differentiation of periodontal ligament (PDL) mesenchymal cells into osteoblasts(Ob)-like cells to regenerate alveolar bone for the implant dentistry.
Materials and Methods: We isolated mesenchymal cells from the rat molar PDL (Wistar rats; male, 120 g; n=40). The cells were cultured until confluence (37 ℃, humid air with 5% CO2), and then maintained in MINIMUM ESSENTIAL MEDIUM EAGLE (SIGMA; added with AA + 2mM L-glutamine) by adding different combinations of growth factors for 7, 14, 17 or 21 days. Growth factors of 100/200 ng/ml BMP-2, 100/200 nm dexamethasone (DEX), 100 ng/ml IGF-1 and 50 ng/ml bFGF were used. Cell proliferation and differentiation were evaluated by cell counting and alizarin red S staining for the osteogenic cultures. Moreover, we examined the existence of anti-Runx2 and anti-osterix immunoreactive (IR) cells in the cultures by immunohistochemistry.
Results: 1) The 100 ng/ml BMP-2 + 100 nM DEX group: the alizarin red S-stained cells significantly increased in number at the day 14 of culture. 2) The 50 ng/ml bFGF group: the cells were significantly proliferated, but were not induced to differentiate into Ob-like cells until the day 21. 3) The 100 ng/ml IGF-1 group: the cells were induced to differentiate into Ob-like cells after 17 days of culture.
Conclusion: A cocktail of BMP-2 and IGF-1 significantly induced the PDL mesenchymal cells to differentiate into osteogenic Ob-like cells.

(COI: No)

P2-088

Micro-CT-based volume rendering of the lingual muscle in developmental- and postnatal-stage mice and in other animals

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Purpose: The writers already announced that a method to observe a soft tissue in three-dimension (3D) using MicroCT was effective. However, in the case of tongue muscles, the conventional image processing of the 3D information stops in the display example of a tomogram, and a simple 3D image. Only an organ example in the developmental stage was reported in the data. We prepared a mouse for the developmental stage. A postnatal mouse, a cattle tongue, a toad tongue, a tortoise, and a bird in normal laboratory levels were used as the examples this time. The fructification that tried the volume rendering of the muscle unit is reported.
Method: Each tissue was fixed with a 4% neutrality Formalin for one week. The tissue was then freeze-dried according to reduction to a single unit after the decalcification with a Plank-Rychlo liquid for 12 hours. The other part of the tissue was fixed with a 1% osmic acid for 12 hours, and a paraffin preparation was made according to reduction to a single unit. These preparations were photographed in MicroCT. The image processing software performed the image processing.
Fructification: 1. The lingual muscles were showed by the volume rendering images. 2. A lingual muscle in a phylogenetic viewpoint enabled the observation. 3. Beneficial information to study the murine lingual muscle morphosis was obtained.

(COI: No)

P2-089

Effects of mild hyperbaric oxygen on macrophage infiltration during rat skeletal muscle regeneration

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Although hyperbaric oxygen at over 2 atmospheres absolute with 100% O_2 promotes healing of skeletal muscle injury, it is not clear whether mild hyperbaric oxygen less than 2 atmospheres absolute with normal air is equally effective. The purpose of the present study was to investigate the impact of hyperbaric oxygen at 1.25 atmospheres absolute with normal air on muscle regeneration. The tibialis anterior muscle of male Wistar rats was injured by injection of bupivacaine hydrochloride, and rats were randomly assigned to a hyperbaric oxygen experimental group or to a non-hyperbaric oxygen control group. Immediately after the injection, rats were exposed to hyperbaric oxygen. The cross-sectional area of centrally-nucleated muscle fibers was significantly larger in hyperbaric oxygen group than in control group at the early phase after injury. The number of CD68 or CD206 positive cells and the expression levels of TNA-α and IL-10 mRNA were significantly higher in hyperbaric oxygen group than in control group at the early phase after injury. The number of Pax7 and MyoD, or MyoD and myogenin positive nuclei and the expression level of these proteins were significantly higher in hyperbaric oxygen group than in control group 5 days after injury. These results suggest that mild hyperbaric oxygen promotes skeletal muscle regeneration after injury, possibly due to reduced hypoxic conditions leading to accelerated macrophage infiltration and phenotype transition.

(COI: No)

P2-090

Quantitative observation of flexor hallucis longus muscle by using ultrasonograph

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Using ultrasonograph, we have observed cross-sectional image of the flexor hallucis longus muscle at rest, and have investigated sex difference in six healthy male and six healthy female subjects. We confirmed the presence and location of this muscle in vertical direction, and showed that this muscle existed almost middle third of the lower leg. Besides, this muscle of male was longer than that of female in vertical direction. Cross-sectional image of flexor hallucis longus muscle was the most clearly viewed at the central part of the lower leg, but the border of this muscle and surrounding tissues was not constantly apparent. We therefore attempted to quantify the muscle not by area, but by length. We determined three distinguishable points on cross-sectional image of this muscle as landmarks. Then, we measured medio-lateral length and antero-posterior length in cross-sectional image based on these three landmarks. In all subjects, the flexor hallucis longus muscle was longer in medio-lateral direction than in antero-posterior direction. This muscle of male showed a tendency to be longer than that of female in vertical length and horizontal direction. No sex difference was showed in antero-posterior length.

(COI: No)

P2-091

Interaction between supramolecular organization of sarcomeric proteins and myowater revealed with heat denaturation

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Magnetic resonance (MR) images reflect not only water content, but also water states in the tissue. Details of each water state are, however, not clarified yet. In skeletal muscle, MR distinguishes five water states whose localization has been clarified taking advantage of well-organized crystalline sarcomere structure. As has been reported for monomeric (G-) and polymerized filamentous (F-) actin (Wazawa, 2011), proteins develop additional mode of interaction with surrounding water molecules with the order of supramolecular organization. With this view in mind, we observed heat capacity of skeletal muscle (prepared from sartorius muscle of *Rana Catesbeiana*) using differential scanning calorimetry (DSC). With increase in temperature, water molecules released from any intermolecular interaction absorbed additional heat to form an endothermic peak as in the case of melting of ice. Muscle preparation showed endothermic peaks at -25, -22, 0, 45 and 63℃. Each of the peaks at 45 and 63℃ would reflect irreversible denaturation at specific higher-order structure as general heat denaturation of proteins does. Correspondingly, a 45℃ peak irreversibly diminished later -25℃ peaks, and a 63℃ peak diminished later -22℃ peaks with an increase in the integrated heat capacity from -80℃ to 20℃. These results suggest that molecular or supramolecular organization of muscle sarcomere that is subject to heat denaturation significantly affects their interaction with myowater causing substantial endothermic peaks with temperature.

(COI: No)

P2-092

Non-invasive evaluation of skeletal muscle using the wavelet analysis

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The mechanomyogram (MMG) reflect the "mechanical activity of muscle" is a slight vibration associated with muscle contraction, unlike the EMG to record the electrical activity of muscle contraction, muscle sound is, to exercise it is obtained by recording a fine vibration due to skeletal muscle contraction by measuring by placing a vibration sensor at a site file. However, the physiological phenomena of muscle properties reflected by the features of the MMG and electromyography(EMG) signals, and mechanisms of contraction are still not fully clear during movements. The non-invasive method using the wavelet transform will gave a useful analysis for low sound of masticatory muscle with complex noisy background during movements. Therefore we try to accomplish analysis the MMG in masticatory function elucidation by performing the position change of the masticatory muscles of voluntary movement. The distance measurement by laser, MMG and EMG were measured to compare skeletal muscle (biceps brachii) and masticatory muscle in this study. As a result, the sound is found in two areas lower the difference in frequency, moreover, depends on the type of muscle, the present analysis methods muscle contraction, the movement of the muscle fibers and fascia seen when tension reflects the function of the muscle possibility has been suggested.

(COI: No)

P2-093

Characteristics of time course of gene expression in muscle atrophy in cast- immobilized rat model

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We analyzed relevant gene expression while atrophic change in model by cast immobilization on rats. According to our previous study we revealed that the blood flow rapidly decreased in the immobilized leg, and then gradually decrease in muscle weight ant muscle fiber cross sectional area occurred observed after the immobilization applied. This trend was found especially in SO-Fiber (Type-I) rich muscle such as soleus muscle. In this study, we experimented on rat model to simulate muscle atrophy, and analyzed the gene expression pattern in the model utilizing immobilization. Male Fisher rats were used in this study. The right hind-limbs of Experiment group rats were immobilized in a plaster cast (left contra-lateral hind-limbs were analyzed as experimental controls). Gene expression was analyzed encyclopedically in DNA micro array at the following time points; 6 hours, 1 day, 4 days, 10 days after the procedure. We focused on 86 probes which showed significant difference between control and experimented side at each sampling point. Then we searched the relationship of the found probes to their roles either of blood flow or muscle volume. It was revealed that blood flow probes had metabolism regulation feature and muscle volume, structure building feature.

From the analysis it seemed that decrease in blood flow induce suppression of metabolism regulation genes, and then gradually suppress structure related genes to lead morphological change.

(COI: No)

P2-094

Shortening velocity of knee extensor in frog; the effects of the contraction of other lower limb muscles on it

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The present study was investigated to know how the shortening velocity of knee extensor was influenced by the contraction of other lower-limb muscles. We measured the force - velocity relationship, *in vivo*, in whole muscle preparations in knee extensor, triceps femoris muscle (TFM), of the frog, *Rana catesbeiana*. TFM consists of three muscles, rectus femoris (RFM), vastus medialis (VMM) and vastus lateralis (VLM). Frogs were anesthetized and the four kinds of preparations mentioned below were made. Their isotonic shortening velocities were measured at various steps of load at 20 ± 0.5 °C. 1) In the first preparations, all the muscles of thigh were contracted by stimulating sciatic nerve. 2) In the second ones, the sciatic nerve to VMM and VLM was exclusively stimulated by cutting all other branches. 3) In the third ones, the sciatic nerve to RFM, VMM and VLM was exclusively stimulated as in 2). 4) In the fourth ones, the sciatic nerve to hamstrings, VMM and VLM were exclusively stimulated as well. The maximum shortening velocity in 1) was the fastest among four preparations, and the shortening velocity at heavier load (0.5-0.9) was about twice as fast as the others. And the output of power in 1) was also the largest. These results indicate that the interaction between muscle contractions have remarkable influence on shortening velocity and power output, suggesting that interaction between muscle would be able to produce higher power.

(COI: No)

P2-095

Medial Pterygoid initiated the Growth of the Mandible through Premature Muscle Contraction

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Craniofacial growth is influenced by the interaction of muscle and bone tissues. The medial pterygoid is one of the muscles of mastication attached to the mandible. The purpose of the study was to investigate the relation between the medial pterygoid and mandible during embryogenesis. Specimens were prepared from thirty fetal mice at embryonic day (ED) 12, 13 and 14. Slides were stained with hematoxylin and eosin and observed under the light microscope. Immunohistochemistry using desmin, a muscle specific marker, as well as tenomodulin, a tendon specific marker, were also carried out. Results showed that at ED 12, the medial and lateral pterygoid and tensor veli palatini were adjacent to one another. At ED 13, the mandible started to form while the medial pterygoid moved towards the developing mandible. At ED 14, the palatine shelves were also seen in a horizontal position. Over time, desmin localization was observed at myotendinous junctions in between the medial pterygoid and Meckel's cartilage as well as in between the medial pterygoid and mandible and finally in the center of the muscle. Tenomodulin first appeared at ED 13 and had formed spaced linear arrays at either end of the muscle fiber by ED 14. The results suggest that although the muscles of mastication were still immature, the premature contraction of medial pterygoid and the positional relationship provide a dynamic change between the development growth of the mandible and the start of the fusion of the secondary palate.

(COI: No)

P2-096

Epac1 mediates masseter muscle hypertrophy induced by chronic stimulation of β_2-adrenoceptor

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To further elucidate the role of Epac (exchange protein directory activated by cAMP) in the signaling mechanisms responsible for the β_2-adrenoceptor (β_2-AR)-mediated phenotypic changes of skeletal muscle, we examined the effects of chronic stimulation of β_2-AR with clenbuterol (CB) (i.p., 2mg/kg/day for 3 weeks), a β_2-AR agonist, on myofiber cross-sectional area (CSA) and fiber-type composition in masseter muscle (the principal jaw closer in rodents) of wild-type (WT) and Epac1-null (Epac1KO) mice. Masseter muscle mass and myofiber CSA were significantly increased by the CB treatment in WT while not in Epac1KO, demonstrating that Epac1 is involved in β_2-AR signaling promoting muscle hypertrophy. In contrast, the CB treatment significantly increased the proportion of type-IIB fiber at the expense of that of type-IID/X in both WT and Epac1KO, indicating that Epac1 did not mediate the CB-induced slow-to-fast fiber-type transition. The inhibition of the CB-induced masseter hypertrophy by Epac1 disruption was associated with the suppression of CB-induced phosphorylation of Akt and its downstream molecules, S6K1, 4E-BP1 and GSK-3β, as well as CaMKII and its target, HDAC4, a negative regulator of MEF2. These results suggest that Epac1 plays important roles in the β_2-AR-mediated masseter muscle hypertrophy without affecting the slow-to-fast fiber-type transition, potentially through subsequent activation of both Akt and CaMKII/HDAC4 signaling pathways.

(COI: No)

P2-097

TRPV1 channel regulates skeletal muscle regeneration and satellite cell differentiation

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PURPOSE: The transient receptor vanilloid type 1 channel (TRPV1) is a member of non-selective cationic channel family. The activation of TRPV1 induces the influx of Ca^{2+}. Previous studies have demonstrated that Ca^{2+} entry is required for myogenic differentiation and satellite cell (SC) fusion. However, the role of TRPV1 in muscle cell and SC is unknown. The purpose of this study was to determine the role of TRPV1 on muscle regeneration and SC functions.
METHODS: SCs were isolated from male C57BL/6J mice (10 weeks). Capsaicin (cap), TRPV1 agonist, (1 μM) was added to culture media. We confirmed that siRNA transfection silenced TRPV1 gene expression. All animals were injured by the injection of cardiotoxin (CTX) into tibialis anterior (TA) muscles to induce muscle damage in vivo study. Daily injection of cap (1 μM) or physiological saline (con) was performed 8 consecutive days from 3 days before and 5 days after the CTX injection. TA muscles were sampled to analyze regeneration by H&E staining at 5 days after the injection.
RESULTS: We observed that cap induced a significant increase in expression of two makers of SC differentiation. TRPV1 silencing reduced the fusion index. In addition, we observed that exogenous addition of IL-4 was able to restore normal fusion in TRPV1 silenced SCs. The fiber area of the muscles with central nuclei isolated from the cap group was significantly larger than that of muscles from the con group.
CONCLUSIONS: TRPV1 may play critical roles in SC differentiation and skeletal muscle regeneration.
(COI: No)

P2-098

Inhibition of junctional membrane-targeting of skeletal muscle L-type calcium channel by point mutation in the junctophilin-binding domain of $Ca_V1.1$ subunit

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Skeletal muscle L-type calcium channels (LTCC) are specifically localized to the junctional membrane (JM) where the sarcolemma are closely apposed to the sarcoplasmic reticulum, and form a functional complex with ryanodine receptors. Although the targeting of LTCC is critical for efficient excitation-contraction coupling, its molecular mechanism has not been clarified. We previously showed that junctophilins (JP) regulate the proper JM-targeting and function of LTCC through binding to $Ca_V1.1$ subunits. Moreover, a GST-pull down study showed that the JP-binding domain (JBD) is located in 1595-1606 amino acid residues in the C-terminus of $Ca_V1.1$. In this study, we first conducted alanine scanning to the recombinant GST-JBD fusion protein, and examined changes in the binding property of the protein to JPs by the pull down assay. Whereas the binding to JPs is preserved by the alanine substitution of E1595, R1596, and G1606, mutations of other residues in JBD attenuated the binding. We next prepared $Ca_V1.1$_R1596A and $Ca_V1.1$_R1600A and transiently expressed them in GLT myotubes. Immunocytochemical analysis revealed that the JM-targeting rate of $Ca_V1.1$_R1600A but not $Ca_V1.1$_R1596A was significantly reduced compared to the wild type. These results suggested that JBD in C-terminus of $Ca_V1.1$ contributes to the proper JM-targeting of skeletal muscle LTCC.
(COI: No)

P2-099

In vitro effects of K^+ ATP channel agonist on LES tone in rats

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The lower esophageal sphincter (LES) is a specialized region of the esophageal circular smooth muscle that allows passages of a swallowed bolus to the stomach. Functional disorder of an esophagus such as achalasia displays a diminished peristalsis in lower esophagus. It has been reported that nitric oxide plays a major role in LES relaxation. However, the detailed mechanism involved in the regulation of LES activity is still elusive. Nicorandil possesses dual properties of a nitrate and K^+ ATP channel agonist, and is known to reduce LES tone. The present study was carried out to clarify the mechanisms underlying the effects of nicorandil on LES. In particular, possible involvement of K^+ ATP channel was investigated. LES tissues of rats were placed in a standard organ bath and activities were recorded using the software Chart Pro v 4.0. After contraction with carbachol, K^+ ATP channel agonists (nicrandil, pinacidil, diazoxide) were added directly to the tissue bath. Ant they caused a significant relaxation of the LES. Further, the K^+ APT ATP channel blocker glibenclamide prevented the LES relaxation caused nicorandil. On the other hand, the nitoric oxide synthase inhibitor L-NAME, the guanylate cyclase inhibitor ODQ and BKCa channel blocker iberiotoxine failed to prevent the LES relaxation caused nicorandil. Immunohistochemistry revealed that Kir6.1, Kir6.2, SUR1 and SUR2B subunit, which compose K^+ ATP channel, were expressed in rat lower esophagus. These findings suggest that nicorandil causes LES relaxation by activating K^+ ATP channel.
(COI: No)

P2-100

Force-inhibiting effect of phosphatase inhibitor on bovine ciliary muscle

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Purpose: Ciliary muscle is a smooth muscle with parasympathetic innervations and characterized by a rapid response to muscarinic receptor stimulation and sustained contraction. We recently reported that okadaic acid (OA), a phosphatase inhibitor, does not impaired carbachol (CCh)-induced contraction in bovine ciliary muscle (BCM). In order to address the regulatory mechanisms of ciliary muscle contraction, we examined the effects of selective PP2A inhibitors on BCM and guinea pig taenia cecum.
Methods: Smooth muscle strips were excised from bovine ciliary body and guinea pig taenia cecum. Muscle strips were contracted with CCh or ionomycin, and isometric tension was recorded. Various concentrations of OA, Fostriecin (Fos) and Rubratoxin A (RubA) were administered to contracted muscle strips.
Results: In CCh-induced contraction, low concentration of OA and Fos caused relaxation in taenia cecum, but not in BCM. RubA impaired contraction both in taenia cecum and BCM. On the other hand, in ionomycin-induced contraction, all three PP2A inhibitors impaired contraction both in taenia cecum and BCM.
Conclusion: These results strongly support the hypothesis that the force inhibiting effect of OA is due to PP2A inhibition but not non-specific activity. Since OA and Fos, but not RubA, failed to inhibit CCh-induced contraction in BCM, CCh may inhibit PP1 more potently in BCM than in any other smooth muscles.
(COI: No)

P2-101

Nucleotides dependence on the accerelating effects of myosin II inihibitors on the smooth muscle relaxation

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Blebbistatin, a myosin II inhibitor, suppress force development of skinned taenia cecum strips from guinea pig at any given Ca^{2+} concentration but had little effects on the phosphorylation of myosin regulatory light chain (Watanabe et al. Am J Physiol Cell Physiol 2010; 298:1118-1126). Also blebbistatin accelerates relaxation by removing Ca^{2+} from contracting preparations (Watanabe, J Physiol Sci 62:S160, 2012). These results suggest that blebbistatin suppressed skinned smooth muscle contraction through disturbing function and/or conformation of myosin heavy chain by the agent. Analyzing kinetics of the relaxation time courses of the skinned taenia cecum indicated that a portion of fast detaching cross-bridges to transfer to latch-bridges dissociating very slowly, and that, 1) blebbistatin suppressed transferring from fast detaching- cross bridges to slow detaching (latch)-bridges, and also 2) blebbistatin accelerated dissociation of the latch-bridges. To explore mechanisms of accelerating effects of blebbistatin on the skinned smooth muscle relaxation in detail, we investigated blebbistatin effects on the relaxation of the skinned taenia cecum in the presence of various nucleotides. In the absence of ATP, blebbistatin did not affect the relaxation process even in the presence of ADP. On the other hand, in the ATP containing solutions, blebbistatin accelerated the relaxation irrespective of ADP. The results suggest that blebbistatin affects conformational changes of myosin from ATP binding to ADP binding states, resulting in acceleration of dissociation of myosin from actin.
(COI: No)

P2-102

A new method for isolation of bovine ciliary muscle cells using Percoll gradient centrifugation

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In bovine ciliary muscle (BCM), stimulation of M_3-muscarinic receptors (M_3R) opens two types of non-selective cation channel with different unitary conductances (35 pS and 100 fS) which serve as major pathways for Ca^{2+} entry during sustained contraction. The molecular entities of these channels are still unknown, mainly because of the technical difficulty of obtaining BCM cells with sufficient purity. We describe here a new method by which one can obtain BCM cells with unprecedented quality and amount.
Methods The ciliary body dissected from bovine eye were treated with collagenase, and the dispersed cells were subjected centrifugation through discontinuous Percoll density-gradient of 1.050 and 1.060 g/mL. Cells were then collected from the 1.050/1.060 interface and cultured for 1-3 days before use. The intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) was monitored using the Fluo-4 fluorophore. Existence and localization of proteins were examined by immunofluorescence microscopy.
Results and discussion In the cultured BCM cell preparations, carbachol (2 μM) applied to the bath evoked a phasic and tonic increase of $[Ca^{2+}]_i$. Caffeine (20 mM) caused a phasic $[Ca^{2+}]_i$ elevation in the absence of extracellular Ca^{2+}. These responses were clearly observed in most cells, which were well stained with antibody against α-smooth muscle actin. Immunostaining also confirmed abundant expression of M_3R and STIM1 in the plasma and endoplasmic membranes. The present method allows us to obtain as many as 5×10^6 BCM cells with clear agonist sensitivity by a single-step centrifugation procedure.
(COI: No)

P2-103

Discovery of novel Salacia-derived components which specifically inhibit the ROK-mediated Ca^{2+}-sensitization of vascular smooth muscle contraction

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Rho-kinase-mediated Ca^{2+}-sensitization of vascular smooth muscle (VSM) contraction contributes to vasospasm. As upstream mediator for such pathological pathway, we identified sphingosylphosphorylcholine (SPC), which induced vasospasm in vivo, and its levels were extremely elevated in vasospastic patients. Furthermore we found that eicosapentaenoic acid (EPA) selectively inhibited the ROK-mediated Ca^{2+}-sensitization of VSM contraction, and clinically prevented cerebral vasospasm. In this study we aimed to identify a substitute for EPA as functional food from plants, because EPA is a component of fish oil, which is readily affected by marine pollution and has unstable supply. Contractile properties were assessed by the effects on Ca^{2+}-dependent contraction and Ca^{2+}-sensitization, which was induced by high K+-depolarization and SPC, respectively. After extensive screening, we found that extracts of Salacia, a woody climbing plant widely distributed in Asia and South America, strongly inhibited the Ca^{2+}-sensitization and very weakly blocked Ca^{2+}-dependent contraction. Liquid chromatography revealed that water-soluble fraction markedly inhibited the Ca^{2+}-sensitization, without affecting physiological Ca^{2+}-dependent contraction, while other fractions strongly inhibited the both types of contractions. These results suggest that Salacia extracts contain protective and therapeutic components for vasospasm.

(COI: Properly Declared)

P2-104

High-frequency sarcomeric auto-oscillations induced by heating in living neonatal cardiomyocytes of rat

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Previously, we developed an experimental system for simultaneous nano-scale analysis of single sarcomere dynamics and Ca^{2+} changes via expression of AcGFP in Z-discs (Shintani et al., J. Gen. Physiol., 2014). Using this system, we found that elevating temperature up to 40℃ by IR laser irradiation immediately generated fast-paced beating and sarcomeric oscillations outpacing the normal beating coupled with Ca^{2+} in a cardiomyocyte. This sarcomeric oscillations occurred independent of Ca^{2+} transients. We named this phenomena Hyperthermal Sarcomeric Oscillations (HSOs). The HSOs frequencies were stable, whereas under the conditions the normal beating is blocked, the HSOs were gradually organized. Therefore the coexistence of normal beating may be needed to induce stable HSOs.

(COI: No)

P2-105

Functional characterization of calcium holes in heart cells

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Calcium ion (Ca^{2+}) is a versatile intracellular signaling molecule that regulates various cellular functions, such as contraction, secretion, and gene expression. Preceding studies of the author indicate the existence of "calcium holes" in heart cells, a Ca^{2+}-deficient intracellular nanodomain that develops on the sarcolemmal membrane in the proximity (about 100 nm diameter) of a plasma membrane Ca^{2+}-ATPase (PMCA) molecule. Although Ca^{2+}-extrusion by PMCA in heart cells have so far been considered negligible, experimental evidence show that PMCA maintains the local Ca^{2+}-level in a calcium hole substantially lower than the global level. The operation of PMCA creates an encapsulated intracellular Ca^{2+}-signaling nanodomain that is distinct from the bulk Ca^{2+}-environment. Here, the author provide further evidence for the functional characteristics of calcium holes in heart cells, using whole-cell clamp and $[Ca^{2+}]_i$-microfluorimetry techniques. Under physiological conditions, inhibition of PMCA enhanced the amplitude of CICR-induced Na/Ca exchange current, and extended the duration of the action potential, suggesting an involvement of calcium holes in the regulation of cardiac E-C coupling and excitability. Possible physiological roles of PMCA and calcium holes in heart cells are also discussed.

(COI: No)

P2-106

Imaging of sarcomere dynamics in rat neonatal cardiomyocytes expressing stress fiber-like structures

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In the present study, we investigated whether sarcomeric dynamics is influenced by the development of stress fiber-like structures, and observed sarcomeres of rat neonatal cardiomyocytes by time-lapse imaging. Ventricular myocytes were isolated from 1-day-old Wistar rats, and cultured on collagen-coated glass bottom dishes. Stress fiber-like structures developed when myocytes were cultured for three to six days in the presence of basic fibroblast growth factor (FGF) or the actomyosin inhibitor N-benzyl-p-toluenesulphonamide (BTS). The magnitude of sarcomeric contractions did not significantly change upon treatment with FGF-2 or BTS. But lengthening velocity of a small number of sarcomere in series was slower than normal numbers. We hereby conclude that in neonatal cardiomyocytes, 1) intracellular stress fiber-like structures develop, and 2) the stress fiber-like structures do not significantly alter sarcomere contraction, 3) the contractility of remained sarcomeres is maintained.

(COI: No)

P2-107

Multipotent differentiation of human skeletal muscle-derived cells (Sk-Cs): Comparison to mouse Sk-Cs

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The differentiation potential of human Sk-Cs was examined, and compared to that of the mouse Sk-Cs. The samples (5-10g) were obtained from abdominal, and several leg muscles of 36 patients (17-79 years-old) undergoing prostate cancer, and leg amputation surgery following the accidents. All patients gave their informed consent to the aim and procedure. The Sk-Cs were isolated by originally conditioned collagenase solution, then, sorted as CD34-/CD45-/CD29+ (Sk-DN/29+) and CD34+/CD45- (Sk-34) cells, similar to the mouse case. The differentiation potentials were examined by cell culture and in vivo transplantation into the severely damaged muscles of athymic nude mice/rats. Interestingly, these two cell fractions could be clearly divided into highly myogenic (Sk-DN/29+) and multipotent stem cell (Sk-34) fractions as different from the mouse case. At 6 weeks of after separate transplantation of both cells, the former dominantly showed an active contribution to the muscle fiber regeneration, but the latter showed vigorous engraftment to the interstitium associate with the differentiation into Schwann cells, perineurial/endoneurial cells, and vascular endothelial cells and pericytes, as wholly corresponded to the previous mouse cases. Therefore, it was suggested that the human Sk-Cs was potentially applicable to the therapeutic autografts, expecting their multiple differentiation potential in vivo.

(COI: No)

P2-108

Enhancement of myosin heavy chain class I (MHC I) mRNA expression in C2C12 myocyte by multivalent cations

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Calcineurin is a protein phosphatase known as calcium-dependent serine-threonine phosphatase. We have previous reported that La^{3+} in the culture medium upregulated mRNA expression of myosin heavy chain class I (MHC I) and skeletal muscle modulators including interleukin-6 (IL-6) and heat shock protein 70 (HSP70) through the activation of calcineurin without increment of intracellular Ca^{2+}. In the present study we examined the effects of other multivalent cations, such as Gd^{3+} and Ni^{2+}, on expression of MHC, IL-6, and HSP70 mRNAs in C2C12 cells using real-time RT-PCR method. C2C12 cells were induced to differentiate to myotubes by medium exchange to D-MEM containing 2%FBS. The cells were incubated in D-MEM containing 2%FBS with multivalent cations, with or without cyclosporine A at the beginning of differentiation and removed after 24hr, and were maintained in differentiation medium. Our results are as follows: (1) The MHC I, IL-6, and HSP70 mRNA expressions were significantly increased by La^{3+}, but were decreased by cyclosporine A with or without La^{3+}. (2) The MHC I mRNA expression was significantly increased by the application of Gd^{3+} or Ni^{2+}, although the IL-6 and HSP70 mRNA expressions were not significantly upregulated by these cations. These results indicate that multivalent cations flowing into the cytosol may upregulate MHC I mRNA in calcineurin-dependent manner.

(COI: No)

P2-109

Spin-spin relaxation of 1H NMR signals from myosin filaments suspension with or without ATP

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The dynamic changes of water molecules structure surrounding contractile proteins might play an important role in cross-bridge cycling during contraction. The spin-spin relaxation process of 1H-NMR signals from suspension of myosin filaments prepared from rabbit could be well represented by the summation of several exponentials indicating that water molecules in the suspension could be conveniently grouped into several components based on the relaxation time constant (T2). The slowest two components (T2 around 0.4s and 0.15s) dominated over faster relaxation components. This may suggest that the potential of the water molecules existing around myosin filaments is high.

(COI: No)

P2-110

Analysis of novel protein in striated muscles that transcribe from the contiguous region of connectin gene

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Connectin is the largest protein that connects between Z-line and M-line of the sarcomere and functions as a molecular spring of vertebrate striated muscles. At the contiguous region of connectin gene on mammalian genomes, there is a gene for protein that function remain unknown. We found that this gene is expressed in various tissues including heart and skeletal muscles by RT-PCR experiments, and multiple splicing isoforms are produced from this gene. We also found that the protein (about 150kDa) from this gene is existed in heart and skeletal muscles by western blot test using newly produced antibody, and localized in the intercalated disk and the Z-line of sarcomere in heart muscle and the M-line of the sarcomere in skeletal muscles. The Z-line localization also confirmed by transfection of GFP-fusion 150kDa protein into muscle tissues and cultured muscle cells. To know the functions of the 150kDa isoforms in sarcomere genesis, we are now investigating the overexpression and restriction effects of them in cultured skeletal muscles.

(COI: No)

P2-111

Insulin-Growth Factor I Affects the Expression of Irisin

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Objective: Irisin was discovered from skeletal muscles and acts as a hormone to cause heat production by fat combustion. Therefore, irisin is considered to work similarly to the brown adipose tissue. In this study, we analyzed theeffect of insulin-like growth factor I (IGF-1) transferred to cultured muscle cells on the production of irisin.
Methods: IGF-1 gene was transferred into C2C12 cells, a mouse skeletal myoblastic cell line, by an electroporation method. Control (normal) and IGF-I-transferred CeC12 cells were cultured 1.0×10^5 cells / well. We observed every 12 hours until 48 hours. After examining the expression of fibronectin type III domain containing 5 (fndc5) gene at the molecular level by Lightcycler, we assessed potential correlations of irisin. In addition, we also measured isoform myosin heavy chain (MyHC), is the protein of muscle contraction were searched for association with irisin and MyHC.
Results & Discussion: The mRNA of fndc5 in the transferred group was expressed at 12 hours, but it was not expressed thereafter. MyHC-2d (flexible type), in the transferred group, was highly expressed at 12 hours. Therefore, we were considered that the transgenic IGF-1 changed to MyHC-2d by the surrounding environment. In addition, it was suggested that irisin was expressed in order to maintain the homeostasis.

(COI: No)

P2-112

Molecular genetics of type IV intermediate filament, synemin

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Intermediate filaments (IFs) are one of major components of the mammalian cytoskeleton. Synemin, a type IV IF protein, is known to form filaments with desmin and reinforce connection of Z disks and sarcolemmal proteins in skeletal muscle. But there is little knowledge about physiological functions of synemin. For investigating the functions of synemin in vivo, we generated synemin knockout (KO) mice. By observation of haematoxylin-eosin stain of several tissues, we could not find significant difference between wild type (WT) and KO mice. Furthermore we observed the relevance of synemin in muscular structure by immunofluorescence of myofibril markers, but there is no significant morphological change. After treadmill exercise, we observed muscle damage of WT and KO mice by measurement of the serum creatine phosphokinase level and staining of Evans blue, but there is not a significant difference. These results suggest synemin is not required for muscle development and maintenance of muscular structure.

(COI: No)

P2-113

Effects of disease-associated mutations in the central region on the RyR1 channels

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Type 1 ryanodine receptor (RyR1) is a Ca^{2+} release channel in the sarcoplasmic reticulum and the major target for muscle diseases, e.g., malignant hyperthermia (MH) and central core disease (CCD). It is widely believed that MH and CCD mutations cause hyperactivation of the Ca^{2+}-induced Ca^{2+} release (CICR), resulting in abnormal Ca^{2+} homeostasis in skeletal muscle. However, it remains unclear how the disease-associated mutations affect CICR. We have recently characterized several disease-associated mutations in the amino-terminal region by live-cell Ca^{2+} imaging and [^{3}H]ryanodine binding and found that these mutations divergently affect the gain (i.e., peak activity) and the sensitivity to activating Ca^{2+} of CICR. In this study, we extended this approach to 15 MH and MH/CCD mutations in the central region (1592-2508). The disease-associated mutations increased the gain and the sensitivity to activating Ca^{2+} in a site-dependent manner. The calculated CICR activity strongly correlated with the ER Ca^{2+} level, an index of Ca^{2+} leak. Importantly, the accelerated sensitivity to activating Ca^{2+} was linked to pathogenesis of CCD. Overall, the effects were similar to those of the amino-terminal mutations. The underlying molecular mechanism will be discussed.

(COI: No)

P2-114

Muscle glycogen fails to affect ryanodine receptor function and myofibrillar Ca^{2+} sensitivity in rat fast-twitch muscles

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Although the mechanisms by which decreases in muscle glycogen lead to muscle fatigue are not as well understood, some of previous studies have suggested that muscle fatigue induced by decreased glycogen is mediated through excitation-contraction uncoupling. The purpose of this study was to examine whether muscle glycogen depression causes impaired function of ryanodine receptor (RyR), a Ca^{2+} channel of sarcoplasmic reticulum, and/or reduced myofibrillar Ca^{2+} sensitivity. Wistar rats were randomly assigned to exercise or control groups. The rats in exercise group run on the rodent treadmill, then were subdivided into exercise-glycogen (EG) or exercise-fast (EF) groups and were allowed to rest for 2 h after exercise. During recovery, the EG rats were given 5% sucrose in water whereas the EF rats were given water only. Following recovery, the superficial regions of gastrocnemius muscles were excised and used for skinned fiber and biochemical experiments. The EG muscles exhibited a 1.5-hold higher glycogen concentration than the EF muscles. Skinned fiber and biochemical experiments indicated that there were no differences between the EF and EF muscles with regard to myofibrillar Ca^{2+} sensitivity and caffeine threshold of the RyR. These results suggest that changes in the muscle glycogen content may not affect the RyR and myofibrillar function.

(COI: No)

P2-115

Biomagnetic vector fields of gut functional syncytium

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Magnetic field detection of biological electric activity would provide a convenient estimate of the functional state of cellular organization, namely syncytium constructed with cell-to-cell electric coupling. In this study, we show the first real-time measurement of magnetic vector fields induced by biological propagating current in gut musculature, a typical functional syncytium, using an improved magnetoimpedance (MI) gradio-sensor with an amorphous metal wire core and a pair of detector coils. Biomagnetic waves of up to several nT were recorded in the magneto sensor placed ~1 mm below the sample under control conditions. The direction of magnetic waves altered depending on the rotation of the muscle layer and magneto sensor, indicating the existence of propagating intercellular currents. Tetraethyl ammonium (TEA) facilitated and nifedipine suppressed magnetic waves reflecting electric activity in smooth muscle, respectively, suggesting that L-type Ca2+ channels are responsible for the propagating current. The magnitude of magnetic waves rapidly decreased to ~30% by the initial and subsequent 1 mm separations between sample and sensor. The large distance effect is attributed to the feature of bioelectric circuits constructed by two reverse currents, i.e. intercellular propagating current and extracellular return current, separated by a small distance. We anticipate that the amorphous metal-based magneto sensor technology would make biomagentic fields a more realistic aspect in our lives, because these sensors are operated at ambient temperature without a magnetic shield.

(COI: No)

P2-116

Hydrogen sulfide inhibits motor activity of esophageal striated muscle in rats

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Inhibitory effects of hydrogen sulfide (H_2S) on the smooth muscle motility of the ileum and the colon have been reported. However, it is unclear whether H_2S can affect the esophageal motility. Therefore, the aim of the present study was to clarify the effects of H_2S on the motility of the esophageal striated muscle in the rat. An isolated segment of the rat esophagus was placed in an organ bath and the mechanical responses were recorded using a force transducer. Electrical stimulation of the vagus nerve evoked the contractile response in the esophageal segment. The vagally mediated contraction was inhibited by application of a H_2S donor, NaHS. NaHS did not affect the contraction induced by electrical field stimulation, which directly can excite the striated muscle not via vagus nerves. This shows that H_2S can influence not directly the striated muscle but neurons. RT-PCR revealed the expression of CBS and CSE mRNA in the esophageal tissue. These findings suggest that H_2S might be produced in the esophageal tissue and might regulate the motor activity of the esophageal striated muscle.

(COI: No)

P2-117

A genome-wide screen for genes involved in Helicobacter pylori-induced gastric carcinogenesis

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Helicobacter pylori (H. pylori) is strongly associated with atrophic gastritis, peptic ulcer, and gastric cancer. Its major oncoprotein CagA (Cytotoxin-associated gene A) translocates into gastric epithelial cells and disrupts host cell polarity and deregulates cell signaling, such as Receptor Tyrosine Kinase signaling and Wnt signaling. However, the molecular mechanism in which CagA develops gastric cancer is not fully understood. In this study we developed a transgenic Drosophila model in which CagA expression in the larval and adult eye induces eye defects. From a genome-wide overexpression screen of about 7,000 GS lines (from Drosophila Gene Search Project), we identified 30 genes whose forced expression strongly suppressed the CagA-induced rough eye phenotype. Some of these genes were found to be related to Ras-MAPK (Mitogen-activated Protein Kinase) signaling, Wnt signaling and signaling implicated in the establishment and maintenance of cellular polarity, which validates the findings of this screening. Of particular interest is that some gene products have been shown to function in gastric mucus secretion, which could suggest that the mucus secretion pathway may be inhibited by CagA. We will present some findings on the molecular mechanism of the inhibition.

(COI: No)

P2-118

Functional analyses of dipeptidase-1 (DPEP1) using a colon/gastric cancer-derived cell line, HCC56

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DPEP1, a glycosylphosphatidylinositol (GPI)-anchored protein, is highly expressed in colon cancer and thus proposed as a prognostic marker, but its function has yet to be elucidated. We found that HCC56 cells, a colon/gastric cancer cell line, expressed DPEP1 much higher than other colon cancer-derived cell lines, such as LoVo, RKO, HT29, SW480, CaCO2. In colonies of HCC56 cells, immunofluorescence signal for DPEP1 was often observed along the cell surface as dot appearance, and was well colocalized with another GPI-anchored protein, CD59. DPEP1-knockdown in HCC56 cells did not affect cellular activities of proliferation in both culture and xenograft models. However, DNA damage after the oxidative stress induced by disodium hydrogen arsenate heptahydrate was more severe in the DPEP1-knochdown cells than in control cells. These results suggest that DPEP1 expression confer tolerance against oxidative stress probably by its ability to cleavage cysteinylglycine, a glutathione metabolite.

(COI: No)

P2-119

Morphological and functional studies on the gastrointestinal tract in a mouse model of chronic renal failure

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Introduction: It has been reported that mice with 5/6 nephrectomy-induced chronic renal failure (CRF) have reduced gastrointestinal transit (GIT) and increased fecal moisture content (FMC). We have recently shown that feeding adenine (0.2%, w/w) to mice can be used as a model of CRF. Here, we investigated the possible effects of adenine-induced CRF on the GIT physiology and histology in mice.
Methods: The effects of CRF induced by feeding adenine (0.2%, w/w for 2 or 4 weeks) on the gastric emptying index (GEI), GIT, FMC and bead expulsion test (BET) were investigated.
Results: Feeding adenine for 2 or 4 weeks resulted in CRF. The BET was significantly increased in mice given adenine for 2 but not 4 weeks, while the GEI was significantly increased in mice treated with adenine for 4 but not 2 weeks. No significant differences between control and adenine-treated mice were found in GIT, FMC or the histology of the different parts of the gut. Acetylcholine-induced contractions of the ileum of adenine-treated rats were not significantly different from those of the controls.
Conclusion: Feeding adenine for either 2 or 4 weeks resulted in CRF, but it would appear that this model produces effects on the gastrointestinal tract that are milder than those reported before in animal models with 5/6 nephrectomy-induced CRF.

(COI: No)

P2-120

Lectin-based histochemical mapping of fucosylated glycoproteins in mouse intestinal tract

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Recently, clarification of the biological roles of protein glycosylation is receiving special attention. A glycan moiety of glycoproteins is found to be functionally linked to various biological processes through modulating protein functions, such as signal transduction and molecular interactions. Thus, characterization of proteins carrying an interested glycan is a key to clarify the role of the glycan.
Spatiotemporal distributions of glycans have been investigated by lectin-histochemistry in various organs, tissues and cells. However, histochemical profiles of their carrier proteins are hardly revealed. This is because of difficulty in identification of the carrier proteins and specific detection of a particular protein carrying an interested glycan. To overcome these issues, we utilized an approach integrating glycoproteomic analysis for identification of the carrier protein and in situ Proximity Ligation Assay for histochemical detection of the targeted glycoprotein. Here, we report our recent achievements in the mapping of fucosylated glycoproteins in mouse intestinal tract. Fucosylated glycans expressed by the intestinal epithelial cells are reported to play pivotal roles in maintaining intestinal homeostasis. Based on the protein identification result obtained by glycoproteomic analysis, distribution of a particular fucosylated glycoprotein was examined. Our approach will facilitate unraveling the roles of the intestinal fucosylated glycans.

(COI: No)

P2-121

Vitamin A status in a short bowel rat model

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Short bowel (SB) syndrome causes the malabsorption of various nutrients. Among them, vitamin A is important for many physiological activities. Vitamin A is taken up by absorptive epithelial cells of the small intestine and discharged into lymphatics as a component of chylomicrons and delivered to the liver. We used a rat model of SB syndrome to assess its effects on the expression of genes associated with the absorption, transport and metabolism of vitamin A. In SB animals, the small bowel was resected from a point five cm distal to the ligament of Treitz to a point ten cm proximal to the ileocecal junction, resulting in a 75% resection of the small intestine. In SB rats, intestinal expression levels of mRNAs for cellular retinol-binding protein II (CRBP II, gene symbol Rbp2) and apolipoprotein A-IV (gene symbol Apoa4) were higher than in shams. In SB rats, the ileal retinol content and the jejunal retinyl esters content were lower than in sham rats. These results suggest that the elevated expression levels of Rbp2 and Apoa4 mRNAs in SB rats contribute to the effective esterification and transport of vitamin A.

(COI: No)

P2-122

A mathematical model of glucose absorption in small intestinal epithelium

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It is generally accepted that glucose absorption in small intestine is mediated by Na^+-dependent glucose cotransporter (SGLT) in the apical membrane and glucose transporter (GLUT) in the basolateral membrane. In the present study, we have tried to construct a mathematical model of glucose transport by small intestinal epithelial cell using MATLAB/Simulink. The apical membrane contained $2Na^+$-1glucose cotransporter (SGLT), Cl^- and HCO_3^- conductances, $1Cl^-$-$1HCO_3^-$ exchanger (AE), and Na^+-H^+ exchanger (NHE). The basolateral membrane contained glucose permeability (GLUT), Na^+-K^+ pump, K^+ conductance, Na^+-K^+-$2Cl^-$ cotransporter, AE, and NHE. Both apical and basolateral membranes have CO_2 and H_2O permeabilities. The paracellular pathway contained Na^+, K^+, and Cl^- conductances. The permeability values of those ion channels/transporters/pump were optimized to reproduce reasonable values for intracellular parameters (pH 7.2, Cl^- 40 mM, Na^+ 10 mM, basolateral membrane potential -60 mV) and the published experimental data of glucose transport rate (Hardin *et al*, Gut. 2000). In the constructed model, when luminal glucose was elevated from 5 to 10 mM (basolateral glucose was kept at 5 mM), intracellular Na^+ increased from 9.7 to 13.7 mM, intracellular glucose increased from 5.01 to 5.04 mM, and basolateral membrane was depolarized from -67.3 to -59.7 mV, while the rate of glucose absorption increased by ~5 times from 2.5 to 12.3 nmol cm^{-2} min^{-1}. The apparent K_m for luminal glucose was 26.9 mM. Our mathematical successfully reproduced glucose absorption in small intestinal epithelium.

(COI: No)

P2-123

Morphological changes in tunica muscularis in adenoma regions of the small intestine in *Apc*$^{Min/+}$ mice, with special reference to the interstitial cells of Cajal

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Apc$^{Min/+}$ mouse, a mouse model for familial adenomatous polyposis, spontaneously develops numerous adenomas in the small intestine. We noted that the thickening of tunica muscularis and the increase of interstitial cells of Cajal (ICC) occurred in the adenoma region of *Apc*$^{Min/+}$ mice. ICC have been widely acknowledged as being essential for the normal function of the digestive tract, acting both as pacemaker cells and as mediators between nerves and smooth muscle cells. The present study has been designed to clarify the morphological changes in tunica muscularis in the small intestinal adenoma regions together with the morphological characteristics of ICC. Male C57BL/6J-*Apc*$^{Min/+}$ mice aged 7 months were used. Short segments including adenoma regions were observed by the immunohistochemistry and electron microscopy. In the adenoma region, the increase in the mass of intercellular substances caused the thickening of the tunica muscularis, resulting in loss of attachments among smooth muscle cells. Dense distribution of ICC was observed to associate with the myenteric plexus (ICC-MP). Cytoplasmic processes of ICC-MP elongated into circular and longitudinal muscle layers. These processes were closely associated with the nerves and showed the gap-junction protein expression. These results may suggest that ICC compensate the lack of connections among smooth muscle cells with their mediator function.

(COI: No)

P2-124

Expression of muscarinic acetylcholine receptors on ICC and fibroblast-like cells of the mouse gastrointestinal tract

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Acetylcholine is the major neurotransmitter that induces gastrointestinal (GI) smooth muscle contractions. Cholinergic innervation is mediated by M2 and M3 muscarinic acetylcholine receptors expressed on the smooth muscle cells. In addition, we have reported that the interstitial cells of Cajal (ICC), regulatory cells of the GI motility, also express M2 receptor. In the musculature of GI tract, another type of interstitial cells called fibroblast-like cells (FLC) exists. Recent studies have suggested that FLC also contribute to mediate the neurotransmission to smooth muscle cells. In the present study, we examined the expression of M2 and M3 receptors on ICC and FLC. We first isolated these cells from the muscle layer of the small intestine by FACS sorting using KIT-GFP (for ICC) and PDGFR α-GFP (for FLC) mice. The ratios of ICC and FLC to total cells of the muscle layer were 1% and 4-5%, respectively. Quantitative RT-PCR analysis showed that M2 and M3 mRNA were highly expressed in isolated ICC and FLC, whereas the expression of M1, M4 and M5 were not detected in both cell types. By immunohistochemistry, M2 receptor immunoreactivity was detected in ICC and FLC. These M2 receptor-immunoreactive cells were associated with cholinergic nerve bundles. In addition, by using analysis of microarray data, isolated ICC and FLC highly expressed inositol transporters related with M2 receptor. These results suggested that FLC as well as ICC expressed muscarinic acetylcholine receptors and were responsible for cholinergic neurotransmission in the muscle layer of GI tract.

(COI: No)

P2-125

Regional differences in the structure of rat intestinal villi

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The structural differences between the rat duodenum, jejunum, and ileum were investigated by scanning electron microscopy (SEM) and light microscopy (LM). Male Wistar rats, eight to thirteen weeks of age, were used. The animals were fixed by perfusion of 4% paraformaldehyde, followed by the removal of the duodenum, jejunum and ileum. Some of these tissues were further immersed in 2% glutaraldehyde and prepared for SEM, while some others were used for LM. The shape of rat intestinal villi was leaf-like, but they tended to be rather thick in the duodenum. The height of villi was about 460 μm in the jejunum and about 290 μm in the ileum. The width was about 70 μm in the jejunum and 50 μm in the ileum. Paneth cells were observed mainly in the ileum. Filamentous microorganisms (presumably bacilli) were often embedded in iliac villi, but not found in the starved rat. Further structural details will be discussed especially on the shape and arrangement of lymphatics and blood vessels.

(COI: No)

P2-126

Tenascin C producing cells in the gastrointestinal tract of adult mice

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Tenascin C (TnC) is an extracellular matrix (ECM) protein that is expressed during embryogenesis, wound healing and tumorigenesis. TnC promotes the de-adhesion of cells to ECM, and up-regulate cell migration and proliferation. On the other hand, little is known about the expression of TnC in healthy adult tissues. We reported the expression of TnC in the gastrointestinal (GI) tract of adult mice at the last annual meeting. We have revealed organ-specific TnC expression in the GI tract. In the present study we identified the TnC producing cells in the normal adult GI tract using TnC-lacZ transgenic mice. TnC producing cells were detected by immunohistochemistry using anti-β-gal antibody. TnC molecules were widely distributed in the extracellular space of mucosa and muscle layer throughout the GI tract. Fibroblast marker and β-Gal double-positive cells were observed in the lamina propria and within the muscle layer. Smooth muscle cells of the lamina muscularis mucosae in stomach, and longitudinal muscle layers in stomach and colon show dense β-Gal immunoreactivities (IR). In addition, moderate β-Gal IR were observed in smooth muscle cells in circular muscle layers throughout the GI tract. No co-localization of β-Gal and neural cell marker was observed. Some KIT immunopositive ICC within the myenteric plexus region seemed to possess β-Gal IR. In conclusion, fibroblast, smooth muscle and ICC produce TnC. TnC can effects the cell adhesion property of the connective tissue, so they may produce organ-specific micro-environment in the GI tract.

(COI: No)

P2-127

Studies on neurogenesis of enteric neurons in *c-kit* mutant mouse after benzalkonium chloride-induced neuron injury

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Interstitial cells of Cajal (ICC) are mesenchymal cells localized along the gastrointestinal tract and they have close interactions with enteric nervous system (ENS) both morphologically and functionally. To reveal an implication of ICC in ENS regeneration, we used ileum of *c-kit* mutant mice (WBB6F1/Kit-*Kit*$^{W/Kit^{W-v}}$/Slc: *W/W*^{v}), which are known as ICC deficient mice. After ENS injury by benzalkonium chloride (BAC), the neurons in myenteric plexus (MP) were disappeared both in wild type (C57BL/6NcrSlc: WT) and *W/W*^{v} mutant, however other structures such as mucosal epithelium and smooth muscle remained as normal. Two weeks after the injury, the recovery of MP was not observed in neither WT nor *W/W*^{v}, however the elongation of nerve fibers along the longitudinal muscle layer were detected in both animals. In addition, especially in *W/W*^{v} mice, ectopic NADPH positive cells were observed in the longitudinal muscle layer or the subserosal layer, where usually enteric neurons were not observed. These cells were also labeled by PGP9.5 antibody, and therefore these were considered as neurons. Although neurogenesis is hardly observed under normal condition of wild type intestine, the apparent ENS neurogenesis is seen in ileum of *W/W*^{v}. These results may suggest that a deletion of ICC or some factors associated with *c-kit* are contributed to the ENS neurogenesis after injury.

(COI: No)

P2-128

Immunohistochemical study of a Membrane Skeletal Protein, Membrane Protein Palmitoylated 6 (MPP6), in Mouse Small Intestine

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Membrane protein palmitoylated 1 (MPP1) is a membrane skeletal protein that interacts with a 4.1 family protein, 4.1R, in erythrocytes. We already identified another MPP family MPP6 interaction with 4.1G in the mouse peripheral nervous system. In this study, we examined immunolocalizations of MPP6 in mouse small intestines, and compared them with those of 4.1B that we had already reported in intestinal epithelial cells. Cryosections or paraffin sections of small intestines of wild-type or 4.1B-deficient mice were immunostained for MPP6, 4.1B, and E-cadherin. In the small intestines, molecular weight of MPP6 was about 60kD with Western blot, and it was immunostained at lateral portions of all epithelial cells from crypts to intestinal villi, as well as in Auerbach's plexus probably reflecting enteric glia. In the epithelial cells, immunostained areas of MPP6 were slightly different from those of 4.1B whose immunolocalization was restricted in the intestinal villi. The immunostaining pattern of E-cadherin in epithelial cells was similar to that of MPP6. The MPP6 immunolocalization in small intestinal epithelial cells of the 4.1B-deficient mouse was similar to that of the wild-type mouse. Thus, we demonstrated the MPP6 immunolocalizations in mouse small intestines, suggesting that 4.1B in the intestinal epithelial cells was not essential for the MPP6 sorting.

(COI: No)

P2-129

Visualization of the entire differentiation process of murine M cells: suppression of their maturation in cecal patches

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The microfold (M) cell residing in the follicle-associated epithelium (FAE) is a specialized epithelial cell that initiates mucosal immune responses by sampling luminal antigens. The differentiation process of M cells remains unclear due to limitations of analytical methods. Here we found that M cells were classified into two functionally different subtypes based on the expression of Glycoprotein 2 (GP2) by newly developed image cytometric analysis. GP2-high M cells actively took up luminal microbeads whereas GP2-negative or low cells scarcely ingested them, even though both subsets equally expressed the other M-cell signature genes, suggesting that GP2-high M cells represent functionally mature M cells. Further, the GP2-high mature M cells were abundant in Peyer's patch but sparse in the cecal patch: this was most likely due to a decrease in the nuclear translocation of RelB, a downstream transcription factor for the receptor activator of NF-κB signaling. Given that murine cecum contains a protrusion of beneficial commensals, the restriction of M-cell activity might contribute to preventing the onset of any excessive immune response to the commensals through decelerating the M-cell-dependent uptake of microorganisms.

(COI: No)

P2-130

Short-chain fatty acid activates bicarbonate absorption or proton secretion in rat rectal colon

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Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate are synthesized from dietary carbohydrate by colonic bacteria fermentation. These SCFAs are considered to contribute not only to an energy source or prevention of cancer but also in regulating ion transport. However, the SCFA's effects on ion transport and intracellular environment remain unknown. In this study, I showed the effects of a 30 mM butyrate application on rat colon with short-circuit current (I_{SC}) measurements. Butyrate application shifted I_{SC} toward negative direction. Because electroneutral monocarboxylate transporters (MCT1 and/or MCT4) co-transports proton with SCFAs including butyrate, it could be considered that this I_{SC} shift was occurred by electrogenic HCO_3^- absorption or H^+ secretion in order to neutralize intracellular environment. In rat colon, there are some channels or transporters which concern with HCO_3^- transport, but only cAMP-activated Cl^- channel, cystic fibrosis transmembrane conductance regulator (CFTR), matched to the direction both HCO_3^- transport and I_{SC} shift. In fact, CFTR inhibitor, CFTRinh172 reduced this I_{SC} shift induced by butyrate application. On the other hand, H^+ secretion on apical membrane also matched this I_{SC} shift to neutralize intracellular pH which was contributed by H^+-ATPase and/or H^+-K^+-ATPase in cooperation with K^+ channel. The aim of this study was to reveal those channel and/or transporters contribution on the condition of SCFA application.

(COI: No)

P2-131

Secretory effects of Xenin on rat colonic epithelia

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Xenin-25 (Xen) is a 25 amino acid neurotensin-related peptide produced by a GIP-producing K cells in the small intestine. In animals, Xen delays gastric emptying, increases gastric motility induces gall bladder contractions and reduces food intake. Many of these effects are known to be mediated by enteric neurons. Xen has multiple actions on gastrointestinal activity, however, there has been no report on the ion transport in the gut. In this study, we have investigated the effect of Xen on ion transport in rat colon. Xen was synthesized by a solid-phase methodology with Fmoc-strategy using an automated peptide synthesizer (Model Pioneer; Life Technologies, CA, USA). The crude peptide was purified by reverse-phase HPLC (Delta 600 HPLC system; Waters, MA, USA). The homogeneity of the purified peptide was confirmed by analytical HPLC, MALDI-TOF mass spectrometry, and amino acid analysis. Smooth muscle removed mucosa-submucosa preparations of rat large intestine were mounted on Ussing flux chambers and short-circuit current (Isc) was measured as an index of transepithelial ion transport. In addition, expression of Xen in the colon was analyzed by PCR. In Ussing chamber experiments, serosal application of Xen (10-9 ~ 10-6 M) concentration-dependently induced a transient increase in Isc in middle colon, distal colon and rectum but not in proximal colon. These results suggest that Xen functions as a mediator on the ion transport in the rat colon.

(COI: No)

P2-132

Kinase activity of TRPM7 regulates lipid metabolism

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Transient receptor potential melastatin 7(TRPM7) is a member of TRP family of cation channels involved in sensory pathways and respond to various environmental stimuli. TRPM7 is a unique fusion of an ion channel and a C-terminus kinase domain. However, the physiological functions of TRPM7 and its kinase activity in vivo remain largely unclear. We generated kinase-inactive mutant mice and analyzed their phenotype. TRPM7 mutant mice show normal ion channel activity without noticeable kinase function in cells isolated from adult animals. These mice have normal body weight, food intake and general locomotor activity. Screening of serum clinical parameters showed that serum Ca^{2+} and Mg^{2+} levels were not altered, but serum triglyceride and total cholesterol were significantly decreased. High-fat diet increased the accumulation of fat in the liver compared to wild type mice. Our findings define TRPM7 kinase activity as a key cell signaling component that regulates lipid metabolism in the liver.

(COI: No)

P2-133

Silencing of Delta-like3 expression by DNA methylation and histone modification in hepatocellular carcinoma cells

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Introduction: Development and progression of hepatocellular carcinoma is caused by a multistep mechanism. Activation of oncogenes and inactivation of tumor suppressor genes by genetic or epigenetic aberrance are involved in hepatocarcinogenesis. We previously reported that Delta-like 3(DLL3)gene, a member of DSL ligands for Notch receptor is aberrantly methylated and DLL3 expression induces cellular apoptosis in HCC cell line, HuH2. (Aim) The aim of this study is to investigate the epigenetic mechanism of DLL3 silencing in HCC.
Materials and Methods: 1. Immunohistochemical study of DLL3 in HCC tissues. 2. Immunohistochemical study of methylated histone H3 lysine 27(H3K27me3) in HCC tissues. 3. Reactivation assay of DLL3 expression with DNA methylation inhibitor (5-Aza-dC), histone deacethylase inhibitor (TSA), and histone methyltransferase inhibitor (DZNep) in HuH2 cells.
Results: 1. DLL3 expression is not observed in 50% (18/36) of the HCC cases whereas DLL3 is expressed in all (9/9) corresponding non-cancerous tissues. 2. H3K27me3 is observed in 60% of the HCC cases and 80% of DLL3-negative HCC cases. 3. DLL3 expression is reactivated by the treatment of 5-Aza-dC and TSA, whereas no effect is observed by the treatment of DZNep.
Discussion: DLL3 expression in regulated by DNA methylation and may influence hapatocarcinogenesis. However H3K27me3 did not affect DLL3 silencing, further experiments are now undergoing concerning the involvement of other histone modifications.
(COI: No)

P2-134

Effect of starvation on the expression of a lipid droplet protein, ADRP, in the mouse liver

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Adipose differentiation-related protein (ADRP) is a major protein associated with lipid droplet in various types of cells. In this study, we analyzed the effect of starvation on the expression of ADRP in the mouse liver. Adult male ddY mice were starved for 24 hours, and then the livers were collected for histological analysis. When the mRNA expression of ADRP was analyzed by *in situ* hybridization technique, it was significantly increased in the liver of the starved mice. Abundant number of lipid droplets was observed in the cytoplasm of hepatocytes in the starved mice, and an intense immunoreactivity for ADRP was found in the membrane of increased lipid droplets. When lipids contents were stained using BODIPY 493/503 or Sudan III on ADRP-immunostained sections, the lipid droplets surrounded by ADRP immunoreaction tended to be negative in reaction for BODIPY or Sudan III, in contrast to lipid droplets without ADRP immunoreactivity. Immuno-electron microscopic observation revealed the specific localization of ADRP on the edges of vacuoles which were not filled with lipid contents. Because the mRNA expression of adipose triglyceride lipase (ATGL), a late-limiting lipase, was significantly increased in the liver of starved mice, the ADRP-positive lipid droplets may be under a lipolytic condition.
(COI: No)

P2-135

Fibroblast growth factor-5 participates in the progression of hepatic fibrosis

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Non-alcoholic steatohepatitis (NASH) is characterized by the presence of steatosis, inflammation, and fibrosis and is believed to develop via a "two-hit process"; however, its pathophysiology remains unclear. Fibroblast growth factors (FGFs) are heparin-binding polypeptides with diverse biological activities in many developmental and metabolic processes. In particular, FGF5 is associated with high blood pressure. We investigated the function of FGF5 in vivo using spontaneously Fgf5 null mice and explored the role of diet in the development of NASH. Mice fed a high-fat diet gained little weight and had higher serum alanine transaminase, aspartate amino transferase, and non-high-density lipoprotein-cholesterol levels. Liver histology indicated marked inflammation, focal necrosis, fat deposition, and fibrosis, similar to the characteristics of NASH. FGF5 and a high-fat diet play significant roles in the pathophysiology of hepatic fibrosis and Fgf5 null mice may provide a suitable model for liver fibrosis or NASH.
(COI: No)

P2-136

Immunohistochemical localization of CD133, nestin, Bmi-1 and mTOR in the pancreas of rat models of type 1 and type 2 diabetes mellitus

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Stem cell-related markers (CD133, nestin and Bmi-1) and mammalian target of rapamycin (mTOR) were detected in the pancreas of rat models of type 1 and type 2 diabetes mellitus (DM). As a model of type 1 DM, Komeda diabetes-prone (KDP) rat and control rat (KND rat) were used. As a model of type 2 DM, spontaneously diabetic Torii (SDT) rat and control rat (SD rat) were used. In each control rat, pancreatic islets were strongly positive for CD133, nestin, Bmi-1 and mTOR. In the KDP rat, CD133, nestin, Bmi-1and mTOR positive cells did not show cellular mass like pancreatic islets. Theses positive cells were associated with invasion by mononuclear cells. In the SDT rat, CD133, nestin, Bmi-1 and mTOR positive cells also did not show cellular mass like pancreatic islets. These positive cells were associated with increased fibrillar element. The number of nestin positive cell was decreased. Present study suggests that nestin may play an important role in the maintenance of pancreatic islet, especially in the SDT rat.

Reference:
Murata E, Matsumoto S, Shuto M and Akita M. J Stem Cells Res, Rev & Rep. 2014; 1(1):6

(COI: No)

P2-137

Inhibitory effects of Saiko-keishi-to (TJ-10) on pancreatitis-reduced pain in a rat nodel of chronic pancreatitis

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Abdominal pain is one of the most important symptoms in chronic pancreatitis, presenting in 80-90% patients during the course of the disease. The aim of the present study was to investigate the role and underlying mechanisms of Saiko-keishi-to (TJ-10) in a rat model of chronic pancreatitis. In the present study, male Lew rats (150-160 g) were employed as induced-chronic pancreatitis (CP) model by injection of dibutyltin dichloride (DBTC) (8 mg/kg BW) into the tail vein. TJ-10 was given daily by mixed in feed at a dose of 10 g/kg of body weight, starting from two weeks after CP induction. The behavioral testing of mechanical response was tested using von Frey filaments. After treatment of TJ-10, rats were sacrificed, and pancreatic tissues, dorsal root ganglia (DRG) and thoracic spinal cord (T9-12) were harvested for investigating the expression of fibrosis/pain-related factors. Treatment of CP with TJ-10 decreased the histological lesion, and reduced the expression of TGF-β1, Smad2 and Smad3, improved the fibrosis and pancreatitis-induced pain. The $\alpha 2\delta$-1 precession of T9-12 in rats with chronic pancreatitis was declined. The present study suggested that repeated administration of TJ-10 daily could reduce mechanical hypersensitivity in the upper abdomen and produce an analgesic effect in a rat model of chronic pancreatitis. The down-regulation of $\alpha 2\delta$-1 calcium channel subunit might be one of the mechanisms underlying the analgesic effect of TJ-10.
(COI: No)

P2-138

Deletion of the tight junction protein claudin 15 causes malabsorption of ologopeptide in murine intestine

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It is known that the claudin family of tight junction proteins is critical in determining paracellular ionic permeability and selectivity. We have shown that loss of claudin 15 results in decreased luminal Na+ concentration and glucose malabsorption in the small intestine. To gain further insight into the relationship between intestinal Na+ metabolism and changes in peptide absorption induced by the loss of claudin 15, we investigated the site of absorption of electrolytes and peptide in claudin 15 knockout (cldn15KO) mice. Mice were fed a powdered diet supplemented with 14C-polyethylene glycol (PEG) 4000 as a non-absorbable marker and 3H-Gly-Sar (non-hydrolyzable dipeptide). Three hours after feeding, the small intestine was isolated and divided into six segments, the luminal contents collected for analysis of Na+, K+, and Cl- concentrations and the level of 14C-PEG4000. Na+, K+, and Cl- concentrations were determined using ion-selective electrodes. Gastric emptying time, assessed by measuring 14C-PEG4000, was decreased in cldn15KO compared to wild-type mice. Total luminal contents in the small intestine were increased in cldn15KO mice and the retention time of digesta in the upper jejunum was increased approximately 3-fold compared with wild-type mice. Robust Na+ secretion and rate of absorption were observed in the upper jejunum in wild-type mice and this was attenuated in cldn15KO mice. The rate of K+ absorption was increased in cldn15KO mice in the lower ileum. Total luminal Gly-Sar were increased, while absorption rates of Gly-Sar were decreased, in the upper jejunum of cldn15KO mice.
(COI: No)

P2-139

Forensic estimation of age at death using synchrotron-radiation micro-CT of human teeth

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Teeth are the hardest parts of the human body and they survive well under adverse conditions and are the basis for several methods for age-at-death estimation. One established method for the estimation of a person's age is based on the observation that dentinal tubules gradually fill with mineral (secondary dentine) over time. This infilling begins at the apex of the tooth root and proceeds towards the crown. The method of age estimation depends upon being able to establish a relationship between the level of secondary dentine formation and the chronological age of an individual. Synchrotron radiation micro-CT offers the possibility of low noise imaging without any beam hardening and has the potential to overcome the problems. In this study, we acquired micro-CT data from 50 single-rooted human teeth from individuals of known ages, detected the limit of secondary dentine formation and calculate fractional change in the volume of the tooth root occupied by this. For consistency of the image analysis, 17mandibular incisors(12 males and 5 females of 40-79 years old) without caries or other abnormalities were used. The ratio between pulp cavity and dentinal volume decreased with increasing age. The coefficient of determination by multiple regression analysis for 17 specimens was 0.459. Age estimation based one age-related changes in pulp cavity to dentinal volume ratio as well as three-dimentional reconstruction of tooth structure has been successful using synchrotoron- radiation micro-CT.

(COI: No)

P2-140

Regulatory mechanism in differentiation of mesenchymal cells during tooth development

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Objective: While previous studies have suggested some signaling molecules involved in tooth development, but mechanism of differentiation of mesenchymal cells remains unclear in detail. Here, we investigated the expression patterns of some signaling molecules which may be involving in formation of blood vessels during tooth development.
Method: Immunohistochemically we stained serial sections of dental tissues with the antibody against the molecules may be involving in formation of blood vessels. And also we performed in situ hybridization by using the probes of above-mentioned molecules.
Result: These molecules were observed in the mesenchymal layers, dental papilla and around the blood vessels in tooth germ. And also the localization of transcripts of these molecules were observed in mesenchymal cells in tooth germ.
Conclusion: In this research, we confirmed the molecules might have involved in formation of blood vessels during tooth development. *This work was supported by JSPS KAKENHI Grant Numbers 22592052, 26462800.

(COI: No)

P2-141

Immunohistochemical Localization of Bmi1 during odontoblast differentiation and regeneration

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Bmi1 is a polycomb protein localized in stem cells and regulates expression of differentiation genes. In this study, to analyze the role of Bmi1 during dentinogenesis, we examined the immunohistochemical localization of Bmi1 during rat tooth development as well as after cavity preparation. Bmi1 localization was hardly detected in dental mesenchyme at the bud and cap stages. After the bell stage, this protein became detectable, being localized in odontoblasts just beginning dentin matrix secretion and in preodontoblasts near these odontoblasts. As dentin formation progressed, Bmi1 immunoreactivity in the odontoblasts decreased in intensity. After cavity preparation, cells lining the dentin and some pulp cells were immunopositive for Bmi1 at 4 days. Odontoblast-like cells forming reparative dentin were immunopositive at 1 week, whereas this immunoreactivity disappeared after 8 weeks. Next, we further analyzed the function of Bmi1 using dental pulp cells in vitro. Following stimulation with BMP-2, Bmi1 expression was elevated. siRNA knockdown of Bmi1 in these cells reduced the expression of odonto- and osteo-blast differentiation marker genes such as Runx2, Osterix, and Osteocalcin. Taken together, these findings suggest that Bmi1 was localized to the odontoblast lineage cells in their early differentiation stages, and might positively regulate their differentiation.

(COI: No)

P2-142

IFT88 plays a role in the ciliogenesis even during mitosis

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IFT88 is known to be required for the ciliogenesis in most of the quiescent cells. We have previously reported that IFT88 regulate the odontoblastic differentiation through primary cilia. Moreover, conventional idea holds that IFT88 chiefly function in mitogenic events during mitosis. However, since we revealed that IFT88 in preodontobastic KN-3 cells functions on the ciliogenesis even during mitosis, we report here. When KN-3 cells were transfected with a retroviral expression vector for *Ift88* shRNA, cell adhesion, formation of lamellipodia, and cell proliferation were impaired. In addition, *Ift88* knockdown decreased the expression of molecular marker for mitosis. In this case, we reproduced the phenotype by inhibiting the mitosis, and obtained a similar phenotype to those inhibited by *Ift88* knockdown. This result suggested that IFT88 take part in the regulation of cell cycle. From another standpoint, while the ratio of cilia was not changed, the intensity of acetylated α-tubulin-positive protruding structures was decreased by *Ift88* knockdown even during mitosis. Moreover, the non-canonical Wnt signal, which is mediated by primary cilia, was suppressed. To recapitulate the phenomena, we inhibited the non-canonical Wnt signal by an inhibitor, and obtained a similar phenotype to those inhibited by *Ift88* knockdown. This result suggested that IFT88 functions on the ciliogenesis even during mitosis. Collectively, IFT88 exerts its effects on mitotic profiles of KN-3 cells not only through extraciliary pathway but also through ciliary pathway.

(COI: No)

P2-143

Effects of the thyroid hormone on tooth development in newts

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Objective: It has been suggested that the thyroid hormone receptor and thyroid hormone are contribute to the development of the teeth and the regeneration of alveolar bone following amputation in newts. However, the relationshipbetween the mRNA transcript levels of THRs in alveolar bone is unknown in blocking the effect of thyroid hormone.
Method: Propylthiouracil (PTU) is a thiouracil-derived drug used to blocking the effect of thyroid hormone. Japanese newts (*Cynops pyrrhogaster*) were maintained in aqueous solutions with or without 100 μg/ml PTU for one month and tracked the sequential development of the tooth cap, the tooth bud, and ultimately the maturation of the sequential tooth germ. Fifty days after the amputation procedure of the right mandible alveolar bone, the newts were observed the effect of PTU by the expression levels of mRNA of thyroid hormone receptor detected in-situ hybridization and real time RT-PCR.
Result: The amputated mandible sites were observed microscopically. The level of mineralized area of alveolar bone in amputation with PTU treatment was significantly smaller than that in the amputation without PTU treatment (Student's t-test, $p<0.001$).
Conclusion: These data indicate that PTU may affect the tooth germ development and the regeneration of alveolar bone in newts.

(COI: No)

P2-144

Histology and elemental composition of the cervical enamel in human unworn mesiodenses

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Objectives: The purpose of this study is to clear the histological structure and elemental composition of the cervical enamel in human unworn mesiodenses.
Methods: Re-ground surfaces, slightly inclining to the enamel surface, of the cervical part of the mesiodense was prepared, etched with HCl and examined under the scanning electron microscope. The contents of seven elements (mass %) were analyzed quantitatively with an electron probe microanalyzer.
Results: The width of the rod sections at the cervical enamel was larger than that at the incisal edge enamel. The phosphorus, carbon and magnecium contents at the cervical and incisal edge enamels of the mesiodense were higher than those of the canine, while the calcium, oxygen and sodium contents of the mesiodense were lower than those of the canine.
Discussion: It is thought that the cervical enamel of the mesiodense is more easily decayed by dental caries than the incisal edge enamel. It is considered that the cervical enamel of the mesiodense is low calcified than that of the canine. It is thought that more calcium in other condition from hydroxyapatite exists in the cervical enamel of the mesiodense than that in the canine.
Conclusion: The difference was recognized in the histological structure and elemental composition of the cervical enamels among the mesiodense, canine, premolar and molar.

(COI: No)

P2-145

Abnormal enamel formation in thermosensitive TRPV channels knockout mice

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Dental enamel is the hardest tissue in the body and secreted by a specialized ameloblast. Although a great deal of progress in the knowledge of enamel formation has been made, the developmental step with gradual physical hardening remains unclear. Thermosensitive transient receptor potential vanilloid 3 and 4 (TRPV3, TRPV4) were known as non-selected Ca^{2+} permeable ion channels which are activated by warm temperatures. We hypothesized that TRPV3 and TRPV4 channels contribute to the development of ameloblast and calcification of dental enamel, and investigated the influence of TRPV3, TRPV4 on enamel formation in the wild (WT), TRPV3 and TRPV4 knockout (V3KO, V4KO) mouse. We immunohistochemically investigated tooth germs in postnatal day 5 and six-week-old-incisors and molars, using TRPV3 and TRPV4-specific antibodies. We found conspicuous TRPV3 and TRPV4-immunoreactivity in ameloblast layers in the WT tooth germs. There was no significant difference in tooth outlook, size and thickness of enamel between WT and V3KO, V4KO. Under the observation of SEM, we found immature development of the enamel prism and interrod substances in the incisors and molars from V3KO, V4KO mice compared with WT. These observations suggested that TRPV3, TRPV4 are localized in the ameloblast layer of tooth germs, and may affect enamel formation.

(COI: No)

P2-146

Histological and analytical studies on the role of melatonin in the structure and composition of teeth dentin

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The purpose of the present study is to examine the relationship between the structure and composition of teeth dentin and the role of melatonin through the histological and analytics studies. In this experiment, 5-, 6-, and 7-day old SD rats were used. These rats were divided into three groups: 1) a control group; 2) a low-concentration group; and 3) a high-concentration group. In the control group, two dark-staining incremental lines of hematoxylin and one light-staining layer were observed in incisor dentin. In the high-melatonin concentration group, this layer disappeared. The number and size of calcospherites in predentin increased in proportion to the concentration of melatonin administered. The new incremental line was confirmed in the incisor predentin and molar dentin of the melatonin treated groups. Ca and P content were increased in the melatonin treated group. It is considered that melatonin participates in the formation of incremental lines and the calcification mechanism of dentin.

(COI: No)

P2-147

Intercellular Odontoblast Networks via Extracellular Glutamate

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Various stimuli to odontoblasts induce sharp pain. Transient receptor potential (TRP) channels in odontoblast receive these stimuli, which induce the release of ATP to nearby odontoblasts and trigeminal ganglion (TG) neurons and establish intercellular signaling. Recently, it has been shown that odontoblasts express metabotropic glutamate receptor subtype 5. This implies that odontoblasts are capable of receiving extracellular glutamate. However, it remains unclear whether cells, such as neurons and/or odontoblasts themselves, release glutamate in dental pulp. We thus examined intercellular odontoblast-odontoblast and odontoblast-TG neuron signal transduction via glutamate. During mechanical stimulation of an odontoblast, not only the stimulated cell but also the nearby odontoblasts and TG neurons showed increases in intercellular free Ca^{2+} concentration ($[Ca^{2+}]_i$). After application of a cocktail of GluR antagonists, the responses in nearby odontoblasts and TG neurons were suppressed. When we applied each agonist for GluR subtypes to the odontoblasts, $[Ca^{2+}]_i$ increased. These results suggest that a mechanically stimulated odontoblast is capable of releasing glutamate, which activates synchronous intercellular Ca^{2+} signaling via GluR on the cluster of adjacent odontoblasts in an autocrine/paracrine manner. The released glutamate also activates TG neurons in dental pulp as a neurotransmitter. We propose that these inter-odontoblasts and odontoblast-TG neuron networks drive odontoblastic functions such as reactive dentin formation and/or augmentations in sensory signaling.

(COI: No)

P2-148

Ca^{2+} signaling activated by alkaline environment in rat odontoblasts

Kimura, Maki; Sato, Masaki; Kojima, Yuki; Higashikawa, Asuka; Nishiyama, Akihiro; Satou, Ryoichi; Shiozaki, Yuuta; Shimada, Miyuki; Ogura, Kazuhiro; Mochizuki, Hiroyuki; Shibukawa, Yoshiyuki; Tazaki, Masakazu (*Dept Physiol, Tokyo Dent Coll, Tokyo, Japan*)

Alkaline environment provided by calcium hydroxide, which is used for endodontic treatment, induces reparative dentinogenesis, however its precise mechanisms remain unclear. We examined intracellular Ca^{2+} signaling pathway induced by alkaline environment in rat odontoblasts. In dentin sialoprotein-positive acutely isolated odontoblasts, intracellular free calcium concentration ($[Ca^{2+}]_i$) was measured by fura-2 fluorescence. In the presence (2.5 mM) and absence (0 mM) of extracellular Ca^{2+}, application of alkaline solution prepared by adding NaOH increased $[Ca^{2+}]_i$, showing a dependence of $[Ca^{2+}]_i$ on extracellular pH (pH 8.5-10.5). In the presence of extracellular Ca^{2+}, $[Ca^{2+}]_i$ increase induced by the alkaline solution were greater than in the absence of extracellular Ca^{2+}. Alkaline solution-induced $[Ca^{2+}]_i$ increases were dependent on the extracellular Ca^{2+} concentration. Repeated applications of alkaline solution did not induce a desensitizing effect on the increase. In the presence of extracellular Ca^{2+}, $[Ca^{2+}]_i$ increases evoked by the alkaline solution (pH 10) were inhibited by HC030031, a specific antagonist of transient receptor potential ankyrin subfamily member 1 (TRPA1) channels. These results indicate that alkaline stimuli activate Ca^{2+} mobilizations via TRPA1 channels and intracellular Ca^{2+} release in odontoblasts, suggesting that alkali-sensing mechanisms in odontoblasts may play an important role in driving dentinogenesis induced by calcium hydroxide.

(COI: No)

P2-149

Voltage-dependent calcium influx pathway in rat odontoblasts

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Odontoblasts play a role in the sensory signal transduction involved in generating dentinal pain. We have previously reported that depolarizing-stimuli induce $[Ca^{2+}]_i$ increases; however, voltage-dependent Ca^{2+} current activity was not observed. Thus, the voltage-dependent Ca^{2+} influx pathway in odontoblasts is still unclear. Recently, calcium homeostasis modulator (CALHM1), which is able to carry Ca^{2+} and ATP voltage-dependently, has been found. In the present study, we hypothesized that CALHM1 contributes to the voltage-dependent Ca^{2+} influx and aimed to clarify its functional expression in odontoblasts. In acutely isolated odontoblasts, the application of high-K^+ extracellular solution elicited transient $[Ca^{2+}]_i$ increases in the presence of extracellular Ca^{2+}, but not in the absence of extracellular Ca^{2+}. The high-K^+-induced $[Ca^{2+}]_i$ increase showed dependence on extracellular Ca^{2+}. Application of Gd^{3+}, but not Ni^{2+}, inhibited the depolarization induced-increase in $[Ca^{2+}]_i$. The results indicated that membrane depolarization activates the Gd^{3+}-sensitive voltage-dependent transmembrane Ca^{2+} influx in odontoblasts, which was not mediated by Ni^{2+}-sensitive-T/R type voltage-dependent Ca^{2+} channels. The results imply that the voltage-dependent Ca^{2+} influx in odontoblasts might be mediated by Gd^{3+}-sensitive CALHM1, which may play an important role in the generation of receptor potential, elicited by the sensory transduction sequence during dentinal pain.

(COI: No)

P2-150

Involvement of The TRPV4 Channel in gingival epithelia barrier function

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Transient receptor potential vanilloid 4 (TRPV4), a nonselective cation channel, is reported to play a skin barrier function via direct interaction with beta-catenin, a component of cell-cell adhesion complex. We found TRPV4 expression in oral epithelia using RT-PCR analysis. Under immunohistochemical observation, conspicuous TRPV4 immunoreactions are found in junctional epithelium, which makes direct contact with tooth enamel. Junctional epithelium, surrounding tooth like an epithelial collar, supports epithelial attachment to the tooth, and at the same time, it is the location of periodontal inflammation. We found E-cadherin and beta-catenin with prominent actin rings in junctional epithelium in WT mice. However, the staining intensity was weak in TRPV4 knockout (TRPV4KO) mice. To explore whether TRPV4 affect periodontal disease, we investigated in the mice with ligature placement by sterile silk sutures. Under micro CT analyses, the decrease in bone volume in TRPV4KO mice with ligatures was greater than that of WT mice. Based on these results, TRPV4 is suggested to contribute to the epithelial barrier in tooth-epithelial junction and affects periodontal disease progression.

(COI: No)

P2-151

TRPV3 channel contributes to rapid wound healing in oral epithelia via EGFR signaling

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The oral cavity provides an entrance to the alimentary tract and serves as a protective barrier against a drastic variation of stimuli compared with other tissues. Oral mucosa is susceptible to injury, but it shows faster wound healing than the skin and less scar formation. However, the molecular pathways that regulate this wound healing are still unclear. Here we show that transient receptor potential vanilloid 3 (TRPV3), a thermosensitive Ca^{2+}-permeable channel activated by warm temperatures (>33℃), is functionally expressed in oral epithelia. We found delayed closure of wounds after tooth extraction in TRPV3-deficient (TRPV3KO) mice compared with that in wild-type (WT) mice. We also found that TRPV3 activation increased the number of proliferating cells and EGFR phosphorylation in primary cultured oral epithelial cells from WT, but they were not found in the cells from TRPV3KO. Additionally, the number of proliferating cells and phosphorylated EGFR expression in oral epithelia were also markedly reduced in TRPV3KO mice. These results suggest TRPV3 in oral epithelia promotes the proliferation of oral epithelial cells and contributes to rapid wound repair via EGFR phosphorylation.

(COI: No)

P2-152

Merkel cells transduce mechanical stimuli and release neurotransmitters

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Merkel cells (MCs) are thought to form a part of the MC-neurite complex with sensory neurons. However, the mechanism of neurotransmission between the MCs and nerve endings in this complex has not been clarified yet. We therefore prepared co-cultures of MCs and trigeminal ganglion (TG) neurons and recorded intracellular free Ca2+ concentrations ([Ca2+]i) in response to direct mechanical stimulation of the MCs. TG cells were isolated from 5-day-old Wistar rats and cultured in L-15 medium. MCs were acutely isolated from golden hamster (3-5 weeks old) buccal mucosa following intraperitoneal injections of quinacrine 24 h prior to isolation. These were added to the culture dish of TG cells. We identified quinacrine fluorescence-positive cells as MCs. Fura2 fluorescence was used to measure [Ca2+]i. Application of direct mechanical stimuli using a glass micropipette caused an increase in [Ca2+]i, which was dependent on the intensity of the mechanical stimuli and sensitive to antagonists of transient receptor potential (TRP) vaniloid subfamily member (V) 1, TRPV2, TRPV4, and TRP ankyrin subfamily member (A) 1 channels. In the co-culture system of TG cells and MCs, direct mechanical stimulation of the latter induced [Ca2+]i increases not only in the stimulated MC, but also in TG neurons. These results indicated that mechanical stimulation of MCs activates the TRPV1, TRPV2, TRPV4, and TRPA1 channels; sensory signals are then transmitted to the neurons through diffuse chemical substance(s).

(COI: No)

P2-153

Morphological and molecular characterization of microfold cells in nasopharynx-associated lymphoid tissue

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Mouse nasopharynx-associated lymphoid tissue (NALT) located at the base of the nasal cavity is a site for induction of mucosal immune responses against airway antigens. The follicle-associated epithelium (FAE) covering the luminal surface of NALT is characterized by the presence of microfold cells (M cells). M cell is a specialized epithelial cell that delivers luminal antigens to lymphocytes underneath epithelium. Although recent studies are uncovering the molecular aspects of M cell in intestinal Peyer's patch, little is known about NALT M cell. Here, we show that NALT M cells express glycoprotein 2 (GP2), M-Sec, Spi-B and Ccl9, which are fundamental molecules of Peyer's patch M cell. Receptor activator of nuclear factor kappa-B ligand (RANKL) is a strong inducer of M cells in the intestine. We found that RANKL is expressed by stroma cells underneath FAE of NALT, and administration of RANKL increased the number of NALT M cell, suggesting that RANKL regulates the differentiation of NALT M cell. Mouse NALT is considered as an equivalent of human Waldeyer's ring. Our research will contribute to understanding mucosal immune responses in nasopharynx.

(COI: No)

P2-154

Morphological approach for a paracellular fluid transport in salivary glands

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During muscarinic stimulation, the fluid secretion via the paracellular route dominants the whole fluid secretion. We applied multi-directional TEM observation for 3D reconstruction on freeze fracture replica, and quick 3D reconstruction of CLSM images. Using sulfo-rhodamine B or Lucifer Yellow in the perfusate, we observed the intercellular space via the fluorescence and the intercellular canaliculli as much less fluorescence. This system allowed us to obtain 30 sliced images every 2 s and produced 3D reconstructed images (5 Live, Zeiss).

Results: 1) Carbachol/isoproterenol stimulation opened the paracellular passage of Lucifer Yellow. 2) Simultaneously, the cytoskeleton lattice beneath the tight junction became narrower than that during control perfusion. 3) The 3D movie showed the increase in cell surface movement during carbachol stimulation. 4) During stimulation with carbachol, the cell volume decreased, shrinking the cytoskeleton fiber intervals, suggesting an increase in the cytoskeleton movement, and thus the strand particles (claudine) of the tight junction could increase the movement to allow paracellular movement of water and solutes. The present findings suggest that a part of the paracellular transport could be driven by plasma membrane vibration near the intercellular canaliculli. Acknowledgement: JSPS KAKENHI (23590271, 26460308).

(COI: No)

P2-155

Subcellular localization and functional implication of V-ATPase in ductal cells of mouse salivary glands

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V-ATPase, which is composed of V_1 (A-H) domain and V_o (a, c, c', c'') domain, is known to be localized in intracellular membranes of organelles of cells or in their cell membranes to acidify the outside. We previously reported that the B2 subunit isoform of V-ATPase was localized in the ductal cells of the mouse major salivary glands. In this study, we have further studied subcellular localization of V-ATPases and demonstrated that immunofluorecence for a1 subunit tend to be localized in the apical region, and that B2 subunit isoform was found in the apical and the basal regions of parotid ductal cells, and that immuno- transmission electron microscopy for V-ATPase was proved to be localized in the apical, lateral, and basal membranes and basal infoldings of striated duct. Phenotypes analysis of the knockout mice of the a3 subunit isoform (a3-KO mice) revealed that the size of salivary glands and the amount of saliva secretion in the a3-KO mice was reduced. The amount of saliva secreted by pilocarpine injection (i.p.) in the a3-KO mice was significantly less, whereas no difference was detected by isoproterenol. Additionally, intraoral salivary pHs in the a3-KO mice tended to be slightly acidified. These results suggest that the V-ATPase in salivary glands is localized in the apical membrane and the basal region of ductal cells and is involved in salivary pH adjustment and absorption by unknown mechanism.

(COI: No)

P2-156

Bone marrow-derived cells have the ability to differentiate into parenchymal cells of salivary glands

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In recent years, it has been reported that bone marrow-derived cells (BMDCs) were capable of differentiating into multiple cell types of many organs. Therefore the treatment by using BMDCs for the regeneration medicine is expected in the future. We examined whether BMDCs can differentiate into salivary gland cells in the mice. BMDCs from green fluorescence protein (GFP) mice were transplanted into irradiated syngeneic GFP-negative mice. One, two, three and six months after bone marrow transplantation, the salivary glands were removed and immunohistochemical examinations were carried out. Immunohistochemistry (IHC) for GFP showed that GFP positive cells were found in salivary glands. We performed the double-labeled fluorescence IHC staining to identify the cell type of GFP-positive cells, by using following antibodies: AQP as a marker for the acinar cells, cytokeratin 19 as a marker for the ductal cells and α-SMA as a marker for the myoepithelial cells. The double-labeled fluorescence IHC staining demonstrated that GFP-positive cells were detected as secretory cells, ductal cells and myoepithelial cells. These results suggest that BMDCs migrate to salivary glands and differentiate into parenchymal cells of salivary glands.

(COI: No)

P2-157

Putative Bio-Markers in saliva by microRNA pattern depending on secretion systems from salivary glands

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MicroRNAs (miRNAs) are small non-coding RNAs of 18-28 nucleotides that play key roles in the regulation of gene expression. To examine the possibility of miRNA in saliva as a Bio-Marker, expression patterns of miRNAs in submandibular glands (SMGs) and in whole saliva of ICR mice with various hormonal treatments were analyzed by quantitative real-time PCR.
SMGs were investigated for miRNAs and 42 miRNAs were identified. Among 42 miRNAs, miR-21a, miR-141 and miR-143 were much abundant in male mouse. Castration caused remarkable decrease in the expression of these three miRNAs. DHT administration to the castrated animals increased miR-21a, miR-141 and miR-143 such as male. Suggesting, these three miRNAs in the tissues were regulated by androgen.
In the case of exocrine whole saliva collected from mice stimulated parasympathetic nerve, miR-143 was not secreted to saliva, also miR-21a and miR-141 were secreted abundantly but were not dependent on androgen like as SMG tissues. These results suggest that amounts of miRNAs in saliva are not always correlated to ones in SMG tissues, thus depend on specific transport system such as exosome. Also, miR-15a, miR-16, miR-23a and miR-451a were secreted in female mice abundantly. Furthermore, miR-451a secretion was increased by ovariectomy extremely. It is known that miR-451a regulates the drug-transporter protein P-glycoprotein, potentially promoting resistance to the chemotherapy drug Paclitaxel (http://miRBASE.org/). We refer miR-451a in saliva is a putative bio-marker to indicate drug-transport rate for ovarian diseases.
(COI: No)

P2-158

Ultrastructural localization of endogenous peroxidase activity in secretory granule of rhinoceros parotid gland

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A parotid gland (PG) was obtained from a male rhinoceros aged 36 years. Specimens were fixed in 10% formalin for light microscopy. The same specimens were then subsequently fixed with 2% para-formaldehyde-2% glutaraldehyde and 1% OsO_4 buffered with 0.1M cacodylate buffer for electron microscopy. After dehydration, the tissue was embedded in Quetol 653. Using ordinary electron microscopy, acinar secretory granules of PG showed a bipartite structure consisting of the main portion and of the dense bodies (or cores). In the present study, nickel grids with ultrathin sections of Quetol 653 embedded blocks were incubated for 1h in a 3, 3'-diaminobenzidine tetrahydrochloride (DAB)-reaction solution consisting of 0.1% DAB and 0.01% H_2O_2 causing an endogenous peroxidase (PO) reaction. X-ray microanalysis of the DAB-reacted ultrathin sections was performed under an energy dispersive X-ray spectrometry (EDS) attached to a JEM 1400 Plus operated at 80 kv. EDS reflected the presence of moieties caused by the PO reaction. Therefore, the mapping patterns of nitrogen were restricted to the dense bodies. In the rat parotid gland, the PO reaction covered all portions of the acinar secretory granules including the typical serous secreting cells. These results suggest that the parotid gland of the male rhinoceros displayed the morphological characteristics of seromucous secreting cells.
(COI: No)

P2-159

Involvement of MARCKS phosphorylation in lipid rafts in amylase release in parotid acinar cells

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Myristoylated alanine-rich C kinase substrate (MARCKS) is known as a major cellular substrate for protein kinase C. The phosphorylated-MARCKS (p-MARCKS) translocates from the membrane to the cytosol. It has been thought that MARCKS has various cellular functions such as membrane trafficking. MARCKS has been implicated in the actin cytoskeleton regulation through the modulation of phosphoinositide in lipid rafts. In parotid acinar cells, the activation of β-adrenergic receptors provokes exocytotic amylase release. Here, we investigated the involvement of MARCKS phosphorylation in amylase release in rat parotid acinar cells. MARCKS protein in the acinar cells was detected. The β-agonist isoproterenol (IPR) induced MARCKS phosphorylation. IPR induced MARCKS translocation from the membrane to the cytosol. Lipid rafts, which were isolated as detergent-resistant membranes (DRMs), were separated by sucrose density-gradient centrifugation from the acinar cells lysed with 1% Triton X-100. MARCKS was found in monosialoganglioside GM1a-rich DRMs, but that in the DRMs markedly decreased by IPR stimulation. MARCKS-related peptide as the MARCKS inhibitor inhibited the IPR-induced amylase release. These results indicate that MARCKS phosphorylation is involved in amylase release in parotid acinar cells. MARCKS translocation from the lipid rafts to the cytosol may regulate exocytosis.
(COI: No)

P2-160

Morphological study of human submandibular duct: nerve distribution of sublingual caruncula, the common opening area

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Many studies report on the submandibular gland, but detailed structural studies of the human submandibular (Wharton) duct opening are rare. Out of the main salivary glands, the human submandibular gland secretes the most amount of saliva into a sublingual caruncula, an open area shared with the sublingual duct. This common opening area has not been the subject of many reports and its nerve distribution is still unclear. Sialolithiasis is known to be most common in the duct of the submandibular gland, usually causing pain from the sialolith in the duct. For certain, investigating the structure and nerve distribution of the submandibular duct has clinical importance as well. In this study, we conducted an immunohistochemical observation of the common opening area shared between the submandibular and sublingual ducts by using an antibody against anti protein gene product PGP 9.5, a specific marker for neurons, in order to study its nerve distribution. Seven materials were obtained from human adults ranging from age 74 to 93 from the Japanese cadaver collection at Kyorin University School of Medicine. After fixing the removed material in 4%PA/PBS overnight. They were washed with PBS, soak in 20% sucrose, and made into 15 μfrozen sections. Florescence microscope camera was used for observation. Results revealed an abundance of nerve fibers in the opening area of the Wharton duct, as well as an abundance of blood vessels surrounding the duct. We also confirmed the presence of smooth muscle inside the duct wall in the opening area.
(COI: No)

P2-161

Morphological Changes of Myoepithelial Cells in the Rat Submandibular Gland Following the Partial or Total Sialoadenectomy

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Objective: Myoepithelial cells (MECs) surround the basal surface of salivary gland acini. However, their function for salivary secretion is still unclear. Salivary secretion from the residual gland is considered to be accelerated when the glandular tissues were damaged or surgically excised. We analyzed morphological changes of MECs in residual and non-operated contralateral (NOC) submandibular glands after the partial or total sialoadenectomy.
Methods: Male Wistar rats of 8-weeks-old were used. Whole or distal-half of right submandibular gland was surgically excised. After 1, 2, 3 or 8 weeks of surgery, rats were fixed and residual and/or left NOC submandibular glands were prepared for frozen sections and immunohistochemistry using the polyclonal antibody against smooth muscle actin (SMA).
Results: In both residual and NOC glands, number of visible cell-bodies (nucleus and perinuclear region) of SMA-positive MECs and SMA-immunopositive area in serial sections increased significantly after the surgery. Three-dimensional analysis revealed that number, length and thickness of processes covering the acini were enhanced substantially. Complexity of process-branching measured by number of primary and terminal processes clearly was up-regulated in both residual and NOC glands.
Discussion: MECs adapt their morphology of their processes according to the demand of salivary secretion. MECs presumably promote salivary secretion by tightened their grasp on glandular acini.
(COI: No)

P2-162

The interaction between ACh and VIP on parasympathetic blood flow increase in rat sublingual gland

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Previously, we reported that the parasympathetic blood flow increase is evoked by both cholinergic and non-cholinergic fibers in sublingual gland (SLG). The parasympathetic vasodilation evoked by non-cholinergic fibers has been reported in orofacial area such as lip, tongue, masseter muscle, although the precise mechanisms of non-cholinergic parasympathetic vasodilation is still unclear. The SLG secretes mucous saliva including mucin, and it is well known that the vasoactive intestinal polypeptide (VIP) is important in protein secretion from acinar cells. Thus, in the present study, we examined the rule of VIP in parasympathetic blood flow increase of SLG. The urethane anesthetized rats paralysed by pancuronium bromide were artificially ventilated. The cervical vagi and cervical sympathetic trunk were cut in the neck bilaterally. The blood flow of SLG (SLGBF) were analyzed by laser speckle imaging flow meter when the central cut end of lingual nerve (LN) was electrically stimulated (20 V, 20 Hz, 20 s). The SLGBF increase evoked by LN stimulation was completely inhibited by intravenous administration of autonomic ganglion blocker hexamethonium. The SLGBF increase was reduced to 25% by simultaneous administration of atropine and VIP antagonist, although that was reduced to 60% by atropine only. The VIP or ACh administration induced the SLGBF increase, and the value of SLGBF increase was similar to that evoked by LN stimulation. Thus, it was suggested that ACh and VIP are related to parasympathetic SLGBF increase.
(COI: No)

P2-163

Morphological and histochemical features of the intercalated duct in the submandibular gland of mice deficient for the androgen receptor

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The submandibular gland (SMG) of mice has a marked sexual dimorphism, in which a special duct portion called granular convoluted tubule (GCT) develops from the striated duct (SD) preferentially in males. In the androgen-receptor-knockout (ARKO) male mice, the SMG appears similar to that in control and ARKO females. The administration of androgens to ARKO males had no effect on SMG, whereas the administration of thyroid hormone (T4) caused an extensive conversion of SD cells to GCT cells (Adthapanyawanich et al., in press). Another representation of sexual dimorphism in the SMG is the retaining of granular intercalated duct (GID) cells in the adult female mice. GID cells have secretory granules containing submandibular gland protein C (SMGC) and considered to be the remnants of terminal tubule cells that constitute the precursor of the acinar system of perinatal mice. In the present study, we found that the SMG of ARKO male and female mice also have many GID cells that are similar to those in control females. The administration of androgens to ARKO males had no effect on GID cells, but the administration of T4 caused marked decrease in the number of GID cells, as confirmed with immunohistochemistry for SMGC and electron microscopy. These results suggest that the development of GID cells is negatively regulated by AR, but that T4 can overcome the absence of AR by functioning downstream of the signaling pathway of androgens in the mouse SMG.

(COI: No)

P2-164

Dynamic change of PACAP receptor with the development of granular ducts in male mouse submandibular glands

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Saliva secretion is mainly controlled by autonomic nervous system. Pituitary adenylate cyclase activating polypeptide (PACAP) is now recognized as the multi-functional neuropeptide in various organs. We previously compared the distribution of PACAP receptor (PAC1R) in three major salivary glands of young and old male C57BL/6 mice. The distribution of PAC1R in the glands was not different by age. In submandibular gland, PAC1R was detected in the tall columnar epithelial cells, called pillar cells, in granular ducts and some of the cells in the striated ducts. The granular duct is characteristic in rodents. In this study, we examined the expression of PAC1R with the development of mouse submandibular gland. The submandibular glands at 1, 3, 5, 7 days-old and 2, 3, 4, 8 weeks-old male C57BL/6 mice were used for the immunohistochemical detection of PAC1R. Granular duct was not identified until 3 weeks after birth. PAC1R was detected in the striated duct by 2 weeks. At 3 weeks, granular duct was clearly identified and PAC1R was expressed in the pillar cells of the duct. After 4 weeks, PAC1R was more strongly detected at pillar cells than 3 weeks. These results indicated that the distribution of PAC1R was changed from striated duct to pillar cells with the formation of granular duct. A precise study might be necessary to clarify the function of pillar cells by examining the shift of PAC1R-positive cells in the submandibular gland.

(COI: No)

P2-165

Morphology and gene expression profile of the submandibular gland of androgen-receptor-deficient mice

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In the submandibular gland (SMG) of mice, the granular convoluted tubule (GCT) develops preferentially in males dependent on androgens. To clarify the molecular mechanism of androgen action in SMG, we examined the morphology and gene expression profile of the SMG of mice deficient for the androgen receptor (ARKO). The development of GCT and expression of GCT-specific products such as NGF were even lower in ARKO male SMG than in control female SMG. The administration of androgens to ARKO males had no effect on SMG, whereas the administration of thyroid hormone (T4) caused the extensive conversion of striated duct cells to GCT cells with the increase of NGF mRNA. Gene expression profiles in control and ARKO male SMG were analyzed by DNA microarrays, and genes with higher or lower expression in ARKO male SMG were determined. They were then classified into groups according to their responsiveness to the administration of dihydotestosterone (DHT) or T4 to ARKO males. RT-PCR revealed that, while no gene was responsive to DHT, expression of many genes was up- or down-regulated by T4. These results revealed that GCT cell differentiation induced by androgens is dependent on the classical androgen receptor (AR), whereas that by T4 is independent of AR, suggesting that T4 functions downstream of the action of androgens in the signaling pathway leading to GCT differentiation.

(COI: No)

P2-166

Intracortical interaction evoked by periodontal ligament nociception in rat

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The orofacial nociception is transmitted to the primary somatosensory cortex (S1) via the ventral posteromedial nucleus in thalamus. Several morphological studies have demonstrated that S1 is connected to the secondary somatosensory cortex (S2) and the area 5, but the functional organization among them is still unknown.
Recently, it was reported that electrical stimulation of the rat periodontal ligament elicited neural excitation in the region composed of ventral S2 and the insular oral region (IOR), which simultaneously occurred with S1 excitation. However, the physiological relationship between S1 and S2/IOR was unclear yet. To address this issue, we observed intracortical responses evoked by the electrical stimulation of rat periodontal ligament by *in vivo* optical imaging using voltage-sensitive dye.
Electrical stimuli in the periodontal ligament of a incisor evoked the simultaneous neural activation in S1 and S2/IOR. The amplitude and area of the responses increased over the stimulus intensity. The responses in S2/IOR were also observed by the electrical stimulation within the responsive area in S1 without no latency, and vice versa. After confirming those reciprocal interactions, we incised the cortical region between S1 and S2/IOR and then stimulated S2/IOR. Such operation resulted in disappearance of intracortical S1 activation.
These results suggest the existence of a dense intracortical connection between S1 and S2/IOR. This interaction has a possible role in processing the oral somatosensory information.

(COI: No)

P2-167

Catecholaminergic neurons involved in the neuronal system responsible for Cisplatin-induced nausea and/or emesis in the area postrema and the nucleus tractus solitarius

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To clarify if catecholaminergic neurons in the area postrema (AP) and the nucleus tractus solitarius (NTS) were involved in the induction of Cisplatin-induced nausea, we performed immunohistochemical analysis of c-Fos expression and catecholamine synthesis. Male Sprague-Dawley rats (200-300g) received intraperitoneal injection of Cisplatin (10.0 mg / kg body weight) or saline (5.0 ml / kg body weight). Animals were transcardially perfused with fixatives at 2, 6, 12, 24 and 48 hours after injection of Cisplatin or saline. Coronal sections (30 μm thick) were made from the brain removed from skull with a freezing microtome. In each section treated with a c-Fos antibody combined with either DBH (dopamine β-hydroxylase) or TH (tyrosine hydroxylase), immunoreactive neurons in the AP and the NTS were examined under a fluorescence microscope. Immunoreactivities for DBH and TH were found in a certain number of c-Fos immunoreactive cells in the AP and the NTS. These results suggest that catecholaminergic neurons in the AP and the NTS play an important role in drug-induced nausea. In animals fixed at 12 hours after injection of Cisplatin, the number of c-Fos positive AP neurons was significantly smaller than those in other rats fixed at different time course. This result indicates that the acute nausea and/or emesis may switch to the chronic symptom at around 12 hours after Cisplatin injection.

(COI: No)

P2-168

Jaw-position dependent surppression of the low threshold jaw-opening reflex during fictive mastication in rabbits

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The aim of present study is to verify whether the strong suppression of the low threshold jaw-opening reflex (Lo-JOR) at end of jaw-closing phase (end-CL) on the working side is fundamentally depends on jaw positions relative to configuration of jaw movement trajectories, or the physical distance from the intercuspal jaw position. We tested this question by using a removal appliance (splint) that increases inter-incisal distance during fictive mastication. EMG activity of the digastric muscle was recorded with movements of the incisor point of the mandible during fictive mastication. The jaw movement signal was used to deliver the stimuli to the inferior alveolar nerve at half of closing phase (hal-CL) and end-CL. The splint was unilaterally applied on the working side. The magnitude of the JOR suppression was 22.2% of control at end-CL and that was 62.2% at hal-CL under condition without the splint. When the inter-incisal distance was increased by application of the splint, the JOR was tested at a jaw position which was located at end of closing phase, but its physical dimensions were vertically and horizontally same as those of the hal-CL. It was found that magnitude of the JOR suppressions was significantly different between splint (41%) and hal-CL, suggesting that the strong suppression of the Lo-JOR at end-CL is fundamentally depends on jaw positions relative to configuration of jaw movement trajectories. In conclusion, this jaw position-dependent suppression of the JOR is advantageous for production of strong biting force on the working side.

(COI: No)

P2-169

Activation of α_2-adrenoceptors via cervical sympathetic nerve involves β-adrenergic vasodilation in the masseter muscle mediated by sympathoadrenal system

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Sympathetic activity is one of the important factors for the regulation of the hemodynamics of jaw muscles and disturbances in intramuscular blood flow evoked by modulation of sympathetic vasomotor response may be related to jaw muscle dysfunctions. Sympathoexcitation has been reported to cause changes of either increase or decrease of the blood flow in the masseter muscle (MBF). Although the reason for the differences in the effects is unclear, the interaction between neural and humoral mechanisms of MBF regulation may be important for the difference because sympathoexcitation induces activation of both sympathoadrenal system and cervical sympathetic nerves (cSN). We explored this question by investigating the effects of electrical stimulation of the splanchnic nerve (SPLN) on the MBF either intact or sectioning of cSN, and adrenoceptor agonists or antagonists on the responses in anesthetized rats. The SPLN stimulation caused a significant MBF increase and the increase significantly reduced by intravenous administration of propranolol. The MBF increase evoked by SPLN stimulation was almost abolished by cSN section. The SPLN stimulation after cSN section in combination with administration of clonidine significantly increased the MBF, but not phenylephrine. Our results indicate that cSN is involved in β-adrenergic vasodilation in the masseter muscle mediated by sympathoadrenal system, and suggest that the activation of α_2- rather than α_1-adrenoceptors via cSN contributes to this response.

(COI: No)

P2-170

Association of oral fat sensitivity with body mass index and food preference in Japan

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This study was conducted to evaluate the association between oral fat sensitivity and BMI among Japanese adults. We also aimed to evaluate the relation between oral fat sensitivity and taste preferences. The BMI and taste preferences of 25 healthy Japanese adults were investigated using measuring scales and a questionnaire. Sensitivities to prototypical tastants were determined as controls. Oral fat sensitivity was evaluated using oleic acid (OA) in non-fat milk. More than half of the participants detected OA in the non-fat milk at 2.8 mM and the OA detection threshold was associated with BMI. Based on the results, the participants were divided into three groups: super-hypersensitive (SHE), hypersensitive (HE), and hyposensitive (HO) group. No association was observed between the recognition threshold of each prototypical tastant and BMI. The average extent of preference for each prototypical tastant showed that only for sweet preference, significant differences were observed among the oral OA sensitivity groups. Moreover, we found a significantly higher preference for fatty sweet foods than non-fatty sweet foods in the SHE and HE groups but not in the HO group. These findings suggest that OA sensitivity is associated with BMI and the extent of preference for fatty sweet food in Japanese adults.

(COI: No)

P2-171

This poster presentation was withdrawn.

P2-172

Relationship between eating behavior and obesity in humans

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For a long time, obese people were thought to exhibit a different eating behavior (i.e., natural bite size, number of chews per bite, chewing speed, etc.) to normal weight people. However, actual relationship between obesity and eating behavior has not been fully elucidated. Therefore, we investigated the relationship between obesity and several individual eating behaviors (self-reported eating speed; ES, bite size, number of chews until final swallowing per bite; NCS, masticatory performance; MP) in 61 adult subjects (39 males; 22 females; mean age, 23.2 yrs). Fish sausage (FS) and bun (B) were used for the test food. Individual MP was measured by the gluco-sensor. Individual NCS for the FS or the B was counted by the masticatory counter. Body mass index (BMI) was calculated to estimate the individual degree of obesity. There was no significantly correlation between BMI and the MP. There was also no significant correlation between BMI and the NCS. On the other hand, significant positive correlation between the BMI and the bite size, and significant positive correlation between the BMI and the ES were obtained. These results suggest that the natural bite size and the ES closely relate to the obesity in humans.

(COI: No)

P2-173

Role of mitochondria in thymic epithelial cells

Kim, Bongju; Ohigashi, Izumi; Takahama, Yousuke (*Division of Experimental Immunology, Institute for Genome Research, University of Tokushima, Tokushima, Japan*)

Thymic epithelial cells (TECs) play an essential role in supporting T lymphocyte development and selection. TECs are functionally divided into cortical TECs (cTECs) and medullary TECs (mTECs) based on their localization within the thymic cortex or medulla, respectively. Mitochondria play a pivotal role in intracellular Ca^{2+} storage and ATP production. However, the relevance of mitochondria in the function of TECs has been unclear. To understand the role of mitochondria in TECs, we measured mitochondrial mass and their membrane potential in mouse TECs using MitoTracker Green and MitoProbe DiOC2(3), respectively. We found that the mitochondrial mass was larger in TECs than thymocytes in flow cytometry and confocal images. The mitochondrial mass was larger in mTECs than cTECs. Mitochondrial membrane potential was almost intact in cTECs, whereas a large fraction of mTECs had depolarized mitochondria. These data indicate that quantity and quality of mitochondria are different between mTECs and cTECs.

(COI: No)

P2-174

The Effect of Minodronate on Murine Hematopoiesis

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Bisphosphonates (BP) are potent inhibitors of osteoclast-mediated bone resorption, and are classified in the nitrogen-containing BP (NBP) and the non-nitrogen containing BP (non-NBP). We previously showed that one of NBP, alendronate (ALD), decreased the number of erythroid-lineage cells, increased the number of osteoclasts and granulocytes, and enhanced the cell size of osteoclasts *in vivo*, indicating that NBP might have a profound effect on murine hematopoiesis. Minodronate (MIN) is other NBP and is more potent inhibitor of bone resorption than alendronate. The purpose of this study is to clarify and compare the time-kinetic changes in hematopoietic cells and osteoclast by MIN and other non-NBP, clodronate (CLO). MIN (10 μmol/kg) or CLO (160 μmol/kg) were intraperitoneally injected into 8 weeks-old male BALB/C mice. In MIN group, whitish bone marrow and splenomegaly were observed 4days after the injection. Flow cytometric analysis of bone marrow indicated the decrease of the number of Gr-1-/CD11+ macrophage at 1-2days, and the increase of the number of Gr-1+/CD11+ granulocytes, the decrease of the number of TER-119+ erythroid cells and the recovery of the number of Gr-1-/CD11+ macrophages to the control level at 4days after the treatment. Histological study indicated the decrease of the number of $TRAP^+$osteoclasts. These cells were located along the trabecular bones beneath the growth plate. These results indicate that hematopoietic cells are strongly influenced by MIN similar to ALD and suggest the different effect on osteoclasts from ALD.

(COI: No)

P2-175

Genenration and differentiation of iPS cells derived from plasminogen activator inhibitor-1 deficient patient

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Plasminogen Activator inhibitor-1 (PAI-1) is the key regulator of plasminogen activation system. Number of studies have shown the relationship between PAI-1 expression levels and diseases such as thrombosis and poor prognosis of cancers. Recently we have reported a PAI-1 deficient patient having apparent phenotypes of severe bleeding and impaired wound healing, both of which are not seen in the PAI-1 deficient mice. To investigate the intrinsic function of PAI-1, iPS cells from the patient were generated, and are differentiated into endothelial cells (ECs) which PAI-1 is mainly producing and having analyzed its role. After co-cultured with stromal OP9 cells, we isolated ECs by MACS with VEGFR2 antibody. The expression of some of the ECs markers were increased, which was more prominent in PAI-1 deficient iPS cells than in wild type. Furthermore we found that ECs from PAI-1 deficient iPS cells detached from dish bottom earlier than control, when the cells were cultured for longer period of time. These results suggest that PAI-1 plays critical roles in differentiation and maturation of ECs. We are now trying to confirm further authentic roles of PAI-1 in ECs and other kind of cell differentiation and functions, such as adipose cells and platelet/megakaryocytes.

(COI: No)

P2-176

EphA2 receptor and ephrin-A1 ligand expression in the spleen

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The spleen filters the blood, and red pulp macrophages are engaged in the phagocytosis of damaged erythrocytes. Recently it has been revealed that monocytes reside in the red pulp of the spleen more than in circulation and emigrate to inflammatory sites (Swirski et al., Science, 2009). We have studied whether EphA2 receptor and ephrin-A1 ligand in vascular endothelial cells could involve in the transendothelial migration of monocytes/macrophages, certain types of which clearly express these membrane proteins. In the present study we therefore examined EphA2 receptor and ephrin-A1 expression and localization in the mouse spleen to determine whether the organ has niches suitable for studying EphA2/ephrin-A1 functions in the transendothelial migration and colonization of monocytes/macrophages. RT-PCR analysis showed that substantial amounts of EphA2 and ephrin-A1 mRNA were expressed in the spleen of Balb/c adult male mice and significantly upregulated in the mice intraperitoneally treated with clodronate liposomes (FormuMax Scientific), which induce the depletion of phagocytes in vivo. Immunofluorescence analysis showed that (1) EphA2 expression was restricted in the red pulp: clearly in CD144-positive cells (splenic sinus endothelial cells) and ER-TR7-positive cells (red pulp fibroblasts) and (2) ephrin-A1 was expressed clearly in endothelial cells of the red pulp and marginal zone. These findings may indicate that the spleen is a suitable organ to examine EphA2/ephrin-A1 functions in terms of the transendothelial migration of monocytes and the colonization of macrophages.

(COI: No)

P2-177

Regulation of the blood-cerebrospinal fluid barrier permeability by TRPV4

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The blood-brain barrier (BBB) and the blood-cerebrospinal fluid barrier (BCSFB) restrict the diffusion of materials from systemic circulation to the central nervous system (CNS). Choroid plexus epithelial cells (CPECs) of the brain ventricles constitute the BCSFB, and regulate the infiltration of plasma proteins as well as immune cells into the interstitium of the CNS. While some pathological conditions are known to alter the barrier function of BCSFB, the regulatory mechanism is not fully understood. Here, we investigated the function of TRPV4, a polymodally gated divalent cation channel in CPECs. TRPV4 was localized broadly on the apical membrane in swine CPECs. Treatment with the TRPV4-specific agonist, GSK1016790A, induced a robust calcium influx and an immediate serine/threonine protein phosphorylation. In 10-20 min after the agonist treatment, a marked decrease in the amount of filamentous actin, and disintegration of the cell junctions were induced, while the protein levels of some tight junction proteins, ZO1 and Claudin 1, were unchanged. By contrast, inhibition of the basal TRPV4 activity with the TRPV4-specific antagonist, HC067047, reduced the basolateral-to-apical transport of alpha 2 macroglobulin. Overall, this study demonstrated a novel physiological function of TRPV4 in the regulation of BCSFB permeability.

(COI: No)

P2-178

Broad distribution of LYVE-1-expressing endothelial cells and reticular cells with special reference to the reticulo-endothelial system (RES)

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LYVE-1, a receptor molecule for hyaluronic acid, is selectively expressed in the lymphatic endothelium and some macrophage lineages. Besides the lymphatic endothelium, hepatic sinusoidal endothelial cells are known to express LYVE-1 and may function in the uptake of hyaluronate circulating in the body. Our immunohistochemical study revealed more broad distribution of LYVE-1 in the endothelium of the lung, adrenal gland, spleen, and heart (endocardium of auricle) of mice. In addition, reticular cells in the medulla of the lymph node intensely expressed LYVE-1. These cells are largely classified as reticulo-endothelial system (RES) for eliminating foreign particles. The LYVE-1-immunoreactive cells were topographically associated with a dense distribution of macrophages in each tissue: Kuppfer cells in the hepatic sinusoids, alveolar macrophages in the lung, macrophages within both sinusoidal lumen and parenchyma of the adrenal gland, macrophages in the splenic red pulp, and macrophages in auricular wall. Ultrastructurally, the immunogold particles for LYVE-1 were localized on the plasma membrane of all cell types. Function of the LYVE-1-expressing cells may be uptake of hyaluronate circulating in blood and lymph and subsequent degradation in relay with adjacent macrophages. This idea is partially supported by a higher activity of hyaluronidase in some organs possessing LYVE-1-expressing endothelial and reticular cells: the liver, lung, adrenal cortex, and medulla of the lymph node abundantly expressed mRNA of hyaluronidases.

(COI: No)

P2-179

Podoplanin$^+$ cells have roles of wound healing by expressing CCL2 and MMP9

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Recently, we have reported that a large number of stromal cells appeared and showed positive for podoplanin (PDPN), known as a lymphatic marker, in wound areas. In this study, we characterized the PDPN$^+$ cells appearing during wound healing in the mouse tongue to clarify their roles in tissue repair. We made a 1mm-deep laceration with a sharp sterile razor on the tongue in C57BL/6 mice under anesthesia. Tongues were excised and then snap-frozen at various times after injury. Their cryosections of 10-14 μm thickness were made for various morphological analyses. The epitherium completely healed by day5 after injury. The granulation was formed in the submucus where many active fibroblast-like cells were populated and formed fine meshworks without any tubular formation of lymphatic vessels. In addition, these fibroblast-like cells strongly expressed PDPN, but not LYVE-1. To examine the role of PDPN$^+$ cells, sections were multiple immunostained PDPN in combination with CD68, collagen type IV, CCL2 and MMP9. Most of PDPN$^+$ cells co-expressed CCL2 during wound healing and many CD68$^+$ cells migrated around the PDPN$^+$ cells. And PDPN$^+$ cells were stretched their processes towards collagen type IV$^+$ epithelial basement membrane. In addition, these PDPN$^+$ cells expressed MMP9 and the cell projections seemed penetrate through the basement membrane. These results suggest that the PDPN$^+$ cells in the wounded tongue may have some roles in wound healing by recruiting other cells such as CD68$^+$ cells with CCL2 and by expressing MMP9 to remodeling the tissue.

(COI: No)

P2-180

Preserved polycythemia in mice under long-term hypoxia

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Hypoxia is known to induce polycythemia caused by the activation of erythropoiesis mediated by increased erythropoietin (EPO) production. However, the elevation of EPO is limited and levels return to normal ranges under normoxia within one week of exposure to hypoxia, whereas polycythemia continues for as long as hypoxia persists. We investigated erythropoiesis in bone marrow and spleens from mouse models of long-term normobaric hypoxia (10% O_2) to clarify the mechanism of prolonged polycythemia in chronic hypoxia. The numbers of mature erythroid progenitors (CFU-E) in the spleen remarkably increased along with elevated serum EPO levels indicating the activation of erythropoiesis during the first week of hypoxia. After two weeks of hypoxia, the numbers of CFU-E returned to normoxic levels whereas polycythemia persisted for > 140 days. Flow cytometry analysis revealed a prolonged increase in the numbers of TER119-positive cells (erythroid cells derived from pro-erythroblasts through mature erythrocyte stages), especially the TER119 (high) CD71 (high) population, in bone marrow. The numbers of Annexin-V-positive cells among the TER119-positive cells particularly declined under long-term hypoxia, suggesting that the numbers of apoptotic cells decrease during erythroid cell maturation. These findings suggested that decreased apoptosis of erythroid cells during erythropoiesis contributes to presereve polycythemia in mice during chronic exposure to long-term hypoxia.

(COI: No)

P2-181

Ephrin-A1 Signaling in Monocytes/Macrophages Regulates Transendothelial Migration

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Eph receptors and ephrin ligands were membrane proteins that are implicated in cell adhesion and migration. We have investigated EphA2 and ephrin-A1 expressions and functions in monocytes (MOs), macrophages (Mϕs) and endothelial cells (ECs), and found that these mRNAs were expressed in ECs and MOs/Mϕs, and TNF α stimulated EphA2 and ephrin-A1 expressions in ECs. In the present study we examined whether ephrin-A1 signaling in MOs/Mϕs engages in the transendothelial migration. We used a MO/Mϕ cell line J774.1 and human vascular ECs. We established J774.1 cell lines with stable ephrin-A1 gene knock down by the shRNAs (efn-A1-KD J774.1). We found that Protein G-beads coupled with EphA2-Fc or ephrin-A1-Fc (EphA2-Fc-beads, ephrin-A1-Fc-beads) adhered to the ECs surface and ephrin-A1-Fc-beads were mostly invaginated to the cytoplasm of ECs, where actin filaments covered the beads. Moreover, ephrin-A1-Fc-beads induced membrane retraction in ECs. We also found that J774.1 parent cells adhered EphA2-Fc or ephrin-A1-Fc protein-adsorbed surface significantly higher in cell density than Fc protein-adsorbed surface. J774.1 cells on the EphA2-Fc and ephrin-A1-Fc surface formed cytoplasmic microspikes more prominently than those on the control surface although microspikes were less prominent in efn-A1-KD J774.1 cells on the EphA2-Fc adsorbed surface. Efn-A1-KD J774.1 cells seeding on confluent ECs migrated through the EC layer less frequently than J774.1 parent cells. These results may indicate that ephrin-A1 signaling in MOs/Mϕs and EphA2 signaling ECs are deeply implicated in transendothelial migration.

(COI: No)

P2-182

Lymphocyte homing ligand expression profile in the murine celiac and gastric lymph nodes

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Lymphocyte homing is mediated by interactions of L-selectin with their ligands and of integrin α4β7 with mucosal addressin cell adhesion molecule-1 (MAdCAM-1). L-selectin ligands are predominantly expressed on HEV in the cutaneous draining peripheral lymph node (PLN), whereas MAdCAM-1 is primarily expressed on HEV and lamina propria venules in mucosa-associated lymphoid tissue (MALT) such as Peyer's patches (PP) and is also expressed in the gut draining mesenteric lymph node (MLN). Celiac and gastric LNs and other secondary lymphoid tissues of ICR mice were stained immunohistochemically using anti-peripheral lymph node addressin mAb (MECA-79), anti-MAdCAM-1 mAb (MECA-367), and anti-GlyCAM-1 Ab (CAMO2). Celiac and gastric LNs HEVs were stained with MECA-79 and CAMO2, while MECA-367 staining is weak and/or absence on the majority of HEVs, especially in celiac LN. Although celiac LN, which is draining stomach and liver, therefore, belongs to MALT, the homing ligand expression profile is different from that in MLN.

(COI: No)

P2-183

Differential Expression of Toll-like Receptor-2, -4 and -9 in the Various Type of Epithelia Associated with Rat Peyer's Patches

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This study aims to elucidate the characteristic expression of TLR-2, -4 and -9 in follicle-associated epithelium (FAE) and epithelium of follicle-associated intestinal villi (FAIV) in rat Peyer's patches.

MATERIALS AND METHODS: The ileal cryosections with Peyer's patches from 5 male Wistar rats were stained with enzyme immunohistochemical method using anti TLR-2, -4 or -9 antibody.

RESULTS: TLR-2 was immunopositive in the striated borders of epithelia of ordinary intestinal villi (IV), anti-follicular side of FAIV (oFAIV) and follicular side of FAIV (iFAIV), but TLR-2$^+$ columnar epithelial cells in iFAIV were less frequent than those in IV and oFAIV. The immunopositive intensity of TLR-2 in the iFAIV was weaker than those in the IV and oFAIV. TLR-2, -4 or -9 was immunopositive in the apical membranes of many M cells in FAE. TLR-2$^-$ epithelial cells in the apical portions of lymphatic follicles were more numerous than those in the apical portions of IV and FAIV. No TLR$^+$ columnar epithelial cells were observed in the vicinity of TLR-2$^+$, -4$^+$ or -9$^+$ M cells.

DISCUSSION: From the present findings, the differential abilities of recognization to bacterial ligands among 3 types of epithelia and the cellular differentiation into M cells in FAE are discussed.

(COI: No)

P2-184

Anal tonsillar histopathology after the HRP and LPS administration. in the laboratory shrew, Suncus murinus

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It has been elucidated that the laboratory shrew belongs to one of the ancestors of mammals. We reported that the laboratory shrew had tonsil-like structures near urogenitoanale, which were situated at different sites from any mucosal structures, named anal tonsil of mucosal immune structures. In order to research the function of anal tonsils, we administrated horseradish peroxidase (HRP) and Lipopolysaccharide (LPS) to the anal tonsils, and observed the change in the movement of inflammatory cells. After five, ten, fifteen and twenty minutes later of HRP administration, we took out the anal tonsil and made cryostat sections. These sections were reacted with peroxidase, and counted the number of the positive cells. After five minutes, there were several positive cells in the epithelium. We instilled 5mg/kg LPS into anal tonsils, and three days later, did same dosage again. After ten days later, we sacrificed animals, made paraffin sections. These sections were stained by CD3, CD19, CD20, CD1a S-100, FDC, lysozyme antibodies using immunostaining method. Especially, paracortico area of the tonsils, T-lymphocytes, dendritic cells and macrophages were increased.

(COI: No)

P2-185

Role of FABP7 in fat diet induced non-alcoholic fatty liver disease (NAFLD)

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Background: It has become evident that adipose tissue macrophages and liver macrophages (Kupffer cells, KCs) are important not only in local inflammation and tissue damage but also in systemic diseases associated with metabolic abnormalities. Recently, we reported that fatty acid binding protein (FABP) 7, a member of the intracellular lipid binding protein family, is expressed by KCs and regulates cytokine production and phagocytosis against dead cells. In this study, we investigated the role of KC-expressed FABP7 in the high fat diet (HFD) induced non-alcoholic fatty liver disease (NAFLD).

Methods: C57BL/6 (WT) and Fabp7-knock out (KO) mice were fed HFD (60 % kcal from fat) from 7 weeks until 19 weeks. Oil-red O staining was performed to detect lipid accumulation in the liver. To examine the liver injury and/or liver inflammation induced by HFD feeding, plasma ALT levels and mRNA expression of inflammatory cytokine/chemokine (TNF-α and MCP1) in liver were measured. For morphological analysis of KCs, F4/80 immuno-staining was performed.

Results: In KO mice, lipid accumulation in liver after HFD feeding was significantly decreased compared to WT mice. Plasma ALT levels and mRNA expression of TNF-α and MCP-1 in liver were lower in KO mice after HFD feeding than WT mice. The appearance of crown like structures, the aggregated KCs surrounding dead hepatocytes, was decreased in KO liver compared to WT liver.

Conclusion: Taken together, FABP7 expressed in KCs might be involved in the progression of NAFLD by modulating hepatic lipid metabolism.

(COI: No)

P2-186

The study of fat associated lymphoid clusters and hemal node-like structures in critical anemic mice

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The association between adipose tissue and immune system has been discussed, and fat associated lymphoid clusters (FALCs) are considered as a source of immune cells such as NK cells and macrophages. We previously reported induction of hemal node-like structure in omentum of critical anemic mice. Here, we analyzed the relationship between FALCs and hemal node like structure.

Splenectomized mice were treated with nitrogen-containing bisphosphonates to inhibit erythropoiesis in bone marrow, followed by injection with phenylhydrazine to induce hemolytic anemia.

In histological and immunohistochemical examination, numerous lymphoid cells formed clusters in the omental adipose tissue, and the most cells were B220-positive B lymphocytes. The CD3-positive T lymphocytes and F4/80-positive macrophages were dispersedly observed in these clusters. Furthermore, in critical anemic condition, TER119- and/or CD71- positive erythroblasts accumulated in these clusters. In RT-PCR analyses, we detected the expression of some mRNA related in hematopoiesis in hemal node-like structures, and Scf, Mcsf and SDF-1 were also detected in the omentum including FALCs.

These results indicated that the FALCs such as omental milky spots has the potency of supporting the microenvironment for hematopoiesis because of constantly providing the site of hematopoietic cells establishment. Therefore, we consider the possibility that some hematopoietic precursor cells establish and differentiate to erythroid progenitors in the FALCs, which may develop to hemal node-like structures under the emergency condition such as high EPO.

(COI: No)

P2-187

IL-17A inhibits osteoclast differentiation of RANKL-stimulated RAW 264.7 cells

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Periodontitis is a chronic inflammatory disease characterized by alveolar bone resorption. Inflammation-mediated bone loss is a major cause of various bone diseases, such as chronic periodontitis, and is due to an imbalance in bone remodeling that favors resorption. This imbalance is caused by increased inflammatory cytokines. Interleukin-17A (IL-17A) is a proinflammatory cytokine that is mainly secreted by activated T cells. IL-17A stimulates osteoclastic bone resorption via osteoblasts by inducing the expression of the receptor activator of NF-κB ligand (RANKL). However, little is known about the direct effects of IL-17A on the osteoclast precursors. We confirmed that IL-17A suppresses the osteoclast differentiation of RAW264.7 cells in the presence of RANKL in a dose-dependent manner. We also found that treatment with SP600125, a specific inhibitor of c-Jun N-terminal kinase (JNK), significantly inhibits the TRAP activity of RAW264.7 cells, which were stimulated by RANKL. In addition, we found that IL-17A reduces the phosphorylation of JNK and expressions of c-Fos, which were increased by RANKL stimulation. These results suggest that IL-17A-induces inhibition of JNK phosphorylation and that expression of c-Fos may be one of the factors that suppresses the differentiation of osteoclast precursors into osteoclasts.

(COI: No)

P2-188

Electron microscopic observation of a novel cytoplasmic rods and rings structure recognized by autoantibodies from patients with chronic hepatitis C viral infection

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Background and Purpose: Ribavirin binds to cellular inosine monophosphate dehydrogenase (IMPDH) and inhibits DNA synthesis. A combination of ribavirin and interferon-alpha is a standard therapy for chronic hepatitis C virus (HCV) infection. Autoantibodies that bind to a novel cytoplasmic rods and rings structure (RR) are induced in ~20% of the patients receiving this therapy. Ribavirin induces RR in nearly 100% of culture cells within 3h. The morphological feature of the RR was pursued by transmission electron microscopy (EM).
Methods: RR was induced in HeLa cells within 3 hour by ribavirin. Cells were stained with anti-RR/IMPDH (+) HCV sera or rabbit anti-IMPDH2 antibodies and developed using DAB. Slides were then fixed with glutaraldehyde and osmium double fixation. Samples for EM were prepared by epon embedding with a handstand gelatin capsule method.
Results: By immunofluorescence, many rods (3~10 μm length) and rings (2~5 μm diameter) were observed in ribavirin-treated cells, mostly in cytoplasm but some were also seen in nuclei. By EM, RR was morphologically a single paracrystalline array of individual filaments similar to intermediate filaments, without limiting membrane. Some rod structures were observed in the undetermined homogeneous structure in cytoplasm.
Conclusion: The fine RR structure morphologically appears to be a single paracrystalline array of individual filaments similar to intermediary filaments by EM.

(COI: No)

P2-189

Fabrication of tissue-engineered human arterial constructs by cyclic hydrostatic pressure

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Background: Ischemic heart disease is the primary cause of death and small diameter biological artificial vessels has been desired for supplying blood into ischemic lesion. Since hydrostatic pressure has been shown to increase extracellular matrices which are critical for arterial integrity, we aimed to fabricate biological arterial constructs using the apparatus generating cyclic hydrostatic pressure.
Methods and Results: Human umbilical arterial smooth muscle cells (HUSMCs) suspended in 10% fetal bovine serum/DMEM at the density 6.5×10^5 cells/mL, followed by culture in 5 mL syringe. Cyclic hydrostatic pressure was applied to HUSMCs in 5 mL syringe for 18 h. When HUSMCs were exposed to 110kPa-180kPa hydrostatic pressure at 0.002Hz, HUSMCs were self-assembled and exhibited sheet-like construct (2 mm × 4 mm × 100 μm), whereas pneumatic pressure control did not produce HUSMC sheet. The other cycle conditions, i.e., 0.25, 0.05, 0.01Hz, produced smaller- and irregular-shaped HUSMC sheets. In the HUSMC sheets fabricated by 0.002Hz hydrostatic pressure, mRNA expression of elastic fiber-related genes including fibrilin1, fibrilin2, fibronectin, fibulin4, and lysyl oxidase was more than two-times higher than in pneumatic pressure control ($p<0.05$, n=4-8).
Conclusions: hyper-hydrostatic pressure with lower cycle stimulation produced self-assembled human arterial sheets.

(COI: No)

P2-190

Effect of Epac-specific inhibitor, ESI-09 on heart failure model mice

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Although β-blockers are the first-line drugs for heart failure treatment, its overdose often leads to exacerbation. Recently, a downstream effector of β receptor, exchange protein (Epac) directly activated by cAMP emerges as a novel therapeutic target. Our previous studies showed that genetic disruption of Epac1 protects heart from pressure-overload as well as chronic catecholamine stress. Recently, Epac-specific inhibitor ESI-09 has been developed. In this study, we examined the effect of ESI-09 against heart failure. We performed chronic isoproterenol (ISO) infusion via osmotic mini-pump (60 mg/kg/day for 7 days) in C57BL/6 mice to produce heart failure model, and treated them with ESI-09 (1 or 5 mg/kg/day for 7 days) via intraperitoneal injection in one mice group. We found that left ventricular ejection fraction (LVEF) was significantly decreased in ISO infusion (from 65.3 ± 2.9 to 46.5 ± 2.9 %, $p< 0.01$), but ESI-09 did not improve the ISO-induced decrease of LVEF (from 66.4 ± 0.8 to 50.9 ± 2.3% at 1 mg, from 64.4 ± 2.6 to 51.3 ± 5.4% at 5 mg ESI-09). Moreover, injection of ESI-09 was also unable to inhibit ISO-induced cardiac hypertrophy and fibrosis. Rather negative results contrary to knockout study suggest the limitation for usage of this drug. ESI-09 has a non-selective effect to be capable of inhibiting both Epac1 and Epac2. Although we have to await for development of new specific drugs, our present results suggest that ISO-induced progression of heart failure includes a complex pathway, which cannot be simply prevented only by drug inhibition of Epac.

(COI: No)

P2-191

Intermittent hypoxia protects against oxidative stress-induced cell death by alteration of intracellular zinc regulation in adult rat cardiomyocyte

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Intermittent hypoxia (IH) with repetitive hypoxia-normoxia cycles has been shown to exert preconditioning-like cardioprotective effects. There are many findings demonstrated that IH against I/R injury via preserve ion homeostasis, including K^+, Na^+ and Ca^{2+}. Zn^{2+} is an important trace element in cellular physiology which including proliferation, cell signaling, metabolism and survival. However, there are very few literatures reporting the relationship between Zn^{2+} homeostasis and cardioprotection in IH process. The aim of the present study is to determine whether IH process changes intracellular Zn^{2+} homeostasis and which is involved in IH-induced cardioprotection. We investigated the changes in Zn^{2+} homeostasis using the Zn^{2+}-specific fluorescent dye, FluoZin-3. Using 2, 2'-dithiodipyridine (DTDP), a reactive disulphide compound that induce the intracellular release of Zn^{2+} and trigger cell death. In this this study, we found that DTDP release Zn^{2+} from MT, therefore elevated intracellular Zn^{2+} entry mitochondria via Ca^{2+} uniporter. Subsequently, mitochondrial Zn^{2+} increased to induce mitochondrial membrane potential depolarizeation and cell death. However IH increased mild ROS generation time-dependently to release Zn^{2+} from MT gently. This phenomenon lead to IH against DTDP induced cell death. These finding suggest IH induced Zn^{2+} store decrease, which attenuated excess intracellular Zn^{2+} induced cell death in cardiomyocyte.

(COI: No)

P2-192

Mathematical model of Ca^{2+} induced Ca^{2+} release in ventricular myocyte

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A human ventricular cell model including excitation-contraction coupling (HuVEC model) was developed in which the mechanisms of Ca^{2+} induced Ca^{2+} release (CICR) were largely refined. The CICR model is based on Hinch model (2004). In HuVEC model, the steep $[Ca^{2+}]$ gradient near the Ca^{2+}-releasing sites was successfully generated as suggested experimentally. The voltage clamp simulation demonstrated that this local Ca^{2+} accumulation caused interaction among Ca^{2+} releasing units (CaRUs) and realized the graded Ca^{2+} release proportional to LCC. In the normal excitation-contraction coupling, the activation rate of a couplon was low at the onset of AP, and the following rapid rising phase of activation occurred after an apparent delay of a few milliseconds. During this delay, the activation was progressively accelerated through the Ca^{2+} accumulation in junction space in HuVEC model, which corresponded to the spread of individual RyR activations within a cluster of RyRs in full stochastic models of CICR reported recently. The inherently regenerative CICR was terminated through the decline in the activation rate, which was caused by the local Ca^{2+} depletion in SR.

(COI: No)

P2-193

Phase-2 reentry induced from decease in the cardiac sodium channel expression: in silico study

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Ventricular fibrillation in Brugada syndrome is thought to be associated with loss of function mutation in cardiac sodium channels. However, the relation between the fibrillation induction and the loss of function in sodium channels remains unclear. We have recently reported that decrease in sodium channel expression from the lateral surface membrane of each ventricular myocyte in a myofiber model incorporating the electric field mechanism, taking into account the intercellular cleft potentials, leads to the loss of function in the sodium current. Here, we extended the simulation to the initiation of phase-2 reentry in Brugada syndrome. We performed computer simulations of excitation conduction in the myofiber model, and investigated the effects of spatial and subcellular sodium channel distributions on the phase-2 reentry induction. In the myofiber model, the spatial heterogeneity of sodium channel did not reproduce the phase-2 reentry. In the same myofiber model but with specific subcellular sodium channel distribution, markedly decreasing in sodium channel expression of the lateral cell membrane of each myocyte, the spatial heterogeneity of sodium channel resulted in early repolarization followed by phase-2 reentry. Subcellular sodium channel distribution together with the spatial heterogeneity of sodium channel might be responsible for fibrillation induction in Brugada syndrome.

(COI: No)

P2-194

Dynamical Mechanisms of Early Afterdepolarizations in Long QT Syndromes: Insights from slow-fast decomposition analyses for human ventricular myocyte models

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Early afterdepolarizations (EADs) are known to cause lethal ventricular arrhythmias in long QT syndromes (LQTS). The aim of this study was to elucidate the mechanisms of EAD generation in LQTS by slow-fast decomposition analyses based on bifurcation theory. We have developed LQTS type 1 (LQT1) and 2 (LQT2) model cells from the mathematical models of human ventricular myocytes (Kurata et al, Biophys J, 2005; O'Hara et al, PLOS Comput Biol, 2011), assuming the inhibition of the delayed-rectifier K^+ channel current (slow component I_{Ks} or rapid component I_{Kr}). Roles of ionic currents in EAD generation were theoretically investigated by constructing bifurcation diagrams for fast subsystems as functions of slow variables. Slow activation gate of I_{Ks} or slow inactivation gate of the L-type Ca^{2+} channel current I_{CaL} were identified as slow variables (slow subsystems) during EAD generation. Bifurcation diagrams as functions of slow variables for fast subsystems showed stable equilibrium points (EPs) at depolarized potentials and EP destabilization via Hopf bifurcations with increasing the slow variables. Limit cycles, emerging via Hopf bifurcations, disappeared via homoclinic bifurcations as the slow variables further increased. EADs can be regarded as transient oscillations of the full system trajectories around the stable and unstable EPs in the vicinity of the bifurcation points during slow changes of the slow variables.

(COI: No)

P2-195

Electrophysiological characterization of a novel *SCN5A* mutant identified in a Brugada syndrome patient

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Cardiac Na^+ channel encoded by *SCN5A* ($Na_V1.5$) gene contributes to the depolarization phase of an action potential and is essential for maintaining cardiac rhythmicity. Some previously reported mutations of *SCN5A* are reported to cause Brugada syndrome. We identified in a Brugada syndrome patient a novel mutation of *SCN5A* which results in a K817E substitution at the voltage sensor region in the domain II of the channel. It has been reported that other mutations in this region cause myocardial dysfunction and/or fatal arrhythmia. We studied the functional characteristics of the K817E mutant channel, comparing the whole-cell currents in HEK293T cells expressing the wild-type or mutant channel under voltage clamp. The K817E mutation decreased the peak current density (142 pA/pF at a test potential of 0 mV) compared with the wild type channel (268 pA/pF at a test potential of -20 mV). The mutation also right-shifted the voltage-steady-state activation curve by ~21 mV and left-shift the voltage-steady-state inactivation curve by ~3 mV. The modulation of activation kinetics by the K817E mutation limits the availability of the Na^+ channel during an action potential, and this may underlie Brugada syndrome.

(COI: No)

P2-196

Crosstalk between mitochondria and sarcoplasmic reticulum in sinoatrial node cells

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We reported that the mitochondrial Na-Ca exchanger, NCLX, functionally couples with the sarcoplasmic reticulum (SR) Ca pump, SERCA, in B lymphocytes as well as in a cardiac cell line HL-1 (Kim et al., J Physiol, 2012; Takeuchi et al., Sci Rep, 2013; Takeuchi et al., J Physiol Sci, 2014). In the HL-1 cells, the Ca crosstalk between mitochondria and SR via NCLX modulates the automaticity. However, there is little information on the role of NCLX and NCLX-mediated mitochondria-SR crosstalk in the real pacemaker cells, sinoatrial (SA) node cells. In the present study, we performed electron microscopic observation and simulation analyses to examine the role of mitochondria-SR crosstalk in the SA node cells.

In the mouse atrial cells, mitochondria and SR occupied 17.25 ± 2.50 and 4.63 ± 0.78% of total cell area, respectively. On the other hand, it was 14.65 ± 1.40 and 3.86 ± 0.61% in the SA node cells, which were comparable with those in atrial cells. In both types of cells, a considerable fraction of SR localized adjacent to mitochondria. According to the geometry, we modified mathematical models of SA node cell. It was suggested that mitochondria-SR crosstalk affected the firing rate.

(COI: No)

P2-197

Characterization of the cardiovascular anomalies in the *Foxc2* deficient mouse

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Foxc2 gene, one of the forkhead transcriptional factors, is expressed in several embryonic tissues. We found the severity of cardiovascular anomalies in *Foxc2* deficient (*Foxc2-/-*) fetuses had a wide spectrum depending on their backgrounds. In this study, we observed 129 x Swiss Black-*Foxc2-/-* embryos (Winnier et al., 1997) at embryonic day 10.5 and characterized the pharyngeal anomalies. The combining approaches using: Intracardiac ink injection, and serial sections with hematoxylin-eosin staining were performed for the morphological analysis of pharyngeal arches. B6-*Foxc2-LacZ* knock-in embryos at E10.5 were also examined for the gene expression. *Foxc2-/-* embryos showed aplasia of the 4th pharyngeal arch arteries (PAAs), and deformities of the 4th pharyngeal pouch. *Foxc2-LacZ* embryos showed X-gal positive mesoderm cells including PAA endothelia. These data indicate that cardiovascular anomalies in 129xSwiss Black- *Foxc2-/-* mice are due to not only simple aplasia of PAAs and deform of the pharyngeal pouch but also other additional factors which would be concerned.

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(COI: No)

P2-198

Donepezil, Acetylcholinesterase Inhibitor, Attenuates LPS-induced Inflammatory Response in Murine Macrophage RAW 264.7 through Inhibition of NF-κB Translocation

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Introduction: We have previously demonstrated that pharmacotherapy with donepezil suppresses post infarct cardiac remodeling in a murine model of ischemic heart failure. However the precise mechanism is still unknown. Because inflammation is a pathological key event in the cardiac remodeling, we investigated the hypothesis that donepezil acts as an inhibitor of inflammatory mediators.

Methods and Results: RAW 264.7 cells were pretreated with donepezil prior to a pro-inflammatory stimulation by lipopolysaccharide (LPS). Donepezil significantly reduced intra- and extracellular levels of various pro-inflammatory mediators and attenuated nuclear translocation of nuclear factor-kappa B (NF-κB), indicating that donepezil showed anti-inflammatory effect. Acetylcholine (ACh) failed to inhibit the LPS-induced cellular responses. Moreover, ACh receptor blockers were ineffective in the anti-inflammatory effect of donepezil. Other kinds of acetylcholinesterase inhibitors did not show the anti-inflammatory properties. These results suggest that a non-neuronal cholinergic system would not be involved in the donepezil-induced signaling pathway and that the anti-inflammatory effect of donepezil would be independent of its acetylcholinesterase inhibition.

Conclusion: Donepezil attenuates pro-inflammatory response by inhibiting NF-κB activation, thereby may contribute to the cardioprotection during the post infarct cardiac remodeling process.

(COI: No)

P2-199

Effect of aging on the increase in concentration of oxygenated hemoglobin in the prefrontal cortex associated with the Stroop interference task

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We have examined the effect of aging on cognitive function tested with Stroop color-word test (SCWT), which was performed in young (n=9, 23 ± 1 yrs) and elderly (n=9, 64 ± 1 yrs) subjects. The subjects were instructed to answer the displayed color of an incongruent color word. The total time period and the number of errors during SCWT (100 trials) were defined as index of cognitive function. The concentrations of the oxygenated-hemoglobin (Oxy-Hb) were simultaneously measured in 22 sites of the prefrontal brain with a multichannel near-infrared spectroscopy (NIRS) to monitor regional cerebral blood flow (rCBF). The total time period for SCWT was longer in elderly (95± 5 s) than young subject (64± 5 s), and the number of errors tended to be greater (elderly 3.2± 0.6 vs. young 1.7± 0.7). The Oxy-Hb in the dorsolateral prefrontal and lateral frontopolar cortices (Broadmanns areas 46 and 10) increased during SCWT in both groups. However, the magnitude of the Oxy-Hb increase in elderly (0.04-0.06 μM*cm) were less than that of young subjects (0.07-0.10 μM*cm). Furthermore, the Oxy-Hb gradually increased and peaked at the later period of SCWT in elderly, while the Oxy-Hb abruptly increased in young subjects as soon as SCWT was started. These results suggest that the attenuated response in rCBF of the prefrontal cortex in elderly subjects is in good association with the age-related cognitive decline.

(COI: No)

P2-200

Changes of the cardiovascular parameters during 90°Head-up tilt in aging rats

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To elucidate the changes of the cardiovascular parameters in response to the 90° Head-up tilt(HUT) for 30 min in anesthetized aging rats, we measured systemic arterial blood pressure(BP), blood flow in common carotid artery(BF) and heart rate(HR) by analog-digital device(MP36; Biopac, USA). Under anesthesia(urethane, 1.0-1.5 g/kg, ip), we inserted a BP catheter into a right common carotid artery toward the heart, attached a BF probe to an artery(ultrasound flowmeter T206;Transonic, USA), and placed ECG electrodes on a subcutaneous for HR counting. In aging rats, after onset of HUT posture the BP and BF immediately decreased to 73.2 ± 11.8 mmHg and 4.3 ± 0.23 ml/min at 9.1 ± 6.0 sec, respectively, from each value before HUT (83.3 ± 12.4 mmHg, 4.7 ± 0.32 ml/min; n=5, $p<0.05$). On both of the traces, BP and BF turned to values under supine before HUT, nevertheless it spent a lot of time for becoming to the steady state, and the HR gradually increased after HUT;There was statistically difference vs. control at 30 min. These results indicated that initial changes in BP and BF were caused by the hydrostatic pressure gradient as same as adult rats. From the viewpoint of these changes throughout HUT, the aging will lead a smaller compliance of artery and a weaker vaso-constriction, leading the possibility that the baroreflex works inadequately in aging rats, and increase in HR compensate for the smaller vascular resistance to maintain BP.

(COI: No)

P2-201

Sex differences in heart rate variability and circulation in young and elderly volunteers after postural change

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Little is known about the influence of an individual's sex on heart rate variability (HRV) and circulation in various recumbent positions.

The purpose of this study was to evaluate whether there are differences in HRV and circulation at three positions (supine, right lateral, and left lateral decubitus positions) between male and female adults.

We recorded electrocardiograms and measured blood pressure (BP) in the three positions for 10 min in 58 young (23.0 ± 0.6 years) and 50 elderly (74.1 ± 0.8 years) volunteers. For the young group, no significant sex differences were observed for heart rate (HR) or high frequency (HF) components in the supine position. Both systolic BP and the ratio of low frequency to HF components (LF/HF) were significantly higher for men than for women. Although HR significantly decreased after changing position from the supine to the left recumbent position in both sexes, HF components and LF/HF ratios did not change. This phenomenon was also observed in the elderly group. For the elderly group, HR and HF components showed no differences between men and women in the supine position, but the LF/HF ratio was higher in men.

These results suggest that postural change to the left recumbent position has no influence on autonomic nervous activity. Additionally, HR reduction in the left recumbent position occurred equally in men and women, regardless of age.

(COI: No)

P2-202

AT1 promotes oxLDL-induced cell responses through interact with LOX-1

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Objective: LOX-1-mediated actions by oxidized low-density lipoprotein (oxLDL) play a critical role in atherogenesis. Angiotensin II type 1 receptor (AT1) is involved in atherosclerotic development aside from regulating blood pressure. Here, we investigated direct interaction of these two receptors and its role in vascular dysfunction.

Methods and Results: LOX-1 was found to form a complex with AT1 in plasma membrane by a co-immunoprecipitation assay and an in situ proximity ligation assay. Additional expression of AT1 promoted LOX-1-mediated cell responses such as ERK phosphorylation, G-protein activation, and NF-κ B and SRF activation in CHO cells expressing LOX-1 and AT-1 or HUVEC. We also found that AT1 blocker (ARB), olmesartan, suppressed oxLDL-induced ERK phosphorylation in HUVEC. In vivo, SHRSP were given high fat diet and olmesartan (0.1 mg/kg/day) or hydralazine (2 mg/kg/day) for a week from the age of 8 weeks. Arterial lipid accumulation in SHRSP, where LOX-1 is known to be involved, was decreased by ARB-treatment irrespective of blood pressure compared with hydralazine-treatment ($p<0.05$). There was no noticeable difference in plasma total cholesterol, HDL cholesterol, triglyceride, and fatty free acids between two groups.

Conclusion: Interaction between AT1 and LOX-1 might promote modified LDL-mediated vascular reactions.

(COI: No)

P2-203

Critical role of human hepatoblastoma stem cells in tumor angiogenesis

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Tumor angiogenesis play pivotal roles in tumor development, progression and metastasis. Endothelial cells (ECs) in the tumor vasculature are traditionally thought to be derived from normal ECs in existing blood vessels near the tumor. However, Wang et al. and Ricci-Vitiani et al. reported that CD133 positive cancer stem cells (CSCs) in glioblastoma generate tumor endothelial progenitor cells, which further differentiate into tumor endothelial cells. Therefore, we examined the relation between CSCs and tumor angiogenesis in association with CD133, which is a marker of CSCs.

Human hepatoblastoma cells (HuH-6 Clone-5) were cultivated and the fraction of side population (SP) cells was analyzed with flow cytometer. The SP cells were injected subcutaneously into NOD/SCID mice. The digested xenograft tumor fragments were cultured and the tumor sphere assay was performed. The spheres were cultivated using 3D collagen gel culture methods.

Some spheres formed CD133 positive capillary-like structures. TEM images of them confirmed the structures with identifiable lumens. These observations suggest that CD133 positive CSCs in hepatoblastoma differentiate into tumor endothelial cells. The hypoxia inducible factor-1 α (HIF-1 α) has been regarded as the most important transcriptional factor promoting tumor angiogenesis by up-regulating pro-angiogenic genes such as vascular endothelial growth factor (VEGF). We also discuss the relationship between HIF-1 α and VEGF.

(COI: No)

P2-204

Real-time intracellular Ca^{2+} imaging in the mouse heart *in vivo*

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Ca^{2+} imaging in cardiomyocytes is an effective means for analyzing diastolic and systolic states of the heart. We have previously reported spontaneous Ca^{2+} waves / transients in the isolated perfused mouse heart by using Ca^{2+} indicators (e.g., 58th Annual Meeting of Biophysical Society, USA, 2014). In the present study, we attempted to develop a method for real-time imaging of intracellular Ca^{2+} in the beating mouse heart *in vivo*. Namely, the anesthetized open-chest mouse under ventilation was placed on a custom-made microscope stage (25 cm × 35 cm; Olympus). Then, Ca^{2+} indicators were injected into the left ventricle through the apex of the heart, and perfused in the coronary system *in vivo*. Changes in $[Ca^{2+}]i$ were detected by using the microscope equipped with a spinning disk confocal unit (CSU 21, Yokogawa). EMCCD camera (iXon, Andor) was used for signal detection. Ca^{2+} waves were observed in cardiomyocytes *in vivo* when the body temperature of the mouse was lowered. The present system has a wide range of application potentialities, and it will be useful in future studies in wide-ranging fields of cardiovascular physiology.

(COI: No)

P2-205

Regulation of store-operated Ca^{2+} influx by STIM1 phosphorylation

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Background: STIM1 is an endoplasmic Ca^{2+} sensor, which plays a critical role in triggering the store-operated Ca^{2+} influx (SOC). The present study elucidates the role of phosphorylation of STIM1 in the regulation of SOC.
Methods: Porcine aortic endothelial cells (PAECs) and HeLa cells were used. The changes in [$CaCa^{2+}$]i was monitored with a front-surface Fura-2 fluorometry. The phosphorylation of STIM1 was evaluated with Phos-tag SDS-PAGE analysis. The mutants of STIM1 were used to determine phosphorylation sites and the functional significance of STIM1 phosphorylation.
Main results: Thapsigargin-induced depletion of Ca^{2+} store in the absence of extracellular Ca^{2+} caused a stoichiometric phosphorylation of STIM1 in both PAECs and HeLa cells. Subsequent replenishment of extracellular Ca^{2+} induced Ca^{2+} influx; however, the level of phosphorylation remained unchanged. Thrombin induced STIM1 phosphorylation in PAECs, which was reversed upon Ca^{2+} replenishment. ML-9 and wortmannin inhibited thapsigargin-induced STIM1 phosphorylation and sustained component of SOC. Analysis of the truncated mutants of STIM1 revealed the C-terminal 40 residues to contain the phosphorylation site. In HeLa cells expressing STIM1 with the Ala mutation of all four putative phosphorylation sites in this region, thapsigargin-induced Ca^{2+} influx was attenuated; however, the puncta formation of STIM1 was unaffected.
Conclusions: STIM1 is phosphorylated following store depletion. This phosphorylation supports the sustained phase of SOC.
(COI: No)

P2-206

Smooth muscle cells of venules show different responses to various transmitters, compared to those of arterioles

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It has been well known that shape and distributions of smooth muscle cells (SMC) are different between artery/arterioles and vein/venules. Very few data has been available on physiological characters of SMC of vein/venule. We observed [Ca2+]i dynamics of SMC of intact venules to clarify which transmitters can elicit any response of venules, and compered with those of SMC of arterioles. Venules and arterioles were isolated from rats, and loaded with a fluorescent Ca2+-indicator. Ringer solution containing various transmitters/ modulators was perfused around the specimens. SMC of arterioles are fusiform in shape, while those of venules are polygonal. 5-HT, ATP, and angiotensin II elicited an increase in [Ca2+]i in most SMC of arterioles. On the contrary, the [Ca2+]i increase in SMC of venules during 5-HT or ATP stimulation was faint, but NorAd can induce an evident increase in SMC. Response to angiotensin II was also significant. 5-HT and ATP has not been considered as "pressor substances", even though both induced strong vasoconstriction of arterioles. This may mean that not only vasoconstriction of arterioles is a unique factor for increase of systemic pressure. However substances known as "pressor substances" induce SMC response of venules as well as arterioles. The reduction of vascular volume of venous system may play a pivotal role in the increase of systemic blood pressure.
(COI: No)

P2-207

Localization and phosphorylation of focal adhesion kinase (FAK) in splenic sinus endothelial cells

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The endothelial cells that line sinus capillaries in the red pulp in the spleen are structurally different from other vascular endothelial cells. Although the unique structures of the sinus endothelial cells are believed to be formed for the passage of blood cells in the splenic cords surrounding the sinus endothelium, the way the passage of blood cells is controlled in the endothelium remains unclear. The cell-cell junctions of the sinus endothelial cells are constituted of poorly developed tight junctions and predominant adherens junctions. The cell-extracellular junctions is composed of vitronectin, one of extracellular matrices (ECM) and integrin $\alpha v \beta 5$ at focal adhesions, and moreover integrin $\alpha v \beta 5$ is localized not only ad focal adhesions but also in the entire circumference of the endothelial cells. Focal adhesions in endothelial cells are important mediating-sites interacting cytoskeletons and ECM via integrins and integrin-associated intracellular proteins to regulate variety of processes such as cell growth, cell shape changes, cell migration, differentiation, barrier regulation, and so on, and these processes are mediated by a non-receptor protein tyrosine kinase, FAK. Recently, in addition to the roles on cell-ECM signaling events, a role for FAK in regulating cell-cell junctions has come out. Therefore, immnolocalization of FAK and tyrosine-phosphorylated FAKs in the sinus endothelial cells of the rat spleen has been examined by immunofluorescence microscopy. Labeling for tyrosine-phosphorylated FAKs was present at the basal part and the cell-cell junctions of the endothelial cells.
(COI: No)

P2-208

The central pathway between the TMN and NTS increases arterial pressure

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The nucleus tractus solitarius (NTS) is one of the nuclei which receive histaminergic neurons from the tuberomammillary nucleus (TMN) in the posterior hypothalamus. However the physiological role of the TMN-NTS pathway remains unclear. We previously found that NTS histamine plays a role in regulating cardiovascular system via activation of histamine receptor H1. In this study, we investigated whether the TMN-NTS pathway is involved in the central cardiovascular regulation. We electrically stimulated the TMN and found pressor and tachycardiac responses. The pressor responses were partially inhibited by cetirizine, a H1 receptor antagonist, microinjected into the NTS whereas we failed to see the inhibitory effects on the heart rate responses. Moreover the TMN neurons were identified to directly project to the NTS by a retrograde tracer, Fluoro-Gold. Together with our previous reports, these findings further demonstrate that the TMN-NTS pathway is involved in the central pressor responses presumably under high arousal phase such as exercise.
(COI: No)

P2-209

Pathological analysis of atherosclerosis in human disease model ApoE-KO mouse

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Background and Aim: In Japan, atherosclerosis and the related-diseases are increasing with Westernized life style, and the death-related atherosclerosis occupies the higher rank of the cause of death. It is extremely crucial issue for translational research to understand the disease development process using mouse model for atherosclerosis.
Methods: In order to induce atherosclerosis, ApoE-KO mice were administered high-fat diet for 16 weeks, and these lesions were histopathologically examined (H&E and Elastica van Gieson stains).
Results: Many atherosclerosis similar to human lesions were seen, and the lesions were classified into 3 types: 1) early lesion, 2) progressive lesion and 3) combined lesion. Early lesion shows fatty streak which involves foamy cell accumulations (macrophages containing lipid). Progressive lesion is composed of foamy cell accumulations, fibrous cap and lipid-core. Combined lesion is determined with accompanying by calcification and stenosis.
Conclusions: Since atherosclerosis lesions in ApoE-KO mouse are histopathologically similar to humans, it is expected for evaluation of therapeutic efficiency and elucidation of the molecular mechanism.
(COI: No)

P2-210

Roles of CXCL17 and CXCL17-responding MDSCs in angiogenesis during tumor progression

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Recently, Chemokines have been regarded as important targets in cancer therapy, because they participate in tumor metastasis and recruitment of immune suppressor cells. Our previous studies showed that CXCL17 expression by tumor cells enhanced the tumor growth in mice, and demonstrated that CXCL17 also induced migration of myeloid-derived suppressor cells (MDSCs) into tumor sites. In the present study, we focused on tumor blood vessels as one of responsible factors in the progression of CXCL17-expressing tumors to clarify the effects of CXCL17 and CXCL17-responding MDSCs on tumor blood vessels at the same days after transplantation or in the same tumor size. CXCL17-expressing colon26 cells (a mouse colon cancer cell line) injected subcutaneously into BALB/c mice, showed an increase in CD31+ endothelial cell areas and well-maintained blood vessel meshworks compared with CXCL17-nonexpressing control tumors. Moreover, the number of pericytes increased in the tumor vessels of these CXCL17-expressing tumors. Furthermore, the CXCL17-responding MDSCs were separated by their chemotactic activity in the chemotaxis assay using mouse splenocytes. The transplantation of tumor cells together with CXCL17-responding MDSCs enhanced tumor angiogenesis compared with tumor cells transplantation with CXCL17-nonresponding cells. These results suggest that CXCL17 and CXCL17-responding MDSCs may be responsible for the tumor progression by inducing the structural and function changes of tumor blood vessels.
(COI: No)

P2-211

Microvesicles released from two mouse mammary carcinoma cell lines contain premature VEGF-C

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Enhanced expression of VEGF-C in tumor cells and subsequent elevation of lymphangiogenesis has been reported to promote lymphatic metastasis. Cell-derived microvesicles are endogenous carriers transporting proteins between cells. Using two mouse mammary cancer cell lines; BJMC338 cells with low metastatic propensity and BJMC3879 with higher metastatic propensity, we found that these two cell lines released microvesicles (MVs) containing premature VEGF-C (pre-VEGF-C) into the culture medium. Both cell lines released shedding vesicles (500-1,000nm) and exosomes (50-100nm) into media. Western blot analysis of VEGF-C demonstrated that exosomal expression of pre-VEGF-C was stronger than that of shedding MVs. Using mouse endothelial cell line (UV2) which express VEGFR3, tube formation assay demonstrated that the formed-tube area was increased when incubated with BJMC cells-derived MVs. To detect the processing of VEGF-C/VEGFR3 system, immunofluorescent analysis of phospho-VEGFR3, Akt and phospho-Akt in MV-treated UV2 cells were performed. UV2 uptook MVs of both BJMC cell lines, and then expressed phospho-VEGFR3 and phospho-Akt. These results indicated that both MVs of two cell lines enhanced lymphangiogenesis, while the malignancy is different between two BJMC cell lines. More studies are needed to show the target cells of pre-VEGF-C packed in MVs.

(COI: No)

P2-212

Inhibition of Cyclooxygenase Closes Chicken Ductus Arteriosus

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Background: Ductus arteriosus (DA) is an essential fetal artery that connects the main pulmonary artery and the descending aorta. Mammalian DA closes right after birth through vasoconstriction via decreases of circulating prostaglandin E_2 (PGE_2) transferred from placenta. Avian DA also closes after birth although avian has no placenta that is a source of PGE_2 in rodent and mammalian. Previous research demonstrated that PGE_2 signal pathway is not involved in constriction of isolated chicken DA. However, *in vivo* effects of PGE_2 in avian DA are little understood.
Aim: The aim of this study is to elucidate effects of PGE_2 in chicken DA closure.
Method and results: First, we measured blood concentration of PGE_2 in chicken at day 19 embryo by enzyme immunoassay. Blood concentration of PGE_2 in chicken was significantly higher than that of rat at day 21 embryo. Next, in chicken at day 19 embryo, Enzyme immunoassay revealed that PGE_2 in the DA was higher expressed than that of the Aorta. These data suggested that PGE_2 works on fetal chicken DA. Finally, we performed a rapid whole-body freezing method to evaluate DA closure *in vivo*. We measured internal diameter of DA at 2hrs after *in ovo* injection of indomethacin, which is a nonselective cyclooxygenase inhibitor. Indomethacin constricted DA at day 19 embryo *in vivo*, but did not constrict the Aorta. These data suggested that PGE_2 is an important factor in avian although avian has no placenta that is a source of PGE_2.
Conclusion: Inhibition of cyclooxygenase closes chicken DA. Prostaglandin E_2 signal may play an important role in an acute response of chicken DA closure.

(COI: No)

P2-213

Role of TRPC3 channels in cardiac fibrosis

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The cardiac pathological response to sustained pressure overload involves left ventricular hypertrophy and dysfunction along with interstitial fibrosis. Accumulating evidences indicate that activation of small GTP-binding protein RhoA plays a critical role in cardiac fibrosis. Here, we found that an inhibition of diacylglycerol-activated transient receptor potential canonical subfamily 3 (TRPC3) channels suppressed pressure overload-induced cardiac dysfunction and cardiac fibrosis via suppression of RhoA-dependent signaling in rodent hearts. Knockdown of TRPC3 completely suppressed fibrotic responses of rat cardiac fibroblasts induced by mechanical stretch and transforming growth factor (TGF) β. Collagen deposition and cardiac diastolic dysfunction were significantly suppressed in TRPC3-deficient mice compared with wild type (WT) mice. Inhibition of TRPC3 significantly reduced RhoA activity and expression levels of fibrotic genes in mouse hearts induced by pressure overload and rat cardiomyocytes induced by mechanical stretch and TGF β. A tubulin-binding Rho guanine nucleotide exchange factor, GEF-H1, was found to be up-regulated and activated in pressure-overloaed WT hearts. The up-regulation and activation of GEF-H1 were significantly suppressed in TRPC3-deficient hearts. These results strongly suggest that TRPC3-mediated cation influx contributes to pressure overload-induced cardiac fibrosis and dysfunction through mechanosensitive GEF-H1-mediated RhoA activation.

(COI: No)

P2-214

Knock-in mouse model of hypertrophic cardiomyopathy caused by troponin T mutation

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We created knock-in mice with a missense mutation S179F in cardiac troponin T (cTnT), which had been found to be associated with human hypertrophic cardiomyopathy (HCM). Membrane-permeabilized cardiac muscle fibers from these mice showed significantly higher Ca^{2+} sensitivity in force generation than those from wild-type mice, while the maximum force-generating capabilities being not different from wild-type mice. This demonstrated that the mutation S179F does have a Ca^{2+} sensitizing effect *in vivo* on force generation in cardiac muscle, consistent with previous *in vitro* reconstitution studies on other HCM-causing cTnT mutants. The knock-in mice suffered from sudden death frequently, and histological examination of cardiac sections showed significant displacement fibrosis, myocardial hypertrophy, and myocyte disarray. Echocardiography showed that left ventricular (LV) end-diastolic dimension was significantly decreased with no changes in ejection fraction. In vivo cardiac catheter measurements showed a significant increase in $LVdP/dt_{min}$ with no changes in $LVdP/dt_{max}$. These results indicate that the knock-in mouse with S179F mutation in cTnT is a useful mouse model of HCM closely recapitulating the clinical phenotypes of human patients.

(COI: No)

P2-215

Survival benefit of ghrelin in the heart failure due to dilated cardiomyopathy

Zhan, Dongyun[1]; Du, Chengkun[1]; Morimoto, Sachio[2]; Akiyama, Tsuyoshi[1]; Schenke, Daryl[3]; Hosoda, Hiroshi[1]; Kangawa, Kenji[1]; Shirai, Mikiyasu[1] (*[1]Natl. Cereb. Cardiovas. Ctr., Suita, Japan.; [2]Fac. Med. Sci., Kyushu Univ., Fukuoka, Japan.; [3]Otago Univ., Otago, New Zealand*)

Although ghrelin has been demonstrated to improve cardiac function in heart failure, its therapeutic efficacy on the life expectancy remains unknown. We aim to examine whether ghrelin can improve the life survival in heart failure using a mouse model of inherited dilated cardiomyopathy (DCM) caused by a deletion mutation δK210 in cardiac troponin T. From 30 days of age, ghrelin (150 μg/kg/day) was administered subcutaneously to DCM mice, control mice received saline only. The survival rates were compared between the two groups. After 30-day treatment, functional and morphological measurements were conducted. Ghrelin-treated DCM mice had significantly prolonged life spans compared with control DCM mice. Echocardiography showed that ghrelin reduced left ventricular (LV) end-diastolic dimensions and increased LV ejection fraction. Moreover, histoanatomical data revealed that ghrelin decreased the heart-to-body weight ratio, prevented cardiac remodeling and fibrosis, and markedly decreased the expression of brain natriuretic peptide. Telemetry recording and heart rate variability analysis showed that ghrelin suppressed the excessive cardiac sympathetic nerve activity (CSNA) and recovered the cardiac parasympathetic nerve activity. These results suggest that ghrelin has therapeutic benefits for survival as well as for the cardiac function and remodeling in heart failure probably through suppression of CSNA and recovery of cardiac parasympathetic nerve activity.

(COI: No)

P2-216

The cardiac baroreflex sensitivity decreases depending on the intensity and duration of treadmill exercise in cats

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We have reported that central command induces selective inhibition for the cardiomotor limb of the aortic-baroreceptor reflex at the onset of spontaneous motor activity in decerebrate cats (Matsukawa et al. Am J Physiol Heart Circ Physiol 303: H464-474, 2012; Auton Neurosci 179: 75-83, 2013). In this study, we examined whether central command also inhibits the sensitivity of cardiac baroreflex during treadmill exercise for one min (walking speed, 20-70 m/min) in 3 conscious cats. The baroreflex bradycardia response was elicited by brief occlusion of the abdominal aorta repeatedly given before, during (at the onset, 15 s, 30 s, and 45 s) and after exercise. The baroreflex sensitivity (BRS) was estimated from the slope of the mean arterial pressure-heart rate curve. During exercise at 20-40 m/min, the BRS was temporarily decreased at the onset of exercise alone but was maintained throughout exercise. On the other hand, the BRS was decreased at the onset of and during the later period (30-45 s) of exercise at a higher speed of 50-60 m/min. The characteristics of the changes in BRS were similarly observed following denervation of the carotid sinus nerves. These results suggest that the sensitivity of cardiac baroreflex is blunted at the onset and during the later period of treadmill exercise at a higher intensity, probably due to central command.

(COI: No)

P2-217 (AP-7)

Rapid cholinergic and delayed β-adrenergic vasodilatation in non-contracting muscles during one-armed cranking

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We have reported that the rapid cholinergic and delayed β-adrenergic vasodilatation increases blood flow of non-contracting vastus lateralis (VL) muscle during one-legged cycling (Ishii et al. 2013, 2014). It was unclear whether such mechanisms contribute to vasodilatation in non-contracting muscles during one-armed exercise. We examined the influences of atropine and/or propranolol on the blood flow responses of the contralateral biceps and triceps brachii and forearm extensor muscles and VL muscle during moderate one-armed cranking for 1 min (n=7). As an index of muscle tissue blood flow, relative concentration in oxygenated-hemoglobin (Oxy-Hb) was measured using near-infrared spectroscopy. The Oxy-Hb of the muscles increased during one-armed cranking. The increase in Oxy-Hb at the early period of exercise was blunted by atropine, whereas propranolol attenuated the later increase in Oxy-Hb during the exercise. Following combined atropine and propranolol, the Oxy-Hb decreased during the exercise. The influences of the autonomic blockades on the Oxy-Hb response were not different among the muscles. It was concluded that the rapid cholinergic and delayed β-adrenergic vasodilatation increased the blood flows of non-contracting arm and leg muscles during one-armed exercise.

(COI: No)

P2-218

Blockade of glycinergic inputs into the RVLM neurons enhances respiratory modulation of the cardiovascular sympathetic nerve in the in situ arterially-perfused preparation of rats

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It has been known that neurons in the rostral ventrolateral medulla (RVLM neurons) generate activity of the cardiovascular sympathetic nerve (SNA), and receive respiratory modulation from the respiratory center. Recently, we have reported that respiratory-related GABAergic inputs into the RVLM neurons regulate respiratory activity of the SNA. However, it is still unclear whether the other inhibitory inputs, glycinergic inputs, into the RVLM neurons are also related with the respiratory modulation of the SNA. In this study, we evaluated effects of a blockade of glycinergic inputs into the RVLM neurons on respiratory modulation of the SNA in the in situ arterially perfused preparation of rats. We injected a glycine receptor antagonist, strychnine (5 μM, 50 nL), into the RVLM bilaterally, and analyzed the effect on respiratory modulation of the SNA by the phrenic nerve activity-triggered average of the SNA. As a result, blockade of glycinergic inputs into the RVLM neurons elevated the basal SNA and enhanced the respiratory related SNA in the inspiratory phase. This data may indicate that glycinergic inputs into the RVLM neurons tonically inhibits the SNA, and also attenuates the respiratory modulation of the SNA.

(COI: No)

P2-219

Importance of body temperature regulation for the evaluation of cardiac function in anesthetized mice

Tsuchimochi, Hirotsugu; Inagaki, Tadakatsu; Shirai, Mikiyasu (*Dept Physiol, Natl Cereb Cardiovasc Ctr Res Inst, Osaka, Japan*)

Cardiac function is an important determinant of circulatory homeostasis. Although recent technological development enables us to measure it easily and quickly even in small animals, there is considerable variability even in the same parameters across studies, presumably related to the experimental condition, e.g. body temperature. Since little is known how the cardiac function is affected by even relatively small changes in body temperature, we examined the effect of a small change in body temperature on the cardiac function in anesthetized mice. Male C57BL6 mice were anesthetized with isoflurane, intubated and mechanically ventilated. Left ventricular pressure (LVP) was measured by a miniature fiber optic pressure sensor (FISO-LS-PT9: tip diameter 0.9Fr, FISO Technologies Inc.) via the right common carotid artery. The first derivative of LVP ($\pm$dP/dt), heart rate and time constant of ventricular pressure decay (tau) were calculated electronically. Rectal temperature was continuously monitored and fluctuated between 35 and 38 ℃ by an automatic thermo control system (Thermo Plate, Tokai Hit). All measured parameters were strongly correlated with rectal temperature. The temperature-dependent changes in these parameters were attenuated by β1-adrenergic blockade with atenolol. In conclusion, precise control of body temperature is needed for the highly accurate and reproducible evaluation of cardiac function in anesthetized mice.

(COI: No)

P2-220 (AP-5)

In vivo assessment of cardiac autonomic nerve activities and identification of cardioprotective agents for heart failure treatment using atrial microdialysis technique

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Introduction: Sympathoexcitation and vagal withdrawal are causes of heart failure progression. Therefore, sympatho-suppression using beta-blockers has been a gold standard treatment for heart failure. We developed the atrial microdialysis technique to simultaneously assess cardiac sympathetic and vagal activities. Using this technique, we examined the effects of various pharmacological agents on cardiac autonomic nerve activities to identify cardioprotective agents.
Methods: In anesthetized rabbits, a dialysis probe was implanted into the right atrial myocardium near the sinoatrial node and was perfused by the Ringer's solution. Dialysate norepinephrine (NE) and acetylcholine (ACh) concentrations were analyzed as indices of cardiac autonomic nerve activities using high-performance liquid chromatography.
Results: 1) Electrical stimulation of sympathetic nerve or vagal nerve significantly increased dialysate NE or ACh concentration in a frequency-dependent manner. 2) Intravenous injection of medetomidine or guanfacine significantly increased dialysate ACh concentration. Furthermore, medetomidine significantly suppressed sympathetic NE release. 3) Intracerebroventricular injection of ghrelin significantly enhanced vagal ACh release to the heart.
Conclusions: Atrial microdialysis technique enabled us to simultaneously monitor cardiac sympathetic and vagal nerve activities. This technique may be useful for the identification of cardioprotective agents.

(COI: No)

P2-221

Cardiac interstitial small cells co-expressing prion protein (PrP) and cardiac troponin T (cTnT) spontaneously develop into beating atypically-shaped cardiomyocytes (ACMs)

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Atypically-shaped cardiomyocytes (ACMs) is a recently identified novel subpopulation of spontaneously beating heart cells found in the cultures of cardiac myocyte-removed crude fraction cells obtained from adult mouse cardiac ventricles. Most of ACMs are multinucleated and respond to β-adrenergic stimulation on the spontaneous Ca^{2+} transients. In the present study, we demonstrate the efficacy of cellular prion protein (PrP) as a surface marker of ACMs. PrP has been recently reported to serve as a surface marker for isolating cardiomyogenic progenitors from murine embryonic stem cells. Cells expressing PrP at the plasma membrane in the culture of the crude fraction cells were found to develop into beating ACMs by themselves or fuse with each other to become larger multinuclear beating ACMs. Combining PrP with a cardiac-specific contractile protein cardiac troponin T (cTnT) allowed us to identify native ACMs in the mouse cardiac ventricles as either clustered or solitary cells. The results suggest that the PrP- and cTnT-co-expressing cells identified in the mouse heart are native ACMs and their fusion results in the development of multinuclear beating ACMs, while there are some possibilities that the multinuclear cells due to the nuclear division in the ACMs. PrP- and cTnT-marked cells were also found in the interstitial spaces among ventricular myoycytes of adult human hearts, which suggests that the native ACMs exhibit life-long survival in the cardiac ventricles of both mice and humans.

(COI: No)

P2-222

Development of new leukocyte removal column aimed at suppression of the inflammatory response during cardiopulmonary bypass -Biological evaluation in a rat model-

Fujii, Yutaka; Shirai, Mikiyasu; Takewa, Yoshiaki; Tatsumi, Eisuke (*Natl. Cereb. Cardiovas. Ctr, Suita, Japan*)

Extracorporeal life support devices, such as the cardiopulmonary bypass (CPB), preserve the patient's life by providing adequate oxygen supply and blood flow to vital organs. However, previous studies have suggested that the interaction of blood and large artificial surface induces inflammatory response during CPB. As a result of series of chain reactions, the numerous powerful inflammatory mediators, including hormones and autacoids, are formed and released. Therefore, we developed the new leukocyte removal column (LRC) for attenuating the systemic inflammatory response during CPB. Rats were divided into the CPB group and the CPB with LRC group. CPB pump flow was maintained at 80 ml/kg/min. Blood samples were collected before (baseline), and 60 min and 120 min after initiation of CPB. We measured the differential count of leukocytes, pro-inflammatory markers such as (TNF-α) and biochemical markers (LDH, ALT, AST). Moreover, we also measured the wet-to-dry weight (W/D) ratio of the lung 120 min after the initiation of CPB. The increased levels of granulocyte count and pro-inflammatory cytokines in the CPB with LRC group were significantly attenuated than those in the CBP group(TNF-α:CPB group vs CPB with LRC group: 1510$\pm$121pg/ml vs 1112$\pm$160pg/ml). In addition, the level of W/D ratio was lower in the CPB with LRC group than in the CPB group. The data suggest that the new leukocyte removal column is useful for reducing the inflammatory response and lung edema during CPB.

(COI: No)

P2-223

Functional relationships between L-type $Ca_V1.3$ channel and the sustained inward Na^+ current in cardiac pacemaker cells

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The sustained inward Na^+ current (I_{st}) has been suggested to play a crucial role in pacemaker activity in sinoatrial node cells, although the molecular mechanism underlying this current remains unknown. We have recently found that genetic ablation of L-type $Ca_V1.3$ channel resulted in significant decrease in the low voltage-activated component of L-type Ca^{2+} current ($I_{Ca,L}$) as well as nearly complete disappearance of Ist in mouse sinoatrial node cells, indicating that $Ca_V1.3$ is responsible for at least two different current systems carried by Ca^{2+} and/or Na+ during the slow diastolic depolarization. We performed comprehensive analysis of $Ca_V1.3$ transcripts in rat sinoatrial node using second generation sequencing and single-cell PCR, yet no plausible splicing and editing sites remain to be identified in Ca^{2+}-selectivity filter. On the other hand, our patch-clamp recordings of recombinant $Ca_V1.3$ channel heterologously expressed in HEK cells revealed the multi-ion characteristics of the pore under different ionic environments for Na^+ and Ca^{2+}. Based on our experimental data, we will discuss the possibility that I_{st} can be mediated by $Ca_V1.3$ channel without an alteration in the molecular machinery of ionic permeation.

(COI: No)

P2-224

Luminescence imaging of HCN4 expression and phenotypic analysis of HCN4 TET-off mouse

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Among four subtypes of hyperpolarization-activated cyclic nucleotide-gated (HCN1~4) channel, HCN4 is major subtype in sino-atrial node (SAN). It has been reported that homozygous HCN4 knock-out mouse is embryonic lethal. In order to overcome this problem, we generated double knock-in mouse (HCN4Luc/tetA_TRE) that enables visualization of the locus of HCN4 expression with luciferase luminescence, and complete knockdown of HCN4 expression with doxyocycline (TET-off). We could successfully visualize pacemaker cells in vitro and ex vivo. The heart rate of TET-off mouse was significantly lower than WT; intermittent sinus pause or irregular RR interval was also observed. However, the heart rate of TET-off mouse was increased after the intraperitoneal injection of isoproterenol, and was not significantly different from that of WT measured in the same condition. We next compared parasympathetic response of TET-off and WT. For this purpose, we electrically stimulated right cervical vagal nerve. In WT, the heart rate was reduced in reversible manner. In contrast, vagal nerve stimulation in TET-off mice caused complete sinus pause, and recovery from sinus pause was significantly delayed after the termination of nerve stimulation. These finding suggested that HCN4 acted as a limiter for bradicardiac response, stabilizing spontaneous firing of SAN during parasympathetic stimulation.

(COI: No)

P2-225

Novel fish-derived peptide fragments which induce endothelium-dependent and -independent vasorelaxation

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Endothelium-dependent vasorelaxation (EDR) not only regulates physiological vascular tone but also counteracts abnormal vascular hypercontractions which lead to vasospasm and hypertension. Therefore, possible deterioration of these EDR functions of vascular endothelial cells under various pathological states such as aging, oxidative stress and lipid disorders, increases risk of vascular diseases, including heart attack and stroke. For the reliable prevention of such acute and lethal vascular diseases caused by endothelial dysfunctions, we attempted to discover foods or food components which help vascular endothelial cells induce EDR. After extensive screening, we eventually found out fish-derived peptide fragments (FDPFs) as the candidates. They were obtained by eatable enzyme digestion of fish proteins (bulbus arteriosus of skipjack tuna) and strongly induced both EDR and endothelium-independent vasorelaxation (EIR) of the porcine coronary arteries. Subsequently using tandem mass spectrometry and analysis software, Pep Novo, we identified four peptides in FDPFs and deduced other three peptides. All of these peptides are novel and previously unreported, and their synthetic peptides indeed induced both EDR and EIR of porcine coronary arteries. The mediation of NO in the EDR, at least in part, was evidenced by pharmacological studies with an inhibitor of eNOS and the fluorometric measurements of NO production and [Ca2+]i elevation in the endothelial cells.

(COI: Properly Declared)

P2-226

The novel role of calpain in SPC/Fyn/ROK pathway which mediates the signal transduction of abnormal vascular smooth muscle contraction

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Rho-kinase (ROK)-mediated Ca^{2+}-sensitization of vascular smooth muscle (VSM) plays a critical role for abnormal VSM contractions such as vasospasm. Previously we identified sphingosylphosphorylcholine (SPC)/Fyn/ROK pathway as a novel signaling pathway for abnormal VSM contraction. As possible downstream targets of Fyn tyrosine kinase, we identified vimentin by focused proteomics in which tyrosine-phosphorylated proteins were concentrated by anti-phosphotyrosine antibody 4G10 and identified by tandem mass spectrometry. Interestingly, western blot analysis revealed that SPC induced limited proteolysis of vimentin not only in human coronary artery smooth muscle cells (CASMCs) in culture, but also in vascular strips of the porcine coronary artery smooth muscle. Since vimentin is reported as the target of calpain, we examined the possible involvement of calpain. In CASMCs, SPC increased calpain activity, which was blocked by PD150606, a calpain inhibitor. Furthermore PD150606 inhibited the SPC-induced abnormal VSM contraction without affecting high K+-induced Ca2+-dependent contraction. These findings suggest the novel role of calpain in the signal transduction of abnormal VSM contraction.

(COI: No)

P2-227

Analysis of developmentally regulated cardiac titin isoform switch at birth

Hashimoto, Ken; Ujihara, Yoshihiro; Mohri, Satoshi (*First Dept Physiol, Kawasaki Med Sch, Kurashiki, Japan*)

During perinatal heart development, myocardial proteins often undergo isoform switch from fetal to neonatal/adult type, including myosin heavy chain and troponins. This is also observed in titin, a giant elastic protein in muscle sarcomere that critically defines a myocardial passive stiffness and thus ventricular filling in diastole. In mammalian heart, the long and compliant N2BA isoform (3.2-3.7 Mda) mainly expressed in fetus is dramatically replaced by relatively short and stiff N2B isoform (3.0 Mda) in neonate/adult, limiting the distensibility of neonatal/adult heart. Both isoforms are generated by alternative splicing from the single titin gene. Recently Rbm20 protein was identified as a main regulator of titin splicing, but its involvement in the perinatal titin switch is totally unknown. In mammals, the onset of air-breathing at birth leads to abrupt elevation of oxygen tension (PaO_2: 20mmHg in fetus to 100mmHg in adult). In this study, we focused on the involvement of Rbm20 in perinatal titin isoform switch and its possible regulation by elevated O_2 at birth. First we established the SDS-agarose gel electrophoresis system to detect mega dalton-sized N2BA/N2B isoform from mouse heart, and confirmed the perinatal isoform switch did occur. Exposure of fetal cardiomyocytes to atmospheric O_2 resulted in isoform shift from N2BA to N2B, suggesting that the elevated O_2 at birth is an upstream environmental cues that lead to titin isoform switch. We are now exploring the changes of Rbm20 expression and intracellular localization around birth, and its potential as an O_2-sensing molecule.

(COI: No)

P2-228

Electrical stimulation of the insular cortex increases regional blood flow in the mesencephalic ventral tegmental area in anesthetized rats

Liang, Nan; Matsukawa, Kanji; Ishii, Kei; Idesako, Mitsuhiro; Endo, Kana (*Dept Integr Physiol, Grad Sch Biomed Health Sci, Hiroshima Univ, Hiroshima, Japan*)

The mesencephalic ventral tegmental area (VTA) has been suggested to play a crucial role in the central cardiovascular control. In this pilot study, we aimed to clarify whether activation of the insular cortex (IC), a higher brain region that partially participates in the autonomic processing, modulates neural activity in the VTA as well as hemodynamics. In pentobarbital-anesthetized rats, catheters were inserted into the external jugular vein and carotid artery for the administration of drugs and measurement of arterial blood pressure, respectively. Activation of the right or left IC was evoked by an electrical stimulation (0.5-1.0 mA, pulse-width 1 ms, frequency 50 Hz) lasting for 4 or 10 s. A probe of laser Doppler flowmetry was inserted into the ipsilateral or contralateral VTA to measure regional blood flow. Electrical stimulation of the IC induced a pressor or depressor response in an intensity-dependent manner, while the regional blood flow in the ipsilateral VTA was increased consistently. The vascular conductance in the ipsilateral, but not contralateral, VTA was remarkable during the IC stimulation and much larger as compared to that without stimulation. The present results supported the anatomical evidence that connections exist reciprocally between the VTA and IC, and suggested that neural activity in the VTA is modulated by inputs from the ipsilateral IC.

(COI: No)

P2-229

Role of monoamine oxidase on hydroxyl radical production during ischemia/reperfusion in anesthetized rats

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Background: Excessive accumulation of monoamines exacerbates myocardial cell injury during myocardial ischemia/reperfusion. Meanwhile hydroxyl radical (OH) production by enzymatic degradation of monoamine has been suggested to exacerbate myocardial cell injury.
Purpose: To clarify the contribution of enzymatic degradation of monoamine by monoamine oxidase (MAO) to the ·OH production during myocardial ischemia/reperfusion.
Method: Using microdialysis technique with trapping reagent (4-hydroxybenzoic acid) in anesthetized rats, we monitored myocardial interstitial 3, 4-DHBA levels as an index of OH production during myocardial ischemia/reperfusion in the presence (pargyline group) and absence (control group) of MAO inhibitor, pargyline.
Result: In control group, dialysate 3, 4-DHBA concentration at baseline was 2.01 ± 0.26 nM. Dialysate 3, 4-DHBA concentration did not change during ischemia, but significantly increased to 3.80 ± 0.26 nM immediately after reperfusion and then kept this high levels by 60 min after reperfusion. In pargyline group, local administration of pargyline (1 mM) did not change dialysate 3, 4-DHBA concentration at baseline (1.88 ± 0.34 nM) and during ischemia, but significantly suppressed the increase in dialysate 3, 4DHBA after reperfusion.
Conclusion: MAO plays a significant role on hydroxyl radical production during ischemia/reperfusion, suggesting that MAO inhibition can attenuate cardiac ischemia/reperfusion injury.
(COI: No)

P2-230

Ghrelin acts directly on the central nervous system to suppress cardiac sympathetic tone and arrhythmias following acute myocardial infarction in rats

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Cardiac sympathetic nerve activity (CSNA) increases following acute myocardial infarction (MI). This increase adversely triggers life-threatening arrhythmias. Subcutaneous injection of ghrelin prevents the CSNA increase following MI and reduces the occurrence of arrhythmias, improving survival dramatically. The mechanisms by which ghrelin achieves this effect remains unclear. This study aimed to identify whether ghrelin acts directly within the brain to modulate CSNA following acute MI. Rats were anesthetized with urethane and surgically prepared: isolation and recording from cardiac sympathetic nerve, cannulation for systemic arterial pressure measurement, stereotaxic surgery for intracerebroventricular (icv) administering of ghrelin, ligation of the left anterior descending coronary artery (= MI). CSNA were continuously recorded prior to LAD occlusion, and for three consecutive hours following: no manipulation, MI + saline icv injection and MI + ghrelin icv injection. Within three hours of acute MI, untreated rats exhibited: a significant 200% increase in CSNA, a high incidence of arrhythmias and, thus a 34% mortality rate. The ghrelin injection reduced: the CSNA increase (62% increase), the incidence of arrhythmias and, thus, mortality rate (0% within three hours of MI). These results suggest that the direct action of ghrelin on the brain contributes to its suppressive effect on CSNA and arrhythmias following acute MI.
(COI: No)

P2-231

Properties of spontaneous activity in submucosal postcapillary venules of the rat stomach

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Background: We have recently reported spontaneous vasomotion (rhythmic constrictions) of the venules with diameters of 30-130 μm in the bladder, distal colon and stomach. Venular vasomotions may actively regulate tissue metabolite drainage. Here we further examined properties of spontaneous activity of postcapillary venules (PCVs) with diameters of 15-23 μm.
Methods: In the rat gastric submucosa, changes in PCV diameter, intracellular Ca^{2+} signalling of PCV mural cells and their morphology were examined by video imaging, Fluo-8 Ca^{2+} imaging and immuohistochemistry, respectively.
Results: PCV mural cells were α-smooth muscle actin-positive stellate cells and exhibited synchronous spontaneous Ca^{2+} transients associated with vasomotion. Inhibitors of endoplasmic reticulum Ca^{2+}-ATPase (10 μM CPA) or IP_3 receptor (100 μM 2-APB) abolished vasomotion. Nifedipine (1 μM) disrupted Ca^{2+} transient synchrony amongst PCV mural cells and prevented vasomotion. PCV endothelium expressed NO synthase, and tadalafil (1 μM), cGMP-specific phosphodiesterase type 5 (PDE5) inhibitor, abolished vasomotion, suggesting that NO may be continuously released from endothelium to produce cGMP in mural cells.
Conclusion: PCV in the rat stomach submucosa exhibits spontaneous vasomotion. The generation of spontaneous Ca^{2+} transients in PCV mural cells may depend on Ca^{2+} release/uptake of intracellular Ca^{2+} store, while voltage-dependent coupling relying on L-type Ca^{2+} channels appears to be essential for their synchrony. Continuous PDE5 activity that degrades cGMP may be crucial to maintain spontaneous vasomotion.
(COI: No)

P2-232

Relationship between local and overall pulse wave velocity in the aorta preserved well despite of extent and severity of atherosclerotic lesions in heritable hypercholesterolemic rabbits

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We investigated whether relationship between local pulse wave velocity (LPWV) and overall aortic pulse wave velocity (AoPWV) preserved or not despite of extent and severity of atherosclerotic lesions in Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits aged 10-12 and 22-24 months. A catheter-tip transducer was indwelt in the ascending aorta (AA) and another catheter with three pressure sensors at the tip (40 mm intervals) was advanced to the distal end of the aortic arch (Position 0: P.0) under pentobarbital anesthesia (30 mg/kg, i.v.). Pressure waves were recorded at P.0, proximal (P.1), middle (P.2) and distal (P.3) thoracic aortas and proximal (P.4), middle (P.5) and distal (P.6) abdominal aortas simultaneously with those at AA by moving the catheter towards peripherals every 80 mm to calculate LPWV between adjacent aortic positions. There was little difference in averaged LPWV in different aortic segments (avgLPWV) and AoPWV in the normal and KHC rabbit groups aged 10-12 and 22-24 months. Strong positive correlation between avgLPWV and AoPWV was observed in the two strains of the two age groups. Mean error and standard deviation between avgLPWV and AoPWV were very small in Bland & Altman plots in the two strains of the two age groups. We can conclude that the relationship between avgLPWV and AoPWV preserved well regardless of extent and severity of atherosclerotic lesions.
(COI: No)

P2-233

Functional correlation of the arterial structure - a comparative study in the kidney and the skeletal muscle

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The differences of circulation in various organs are well known, but the structural differences are only poorly investigated. The wall structure of arteries was observed in the rat kidney and skeletal muscle. The wall thickness in the kidney was almost equal to that in the muscle about 40 μm in diameter, relatively thin in the larger arteries and thick in the smaller arteries. The smooth muscle cells were regularly arranged in parallel in circular or slightly spiral orientation, whereas those in the skeletal muscle were irregularly arranged in heterogeneous orientations. Extracellular matrices were more abundant in the arterial media in the skeletal muscle than in the kidney. The inner elastic lamina was continuous in the kidney, and longitudinal bundles in the skeletal muscle. The adventitial collagen fibers were abundant and dense in the skeletal muscle, and made scattering small bundles in the fluid filled spaces in the kidney. The arteries in the skeletal muscle were under severe mechanical stress in longitudinal direction during muscle contraction, and dilate dramatically by sympathetic stimuli. Those in the kidney keep the glomerular pressure constant and regulate it slightly. The differences of arterial structure in these organs reflect their functional differences well.
(COI: No)

P2-234

Comparison of heart rate, stroke volume, and blood pressure associated with acupuncture stimulation in supine and sitting subjects

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Acupuncture stimulation (ACP) induces the reduction of heart rate (HR) with the somato-autonomic reflex. Previous studies indicated that ACP responses were reflected by position change on HR. The present study was to investigate the comprehensive change of cardiovascular responses in supine and/or sitting position during ACP. Twenty-two healthy male volunteers participated in this examination. HR, stroke volume (SV), and blood pressure (BP) had been recorded on the both positions in same subjects. ACP had performed to left forearm for 60 seconds that inserted to a depth of 15 to 20 mm after approximately 15 minutes as the rest. Changes of cardiovascular response were compared between both positions. HR and diastolic BP (DBP) were significantly decreased that accompanied with the increase of systolic BP (SBP) and SV in supine position. In sitting position, the decrease of HR and the increase of SV, but not BP, were observed during ACP. Moreover, the decrease of HR in sitting position was stronger than that of supine, and the increase of SV in sitting was lower than that in supine. It seems that the decrease of HR is induced by ACP and increase of SV is dependent on the Starling's law. In sitting position, we are considering the relation of the gravitation and another factor to suppress the increase of SV during ACP.
(COI: No)

P2-235

Acupuncture stimulation induced-changes of hemodynamics

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Acupuncture (ACP) stimulation induces the significant reduction of heart rate (HR) via the somato-autonomic reflex. However, it is unknown whether there are influences on another hemodynamics by ACP stimulation. The present study was to assess ACP stimulation induced-comprehensive change of hemodynamics, which was HR, stroke volume (SV), and blood pressure (BP). Twenty-seven healthy male volunteers were participated in this examination. HR, SV, and BP were recorded at supine position. ACP, using stainless steel needle, was inserted to left forearm at a depth of 15 to 20 mm. ACP was continuously stimulated for 60 seconds after approximately 15 minutes as the rest. Each date were calculated the average value every 10 seconds, and were analyzed before, during and after ACP stimulation. HR had been decreased and SV had been increased throughout ACP stimulation. On the other hands, diastolic blood pressure was significantly decreased only the 20 seconds just after ACP inserting and recovered to control after 40 seconds. These results suggest that ACP stimulation induced-decreasing HR is mediated by somato-autonomic reflex and increasing SV is mediated by Starling's law of the heart. In contrast, although BP is affected by the ACP stimulation-induced somato-autonomic reflex partially, BP may recover to basic value immediately because of the baroreceptor reflex.

(COI: No)

P2-236

Analysis of rate-dependence of drug-induced QT-prolongation in human iPS-derived cardiomyocytes

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Human iPS cell-derived cardiomyocyte (hiPS-CM) is conceptually promising as an unlimited source of human cardiomyocytes for pre-clinical cardiac safety screening. However, the spontaneous activity of hiPS-CM impedes experimental manipulation of pacing rates, and therefore hinders the prediction of antiarrhythmic and proarrhythmic effects of rate-dependent drugs. In order to evaluate rate-dependent cardiac medicine in hiPS-CMs, we here developed a hiPS-CM model with matured phenotypes by transducing ventricular-specific gene X. The gene-X transduced hiPS-CMs ceased spontaneous beating both in single cells and multicellular sheets, but could generate ventricular-like action potentials when triggered by a stimulus and followed at various pacing rates (0.5, 1 and 2 Hz). Using this model, we could demonstrate quantitatively that E4031, a selective hERG blocker, prolongs durations of action potential in single myocytes and extracellular field potential in cardiac sheets, respectively. In the cardiac sheets, the technology enabled us to evaluate a reverse rate-dependence with or without E4031, and the addition of a selective I_{Ks} blocker, chromanol 293B, abolished it, showing a contribution of I_{Ks} to the reverse rate-dependence. Thus, our genetically modified hiPS-cardiomyocytes can be useful especially for evaluating of rate-dependent antiarrhythmic and proarrhythmic drugs.

(COI: No)

P2-237

Lymphatic circulation of cerebrospinal fluid in spinal regions - A morphological investigation and its clinical significance -

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Cerebrospinal fluid (CSF) is a fluid derived from choroid plexuses in the cerebral ventricles. It is generally considered that CSF is absorbed in several sites including venous sinuses via arachnoid villi, capillaries in the choroid plexus and lymphatic systems. Cranial drainage route along the olfactory nerve is one of the best known pathways of lymphatic absorbing systems whereas the regional lymphatic system at the spinal level remains obscure. We therefore aimed to elucidate the spinal CSF drainage route to the regional lymphatic system in Sprague Dawley (SD) rats using various CSF tracers. They were infused into the lateral ventricle with an osmotic pump followed by dissection of vertebral blocks, spinal nerves and regional lymph nodes. In addition, various types of cells of syngeneic rats were labeled with green fluorescent cell linker kit and injected into the cisterna magna. We found that only small tracers collected in lymphatics around the dorsal root ganglions, finally drained into the regional lymph nodes. Lymphoid cells also reached to each lymph node, whereas erythrocytes could not. Vital cells with chemotactic activity could migrate through this way though high-molecular-weight compounds could not pass alone. Hence, this drainage system might play an important role in the defense mechanism. It is highly indisputable that CSF in spinal subarachnoid space is absorbed in epidural lymphatics.

(COI: No)

P2-238

Role of TRPC3/TRPC6 activated by angiotensin II type 1 receptor in the slow force response to sustained stretch in mouse ventricular myocytes

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When cardiac muscle is stretched over several minutes, its intracellular Ca^{2+} transient and twitch force slowly increase, which is known as a slow force response to stretch (SFR). It has been reported that stretch-induced release of angiotensin II have been implicated in the SFR, to raise intracellular Na^{+}, followed by an increase in intracellular Ca^{2+} via Na^{+}/Ca^{2+} exchanger. However, the detailed pathways remain unclear. The activation of angiotensin II type 1 receptors (AT1R) has been reported to induce cation influx via TRPCs (TRPC3/TRPC6), which are known as nonselective cation channels. In this study, we tested the hypothesis that this pathway leads to SFR. A pair of piezo-positioned carbon fibers was attached to each end of an isolated mouse ventricular myocyte. The electrically stimulated cells were perfused in Tyrode solution at room temperature. Passive/active forces were calculated from carbon fibers bending. The stretch by moving carbon fibers led to an immediate increase in twitch force by Frank-Starling mechanism. The force slowly increased by 133.2 ± 2.0% of the force immediately for 300 s after the stretch. The SFR was blocked by AT1R inhibitor, olmesartan (97.6 ± 1.5%). 2-Aminoethyldiphenylborinate (TRPs inhibitor), YM-58483 (TRPCs inhibitor), and Rox-4560 (TRPC3/TRPC6 inhibitor) suppressed the SFR (93.2 ± 4.7%, 98.0 ± 2.4%, and 97.8 ± 2.4%). These results suggest that the activation of TRPC3/TRPC6 initiated by AT1R is involved in SFR.

(COI: No)

P2-239

Age-related effects of dexmedetomidine, an alpha-2 agonist, on coronary vasoactivity and ventricular contraction in guinea-pig hearts

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Dexmedetomidine (DEX) is a potent and selective alpha-2 agonist, and is widely used to produce both sedation, analgesia and anxiolytic effects. It has been reported that DEX sporadically causes bradycardia and hypotension, probably due to its sympatholytic activity. However, precise mechanisms such as involvement of peripheral postsynaptic alpha-2B receptors as well as direct effects on cardiac contractility are not yet clear. This study was carried out in order to evaluate the effects of DEX on coronary vasoactivity and ventricular contraction of guinea-pig hearts using Langendorff perfusion device. The heart was continuously perfused with Tyrode solution, and left ventricular pressure (LVP) and coronary perfusion pressure (CPP) were recorded using a PowerLab. DEX did not affect basal LVP, but markedly inhibited the increase of LVP induced by electro-field stimulation. The finding is consistent with a view that DEC acts on alpha2-receptors at sympathetic nerve terminals. On the other hand, we found that DEX also affected the coronary artery resistance, and that its effects altered with ages. Namely, DEX was without effect on CPP at ages of <4 weeks, but increased CPP at 4-8 weeks. The increase of CPP was more significant at 9-12 weeks, and was inhibited by prazosin, an alpha1/alpha2-adrenergic receptor antagonist. DEX may directly affect coronary vasoactivity via alpha1/alpha2-adrenergic receptors.

(COI: No)

P2-240

Spread of spontaneous transient hyperpolarizations within vascular endothelial cell layer

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The vascular endothelial cells are some 40 μm long and some 7 μm wide, and aligned with their major axes along the vessel. These cells are connected to each other with a lot of gap junctions and the whole endothelial layer is functioning as a syncytium. The electrical resistance seems to be larger in the circumferential pathway than in the axial one because the former includes more gap junctions than the latter. Using the conventional whole-cell clamp techniques, the spread of the spontaneous activities were examined in the endothelial layer acutely dispersed from the guinea-pig mesenteric artery. Two patch electrodes were applied to two individual cells separated either circumferentially or axially along the vessel and the membrane potentials (V_1 and V_2) were observed. The membrane potential was not constant but transient hyperpolarizations randomly occurred. The origin of such a spontaneous activity could be any cell within the syncytium. When one of the patched cells (V_1) is the origin, the amplitude of the hyperpolarization recorded from that cell is expected to be large and the amplitude ratio (V_2/V_1) should be smallest compared to any hyperpolarizations occur in cells other than patched ones. Among many recorded hyperpolarizations, large ones were selected and the distributions of the ratios (V_2/V_1) were compared between the circumferential and the axial directions. Unexpectedly the distributions were not so different between two directions and the electrical signals seem to spread within the endothelial layer into all directions rather equally.

(COI: No)

P2-241

Opening of Rat Ductus Arteriosus is promoted by inflammation

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Background: The ductus arteriosus(DA) is closing at birth. In premature babies, patent DA(PDA) can be fatal. We sometimes experience DAs reopen when the premature babies are exposed to the severe infection. The reopening of DA has not been cleared. We reported NFkB inhibition might facilitate DA closure. NFkB is one of transcriptional factors and relates to some inflammation. We thought NFkB works as an opening factor in the infection.
Methods and Results: We made LPS- stimulated rat models as the infectious models. LPS(100micro gram/kg) were injected to the maternal rats in pregnant day 18th and 19th. In the pregnant day 21st, the maternal rats were performed Caesarean sections under the anesthesia. Rat fetuses were injected IMD- 0354(NFkB inhibitor, IMD), carboxymethyl cellulose and PBS in intraperitoneal administration through maternal uterine wall. Rat fetuses were born and started breathing right after the infection. 30 minutes later, rat fetuses were sacrificed and frozen by liquid nitrogen. LPS injected rat fetuses DA tend to wider compared with nothing injected one. IMD injected after LPS injected rat fetuses DA tend to more narrow than other control models DA.
Conclusion: The present data demonstrated that LPS can open rat DAs and NFkB might facilitate DA opening.
(COI: No)

P2-242

Mechanism of negative inotropic effect on rat left ventricular in hyperthermia: role of TRPV1

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We previously reported that the effects of hyperthermia (42 ℃) on left ventricular (LV) mechanical work and energetics using the excised, cross-circulated rat heart model. We now investigated the effects of capsazepin (a TRPV1 antagonist) on LV mechanical work and energetics in hyperthermia. We analyzed the LV end-systolic pressure-volume relation (ESPVR) and the linear relation between the myocardial oxygen consumption per beat (VO_2) and systolic pressure-volume area (PVA; a total mechanical energy per beat) in isovolumically contracting rat hearts at 300-bpm pacing during infusion of capsazepin (50 μM) under hyperthermic conditions. Downward-shift of LV ESPVR from the control one was observed in hyperthermic-hearts, which was suppressed by capsazepin infusion. The mean slope and the mean VO_2 intercept, which is composed of each myocardial oxygen consumption for calcium handling in excitation-contraction coupling and for basal metabolism, of VO_2 -PVA relations were not significantly different in hyperthermic-hearts. The slope was unchanged but the VO_2 intercept was decreased in capsazepin treated-hyperthermic hearts. Protein levels of SERCA2 and phospholamban (PLB) were unchanged but phosphorylated PLB at threonine 17 was markedly decreased in hyperthermia. The latter decrease was not antagonized by treatment with capsazepin. These results suggested that negative inotropic effect in hyperthermic heart was, at least in part, mediated through TRPV1 signaling pathway.
(COI: No)

P2-243

Expression and functional significance of 5'-nucleotidase in lymphatic vessels

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The cellular expression and physiological function of 5'-nucleotidase (5'-Nase; CD73) in lymphatic vessels was investigated in rats by histochemical methods and in three-dimensional human cell culture system fabricated by layer by layer cell accumulation technique. Intense immunoreactivity of CD73 was predominantly found in the endothelial cells with cellular nuclei immunopositive for Prox1 (a master transcription factor of lymphatic endothelial cells) in specific regions of some large veins and lymphatics sprouting from those at mid-embryonic stage. The CD73-immunoreaction was thereafter demonstrated in several portions, especially in the leading tips, of developing lymphatics and every lymphatic of adult rat. The findings imply that 5'-Nase is preferentially expressed in lymphatic vessels both during vascular development and in mature state to operate on development and function of lymphatic and blood vessels. In addition, our knock down analysis using siRNA for 5'-Nase in 3D-human cell culture system showed an accelerated formation of lymphatic network, it suggesting an inhibitory action of 5'-Nase on control of lymphatic vascular development. In conclusion, it is likely that 5'-Nase regulates development and function of lymphatic vessels repressively in an autocrine fashion.
(COI: No)

P2-244

Glucagon-like peptide-1 (GLP-1) augments the stretch-induced release of atrial natriuretic peptide(ANP) from mouse atria

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Glucagon-like peptide-1 (GLP-1), a member of incretin peptides, has been shown to improve diabetes mellitus and heart failure. GLP-1 receptor is known to be linked with cAMP signaling pathway. Recent studies have shown that GLP-1 receptor is expressed in atrial myocytes. However, it has been controversial whether or not GLP-1 induces the release of atrial natriuretic peptide(ANP). This study was undertaken to clarify effects of GLP-1 on atrial function such as developed force and atrial rate, as well as the relationship between basal tension and ANP release.
Methods: Atria were excised from male mice (C57BL/6J) under deep anesthesia. We measured effects of GLP-1 on developed force, atrial rate, and ANP release from isolated atrial tissues under loads of various basal tensions (0.2-2.0 g).
Results & Discussion: The developed force of mouse atria was augmented depending on the basal tension, while atrial rate was rather decreased. GLP-1 (1 nM) caused significant increase of ANP release from isolated atria. GLP-1 tended to augment the basal tension-dependence of developed force. In sharp contrast, atrial rate was not affected by the application of GLP-1, although recent study has reported that GLP-1 is expressed in mouse sinoatrial node. These results suggest that GLP-1 augments the stretch-induced release of ANP from mouse atria, and that GLP-1 receptor signaling pathway may be linked with distinct regulatory mechanisms of atrial function.
(COI: No)

P2-245

The intracellular mechanisms of the constriction of the guinea pig hepatic veins

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We examined the intracellular mechanisms of the contraction on the guinea-pig hepatic veins. Phentolamin (3 μM) inhibited the contraction evoked by transmural nerve stimulation (TNS). 1 - 30 μM phenylephrine evoked vasoconstriction in a dose response manner. Nifedipine (1 μM) did not have any effects to the TNS-evoked contraction. CPA (10 μM) inhibited the contraction. In the presence of CPA, SKF-96365 (3 μM) increased the amplitude of the contraction. This enhancement effect of SKF-96365 was inhibited by N_ω-Nitro-L-arginine (10 μM). In contrast, the amplitude of contraction evoked by phenylephrine was decreased to only 70 % by 100 μM sodium nitroprusside. Y-27632 inhibited both the TNS- and phenylephrine-evoked contraction. The intensity of the intracellular Ca^{2+} indicator, Fluo-4, was increased in the smooth muscle cells when the cells were stimulated with 10 μM phenylephrine. These results suggest that the adrenergic nerves stimulate both the Ca^{2+} dependent and independent mechanisms to evoke the contraction in the smooth muscle cells of the hepatic vein. The L-type Ca^{2+} channels or the store operated Ca^{2+} channels do not seem to be the main source to cause the intracellular Ca^{2+} increase by the α adrenergic stimulation. The endothelial regulation of the vasoconstriction was limited.
(COI: No)

P2-246

Low birth weight is a predictor of later hypertension risk for both Japanese and Mongolian healthy young adults

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Low birth weight (LBW) was confirmed as a risk of high blood pressure (BP) in later stages of life (Barker DJ et al, 1989). Low-grade inflammation and deterioration of autonomic regulation play an important role in hypertension. However, the associations with birth weight are poorly understood. We examined these relationships in Mongolian and Japanese young adults and investigated whether ethnicity affected these relationships. We measured BP and heart rate variability at rest and during postural change from a supine to a sitting position in 21 Japanese and 16 Mongolian healthy volunteers aged 18-34 years. Blood cell counts, and total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol, triglyceride, and high sensitivity C-reactive protein levels were measured. Mongolians had lower levels of HDL-C than did the Japanese ($p < 0.01$). In Mongolians, the platelet count was higher in the LBW group than in the normal birth weight (NBW) group ($p < 0.05$). Following postural change, systolic blood pressure and heart rate showed no significant increases in the Mongolian and Japanese LBW groups, whereas the NBW groups had normal responses ($p < 0.05$). The Mongolian LBW group displayed a slight decrease in sympathetic nerve activity from a supine to a sitting position, although it increased in the Japanese LBW group ($p < 0.05$). We suggest that LBW is a predictor of later hypertension risks in both Japanese and Mongolian healthy young adults.
(COI: No)

P2-247

Differences in respiratory parameters between controlled $PetCO_2$ and controlled respiratory rate during cycling exercise

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Previous studies have reported that in oxygen uptake kinetics, the time constant increased during transition to heavy cycling exercise in respiratory alkalosis by a controlled end-tidal partial pressure of CO_2 ($PetCO_2$), while the time constant decreased in respiratory alkalosis by a controlled respiratory rate. This study aimed to clarify differences in respiratory parameters between a controlled $PetCO_2$ and a controlled respiratory rate. After $PetCO_2$ was controlled at 20 mmHg (PE) or the respiratory rate at 60 breath/min (RE) for 5 min, a subject performed baseline cycling exercise for 4 min at 10 W, followed by heavy cycling exercise for 6 min at the anaerobic threshold (AT) plus 40% of the difference between the AT and peak pulmonary oxygen uptake intensity. Throughout the experiment, $PetCO_2$ or respiratory rate was maintained at 20 mmHg or 60 breath/min, respectively. In the control experiment (CE), a subject breathed freely throughout the experiment. The breath-by-breath pulmonary gas exchange was measured and heart rate was estimated using an electrocardiogram. At the time of starting baseline exercise, $PetCO_2$ in PE and RE were lower than CE. During baseline exercise, $PetCO_2$ in RE increased, and $PetCO_2$ in PE was lower than RE and CE at the time of starting heavy exercise. Respiratory quotient in PE was higher than RE and CE during baseline exercise, and then decreased to lower than that in RE and CE during heavy exercise. It is possible that these differences between PE and RE have an effect on oxygen uptake kinetics.

(COI: No)

P2-248

Class III/IV POU transcription factors expressed in small cell lung cancer cells are involved in proneural/neuroendocrine differentiation

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One-third of lung malignancies demonstrate a proneural/neuroendocrine phenotype or type of differentiation. However, it has not been clearly elucidated how proneural/neuroendocrine differentiation is controlled in lung cancers. We recently demonstrated that the POU3F2 gene plays a significant role in proneural/neuroendocrine differentiation of lung cancers. Because class III POU genes (POU3F1, POU3F2, POU3F3, and POU3F4) and class IV POU genes (POU4F1, POU4F2, and POU4F3) share similar properties in neural development, we analyzed the association between class III/IV POU genes and a proneural/neuroendocrine phenotype in lung cancers using seven small cell lung cancer (SCLC) cell lines and twelve non-SCLC (NSCLC) cell lines. Class III/IV POU gene expression was generally restricted to SCLC cells. However, the forced expression of class III/IV POU genes in the NSCLC cell lines induced the expression of neuroendocrine-specific markers (neural call adhesion molecule 1, synaptophysin, and chromogranin A) and proneural transcription factors (achaete-scute homolog-like 1, NeuroD1, and thyroid transcription factor 1) in various degrees. Furthermore, each class III/IV POU gene induced other class III/IV POU genes, suggesting the mutual induction of class III/IV POU genes. These findings suggest that the expression of class III/IV POU genes is important for the proneural/neuroendocrine differentiation of lung cancer cells.

(COI: No)

P2-249

Sustained vocalizations suppress expiration of carbon dioxide during *kendo* exercises

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One of the distinct traits of *kendo*, the traditional Japanese martial art of fencing, is the execution of sustained, high-effort vocalizations during actions. The purpose of this study was to determine the effect of these vocalizations on respiratory functions. Respiratory indicators of eight university *kendo* athletes were analyzed using a portable breath gas analyzer during the most intensive *kendo* exercise, *kakari-keiko*, with and without vocalization. Breathing frequency (f_B) increased regardless of vocalization, but in trials with vocalization, f_B and expired minute ventilation were significantly smaller, and expiration time was significantly longer. Components of expired gases were also affected by vocalization: Although there was no significant difference in oxygen uptake, vocalization yielded a reduction in carbon dioxide output (VCO_2) and an increase in fraction of end-tidal carbon dioxide ($EtCO_2$). Thus, we conclude that these vocalizations greatly affect expiration breathing patterns in *kendo*. Moreover, repetition of *kakari-keiko* caused a reduction in VCO_2 and an increase in $EtCO_2$. We consider the possibility that the sustained high-effort vocalizations of *kendo* also increase cerebral blood flow.

(COI: No)

P2-250

Nasal but not tracheal TRPA1 contributes to irritant-induced respiratory slowing

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Transient receptor potential ankyrin 1 (TRPA1), a member of the TRP superfamily, exists in the sensory neurons including trigeminal neurons innervating nasal cavity (Sci Rep 3:3100, 2013) and vagal neurons innervating trachea (Nat Chem Biol 7:701, 2011). Although TRPA1 has been proposed as an irritant receptor of which stimulation triggers respiratory slowing, precise locations of responsible receptor have not been known. Here we examined relative importance of TRPA1 located in upper airway (nasal) and lower airway (trachea). Urethane (1.3-1.5 g/kg)-anesthetized male mice were studied. The trachea was sectioned just caudal to the thyroid cartilage. A cannula was inserted into the rostral sectioned trachea with its tip just passing through the posterior nasal aperture to the nasal cavity for upper airway stimulation. Another cannula was inserted into the caudal sectioned trachea for both ventilation and lower airway stimulation. Flow velocity of spontaneous breathing was measured with a Lilly type flow meter attached to one end of a T-tube inserted into the caudal sectioned trachea. A vapor of one of the TRPA1-stimulants, allyl isothiocyanate (AITC), was introduced into the airline by placing a cotton paper soaked with 20 uL of AITC solution (98%). AITC slowed the respiratory frequency when applied to the upper (ca -30%) but not to the lower airway (ca -5%). No response was observed in TRPA1 knockout mice. These data clearly show that TRPA1 in the nasal cavity is more important than that in the trachea for irritant-induced respiratory slowing.

(COI: No)

P2-251

Blockade of astrocytic activation augments hypoxia-induced depression of ventilation and EEG

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Although mild hypoxia increases ventilation, severe hypoxia disturbs consciousness and decreases ventilation, which makes the subject more hypoxic and eventually causes death. Respiratory motor output is mainly controlled in the brainstem. However, ventilatory response to hypoxia is also dependent on the higher brain status. Recent advances in glial physiology have demonstrated that astrocytes play important roles in information processing in various brain regions. Here we hypothesized that the maintenance of both the higher brain and the brainstem functions are critically dependent on the activity of astrocytes, and analyzed the effects of astrocytic activation blockade on the higher brain function and ventilation. In unanesthetized adult mice the higher brain status and ventilation were monitored by EEG and whole body plethysmography, respectively. Mild (12%) and severe (6%) hypoxia was loaded to mice before and after administration of arundic acid which blocked activation of astrocytes. Severe hypoxia-induced ventilatory depression was accompanied by disturbance of the higher brain that would decrease the central command to the brainstem respiratory center. Hypoxia-induced inhibition of EEG and ventilation was augmented with arundic acid. We suggest that astrocytes importantly contribute to the maintenance of the higher brain function and ventilation under hypoxia.

(COI: Properly Declared)

P2-252

Functional expression of TRPV4 receptor in mouse nasal epithelium

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The nasal epithelium consists of the olfactory and respiratory ciliated epithelia, both of which have sensory properties that are able to respond to various stimuli, such as odorants, carbon dioxide and physical pressures. Transient receptor potential vanilloid type 4 (TRPV4) is known to be a Ca^{2+}-permeable cation channels activated by various physical and chemical stimuli. However, TRPV4 expression in the nasal epithelium has not been previously investigated. We performed RT-PCR, *in situ* hybridization, immunohistochemistry and calcium imaging analysis using wild-type (WT) and TRPV4-knockout (TRPV4-KO) mice to examine the functional expression of TRPV4 channels in the nasal epithelium. TRPV4 mRNA was expressed in the nasal epithelial tissues. TRPV4-positive immunoreactions were observed in the basal cells in both the olfactory and ciliated epithelia. Calcium imaging analysis showed that 4 α-PDD, a TRPV4 agonist, increased an intracellular calcium concentration in a subset of dissociated nasal epithelial cells, indicating the presence of functional TRPV4 channels in the nasal epithelium. Taken altogether, functional TRPV4 is specifically expressed in the basal cells in both the olfactory and respiratory ciliated epithelia. Since TRPV4 is present in the basal cells of some stratified epithelia, such as the urothelium and esophageal epithelium, TRPV4 receptor may be a universal regulator of Ca^{2+}-dependent signaling pathways linked to cell proliferation, cell survival, ATP release, and cytokine production in the basal cells.

(COI: No)

P2-253

Loose regularities of calcium bursting sequence among inspiratory cells in the pre-Botzinger complex during rhythmic burst

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Spontaneous respiratory rhythm is essential for our life activity. Medullary slices containing the pre-Botzinger complex (preBotC) can preserve the spontaneous respiratory rhythmic activity. In the preBotC, respiratory cells including pacemaker cells activate stochastically with each rhythmic burst. In spite of the importance to understand the neuronal network structure and function, the manner of bursting sequence among respiratory cells has not been studied well. Here, we investigated bursting sequences among inspiratory cells in the preBotC during rhythmic bursts using wide-field calcium imaging. For the evaluation of rhythmic calcium bursting sequence, we adopted onset timing and peak timing of calcium fluctuation during rhythmic burst in individual cells. These two timings had weak correlation, which suggests that the parameters might reflect different physiological events. The sequences of both timing changed flexibly at each individual rhythmic burst, and the two kinds of sequences were also different from each other even in the same sequence. In addition, a subset of inspiratory cells covered the earliest activations in the sequences of both timings. These results suggest that the sequence of rhythmic calcium bursts has some loose regularities but is not invariable.

(COI: No)

P2-254

Light and Electron Microscopical Study on the Particulate Bodies Found in Epithelium of Rat Airway

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We found particulates in the epithelium of rat larynx and trachea, but the nature has never been clarified. In this study, we aimed to clarify the characteristics of these particulate.

MATERIALS and METHODS: Larynges, tracheae and lungs from 5 male Wistar rats were observed under a light microscope, and tracheae from 3 male Wistar rats were observed under transmission electron microscope.

RESULTS: The particulate in the epithelium of larynx and trachea were strongly positive for periodic acid Schiff reaction (PAS) and poorly stained with hematoxylin-eosin staining. Particulates with similar stainabilities were also found in the food. The particulates were surrounded by a flattened epithelial cell and frequently found in the ventral epithelium of larynx and in the dorsal epithelium of trachea. The epithelial cells which surrounded the particulates were non-ciliated cells. The microvilli were found in the surface face to the particulate. The secretory granules showing similar electron density with the particulates were found in the cytoplasm of the epithelial cells. The fusion of the particulate with the secretory substances secreted from epithelial cells was rarely found.

DISCUSSION: From the present findings, the origin of particulate bodies is discussed: the exogenous food-derived particulates inhaled into the airway or the intrinsic substances secreted from epithelial cells.

(COI: No)

P2-255

Simultaneous imaging of hemodynamics and hypoxia signals in lung tissues of mouse melanoma metastasis with in vivo cryotechnique

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Cancer cells in metastatic foci need blood supply to proliferate and develop, as those in their primary nest. In this study, we have clarified dynamic structures of blood vessels and circulation in lung metastatic tumor masses (mouse melanoma B16-BL6 cells). The relationship between blood supply and appearance of ischemia signal, HIF-1 α, was compared in primary tumor nests and metastatic tumor masses with in vivo cryotechnique (IVCT), which can capture animal cells and tissues without anoxia. The lung metastatic tissues could be categorized into three areas: (1) tumor masses with poor blood vessels, (2) tumor cells around large blood vessels, and (3) tumor tissues with abundant blood capillaries. In the area (1), melanoma cells were often localized near pleura, and their HIF-1 α was expressed equally in each nucleus, similar to the primary melanoma cells. In the area (3), blood capillaries were accompanied with elastic or collagen fibers, endothelia and type I epithelial cells, resembling the alveolar septum. Horseradish peroxidase injection also revealed that blood circulation was well maintained in these areas. Immunoreactivities of HIF-1 α were varied in the tumor masses, relating to surrounding type I epithelial cells. Therefore, oxygen of some lung metastatic tumors is supplied by original alveolar capillary structures, different from that in their primary nests.

(COI: No)

P2-256

The pulmonary artery remodeling during recovery phase in the hyperoxia-induced newborn lung injury ~Angiopoietin-1 might be therapeutic strategy in the injured developing lung~

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Bronchopulmoany dysplasia (BPD) is a chronic lung disease of premature infants associated with pulmonary alveolar and microvascular injury, and causes the long-term respiratory cardiac problems. In the previous study, we demonstrated that injured developing lung continues to have an abnormal alveolar and microvascular structure even after recovery period, and that Angiopoietin-1(Ang-1) might improve those structural changes. But we don't have enough information about pulmonary artery remodeling (PAR) in those models. And therefore we hypothesized that injured developing lung is associated with continuous abnormal PAR and Ang-1 treatment improves those changes, we investigated PAR with the parameter of medial thickness (MT), adventitial thickness (AT), and total wall thickness (WT) for stereological analysis, by using a hyperoxia-exposed mouse model of BPD. The recovery models still had increased distal air-space area, decreased abundance of secondary septae and thick blood-air barriers. MT%, AT%, and WT%, which increased in the hyperoxic lung, still continued to be larger in recovery models. On the other hand, Ang-1 treated lung partially improved those changes. Those results suggest that injured developing lung continues to have abnormal structure even in recovery period, which is correlated with high morbidity and mortality of BPD patients. Ang-1 might be some therapeutic approach in BPD.

(COI: No)

P2-257

Primary cilia localization of Nphp3 is responsible for renal function and morphology

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Nephronophthisis (NPHP) is an autosomal recessive cystic kidney disease. More than a dozen genes have been identified that cause NPHP. The gene products of NPHP, nephrocystins, are presumed to function in the same pathway. Most of their products localize to cilium or centriole. Nephrocystins-1, -4, -5, -6 are localized to the transition zone of cilium or centriole, and proposed to function as ciliary barrier. Nephrocystin-2/inv, nephrocystin-3 and nephrocystin-9/nek8 are localized the proximal segment of the ciliary shaft named "Inv compartment". We previously reported that Nephrocystin-2/inv acts as an anchor for nephrocystin-3 and nek8. Therefore, it is likely that inv, nephrocystin-3 and nek8 make a functional complex. Although nephrocystins are localized in the cilia or centriole, it is still unknown if the localization of cilia or centriole is essential for their functions. N-terminal second glycine is also essential for trafficking of NPHP3 into the cilia. Replacement of the second glycine to alanine abolished ciliary translocation of nephrocystin-3. To understand the function of nephrocystin-3 in the cilia, we generated Nphp3 Gly2Ala (G2A) knock-in mouse. Two ES cell lines line 35 and 88, carrying the mutation, were generated and chimeric mice were made. The chimeric mice were mated with CAG-cre mice to remove the loxP-flanked neomycin selection cassette. We are going to present the phenotypes of the knock-in mouse mutants.

(COI: No)

P2-258

Involvement of podocin on abnormal receptor-response and mechanosensitivity of mutant TRPC6 channels associated with familial focal segmental glomerulosclerosis (FSGS)

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The mutations of transient receptor potential cation channel 6 (TRPC6) are causative to some hereditary forms of focal segmental glomerulosclerosis (FSGS). We previously investigated the functional impacts of murine TRPC6 FSGS mutations near its N-terminal ankyrin repeats (G108S, P111Q, N124S, M131T, N142S, R174Q) in heterologous expression system with Ca^{2+} imaging and patch clamp techniques, and found that some of these mutants showed enhanced receptor responses to carbachol and different mechanosensitivity. In this study, we investigated the role of podocin, a slit diaphragm protein and a putative mechanosensor, on the receptor responses and mechanosensitivity of TRPC6 FSGS mutants. Co-expression of podocin with TRPC6 in HEK293 cells enhanced responses to low-concentration of carbachol (1 μM) in wild-type, P111Q and M131T, but not in N142S. Mechanical responses cause by a membrane-expanding agent 2, 4, 6-trinitrophenol in these mutants were differently affected by podocin. We also studied the effects of overexpression of TRPC6 FSGS mutants in mouse-derived podocyte cell line which is abundant with podocin. M131T-overexpressed podocytes showed higher basal Ca^{2+} levels than wild-type. These observations indicate that podocin differently modulates the receptor activation and mechanosensitivity of TRPC6 FSGS mutants. Such differences may reflect the different etiology of FSGS patients such as the onset age of diseases, where disruption of filtration barrier by the degeneration of podocytes play a crucial role.

(COI: No)

P2-259

Dynamic fluctuation of glomerular fenestrated endocapillary

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Fenestrated endocapillary regulates transduction of nutrients and humoral information between the systemic and peripheral cells via micropore. In this study, we focused on the morphological changes of the micropores of glomerular fenestrated endocapillaries according to relative hypoglycemic stress induced by chronic continuous subcutaneous insulin injection. Male mice were divided into three group; free-feeding and saline-injected group as a control (CS), free-feeding and insulin-injected group (CI), and scheduled-feeding and insulin-injected group (SI), respectively. The changes in body weight, daily amount of feeding, urinal protein exclusion, and rectal temperature, blood glucose variation and blood pressure were also obtained. Subsequently, all mice were perfused and removed the kidney samples for the microscopic investigation. All statistical significance was set at $p < 0.05$. As a result, significant elevations were observed in CS compared to CI of the parameters of body weight, daily feeding and urinal protein exclusion, blood glucose variation, rectal temperature and blood pressure. On the other hand, the elevation of the rectal temperature and blood pressure and the reduction of the blood glucose variation were confirmed significantly in SI compared to CI, respectively. In the electron microscopic imaging analysis, we ascertained the significant increment in the mean diameter of the micropores in CI and SI compared to CS. SI had outclassed CI in the magnitude of diameter of the micropore significantly.

(COI: No)

P2-260

Newly characterized structure of podocytes revealed by three-dimensional analysis using block-face scanning electron microscopy

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Block-face imaging is a novel SEM technique which enables easier acquisition of serial ultrastructural images directly from the surface of resin-embedded biological samples with a similar quality to TEM images. In the present study, we analyzed the three-dimensional architecture of podocytes using serial block-face imaging. When the reconstructed podocytes from their basal side were viewed, the most proximal portion of the foot processes were connected to each other via a tortuous ridge-like prominence, which was formed on the undersurface of the primary process and was similar to the usual foot processes in structure. We termed the ridge-like prominence a "connecting foot process", and to distinguish the connecting foot processes from the usual ones, we further named the latter as "peripheral foot processes". The connecting foot process anchored the primary process to the glomerular basement membrane, and connected the primary process and the peripheral foot processes. In conclusion, serial block-face imaging is a powerful tool for understanding the three-dimensional architecture of podocytes through its ability to reveal novel structures which were difficult to determine by conventional TEM and SEM alone.

(COI: No)

P2-261

Segment-specific expression of tight junctional proteins, claudins, is regulated by osmotic stress in renal tubular epithelial cells

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Tight junctions form the closest contact between adjacent cells. Claudin-2, a transmembrane protein of tight junction, is expressed in the proximal tubules where maintain an isotonic osmolarity, whereas claudin-4 is expressed in the collecting ducts where maintain a high osmolarity in the fluid of the kidney. In this study, we examined the effect of hyperosmolarity on claudins expression. Hyperosmolarity increased claudin-4 expression, whereas it decreased claudin-2 expression in MDCK II cells. Claudin-4 expression is up-regulated by a MEK/ERK pathway under the hyperosmotic conditions, whereas claudin-2 expression is not down-regulated. We found that the hyperosmolarity-induced decrease in claudin-2 is inhibited by a PKCβ specific inhibitor in MDCK II cells, rat renal slices, and HK-2 human proximal tubular cells. Hyperosmolarity decreased the expression of nuclear GATA-2, which was inhibited by Go6983 and PKCβ inhibitor. Chromatin immunoprecipitation assay showed that GATA-2 bound to the promoter region of claudin-2. Hyperosmolarity may decrease claudin-2 expression mediated by a decrease in PKCβ-dependent GATA-2 transcriptional activity in renal tubular epithelial cells. We suggest that the decrease in claudin-2 expression prevents excess paracellular transport of electrolytes under the hyperosmotic conditions.

(COI: No)

P2-262

Three-dimensional morphological analysis of Alport syndrome and thin basement membrane nephropathy by low vacuum scanning electron microscopy

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We introduce a novel approach to the histological diagnosis of Alport syndrome (AS) and thin basement membrane nephropathy (TBMN) in the present study. These diseases are caused by mutations of the type IV collagen genes. We investigated the three-dimensional ultrastructure of the glomerular basement membrane (GBM) of AS and TBMN under low vacuum scanning electron microscopy (LVSEM). Conventional renal biopsy paraffin sections obtained from 4 cases of AS and 6 cases of TBMN patients were stained with periodic acid methenamine silver (PAM) and observed under LVSEM. The PAM-positive GBM was clearly visible under LVSEM through the overlying cellular components without removing of cells. The GBM showed characteristic coarse meshwork appearances in AS, and thin and sheet-like appearances in TBMN. Moreover, at the cut side view of the capillary wall, the GBM in AS appeared as fibrous inclusions between podocytes and endothelial cells. These different findings of GBM between AS and TBMN were considered to be depending on the different constitutions of alpha chains of collagen type IV. The present three-dimensional morphological analysis of GBM by LVSEM using conventional paraffin sections is probably useful to the histological diagnosis of AS and TBMN, including in retrospective investigations.

(COI: No)

P2-263

Search results for novel biomarker in acute kidney injury

Sugiyama, Noriyuki[1]; Murata, Shinya[1]; Adachi, Takaomi[2]; Otsuki, Yoshinori[1] (*[1]Anat. & Cell Bio. Osaka Med. Univ., Takatsuki, Japan; [2]Nephrol. Med. Kyoto Prefect. Univ. Med., Kyoto, Japan*)

Acute kidney injury (AKI) is caused by various events, for example ischemia-reperfusion and drug administration, and despite the advances in renal replacement therapy, the mortality rate still remains high. Definitions of AKI have relied on either an abrupt increase in serum creatinine or an abrupt decline in urine output within 48h. Multiple studies have focused on the developement of the selective and specific biomarker in the early checkup of AKI. However, it was not reported in most biomarkers to predict a renal prognosis. The aim of the present study was searched novel biomarkers for prognosis prediction in AKI. Mice were subjected to 45 and 60 min of unilateral IRI. Kidney weight/body weight (KW/BW) in the 45-min IRI groups were the same as that of the sham group, whereas KW/BW in the 60-min IRI groups were significantly lower than that of the sham group at 28 days after IRI. From these results, we selected the kidneys that received 60 min of ischemia as the atrophic kidney model, and the kidneys that received 45 min of ischemia as the repaired kidney model. Next, we compared the two models in cDNA microarray method. Microarray experiments were performed using amplified RNA from two IRI mouse kidneys and sham control tissue at 1 day after operation. Number of the up-regulated gene is 177 in the atrophic model only. We have been made the gene expression profiles. It is expected that the novel biomarker is finding out.

(COI: No)

P2-264

Protective effect of renal denervation on renal ischemia-reperfusion injury in rats

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This study was carried out to investigate the involvement of renal innervation in renal injury and repair after ischemia-reperfusion (IR). IR-induced acute kidney injury was induced by unilateral clamping of the renal artery and vein for 30 min followed by reperfusion for 1, 2, 4, and 7 days, after the contralateral nephrectomy. Unilateral renal denervation was performed by cutting large visible nerves and applying phenol. IR significantly increased necrosis of tubular epithelial cells, interstitial expansion, collagen deposition, at the cortico-medullary border. IR also increased the formation of reactive oxygen species in the same region as detected by dihydroethidium staining. Renal denervation improved histological damage and decreased the formation of reactive oxygen species. These results suggest that renal nerves play an important role in the regulation of the progression of IR injury, and this regulation is associated with the attenuation of oxidative stress.

(COI: No)

P2-265

The localization of vacuolar H+-ATPases (v-ATPases) in proximal tubules

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We previously reported that increasing basolateral CO2 from 1.5 to 5% at the constant HCO3- concentration induced slight increases in the cytosolic pH (pHc), the luminal fluid in the proximal tubule (pHTF), and the fluorescence intensity of acridine orange in acid vesicles. Bafilomycin at 10-6 M inhibited these changes. To clarify the mechanism of pH regulation by the v-ATPase and the CLC-5 (H/Cl exchange transporter 5), we examined the reactivity of anti v-ATPase and CLC-5 antibodies in bullfrog proximal tubules. Localized signals were observed, both for the v-ATPase and the CLC-5, on the cytoplasmic membrane and cytosolic vesicles. In response to the 5% basolateral CO2, however, localized antibody signals did not change. Furthermore, mRNA and protein levels remained unchanged. These results suggested the activity of v-ATPase may be responsible for the v-ATPase-dependent pH regulation in proximal tubules.

(COI: No)

P2-266

Properties of expression of exogenously transfected ROMK K+ channels with or without PDZ-binding motif in the polarized and non-polarized membranes of cultured M-1 cell

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ROMK1 K+ channels are expressed in apical side of cortical collecting duct principal cells and play an essential role in K+ secretion to maintain body fluid potassium homeostasis. In this study, to understand the mechanism of specific expression of ROMK1 channel on apical membrane, we focused on PDZ-binding motif at C-terminus of ROMK1 channel. PDZ binding motif has been known to be important for interaction with PDZ scaffold proteins regulating ion channel expression on cell membrane. We constructed EGFP fused ROMK1 deleted PDZ binding motif and transfected to cultured M-1 cells. We applied two types of culture conditions. One was M-1 cells cultured on conventional glass dish, and the other was cells cultured on membrane insert to form apico-basolateral cell polarity. Cell attached mode of patch clamp analysis confirmed that ROMK1 activity was observed in EGFP positive M-1 cells, but not EGFP negative cells. No obvious difference between WT and PDZ binding motif deletion mutant in cellular localization of EGFP fluorescence and detection of frequency of ROMK1 current in polarized M-1 cell. On the other hand, frequency of ROMK1 current acquisition was significantly decreased in deletion mutant compared with WT in non-polarized cell. These results suggest that WT and deletion mutant, both of which have high affinity to polarized apical membrane.

(COI: No)

P2-267

Myogenic damage underlies dysfunctional urethral closure in a rat model of urinary incontinence

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Purpose: During pregnancy and/or after childbirth, many women suffer from stress urinary incontinence (SUI). Various animal models exist that have contributed to finding a suitable therapeutic method to investigate SUI pathophysiology. However, few anatomical studies have provided detailed information on the structures involved. The aim of this study was to examine the effect of simulated birth trauma on the urethral wall musculature including both striated and smooth muscle layers using the rat vaginal distention (VD) model of SUI.

Methods: Female, 8-week-old Wistar rats were divided into control and VD groups. In the VD group, a urinary catheter was placed into the vagina and the balloon was inflated for 4 hours to simulate labor. After 4 weeks, urethral tissue samples were collected for histochemical analysis. Immunofluorescence staining was conducted to detect striated and smooth muscle fibers in the urethral wall using antibodies for myosin heavy chain isoforms (type I, type II) and α-smooth muscle actin.

Results: In general, the most outer part of urethral wall contained a circular striated muscle layer predominantly consisting of type II fibers. Just beneath this layer, a thin circular smooth muscle layer enclosed the mucosa. The VD group showed a marked decrease in the urethral wall musculature of both skeletal and smooth muscle tissues compared to that in the control group.

Conclusion: The VD rat model is useful for examining myogenic damage associated with SUI in humans.

(COI: No)

P2-268

Pentraxin3 expression in a rat model of peritoneal dialysis

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Background: Continuous exposure to peritoneal dialysis (PD) fluid is associated with micro-inflammation that leads to tissue fibrosis of the peritoneum and failure of peritoneal membrane ultrafiltration. Pentraxin3 (PTX3) is a multifunctional soluble pattern recognition receptor modulating the immune-inflammatory responses. PTX3 is produced at sites of inflammation by various cell types. We investigated whether PTX3 could be a marker of inflammation for the peritoneum.

Methods: The rats were instilled with 20 ml of lactate-buffered PD fluid containing 3.86% glucose (n=7) or saline (n=6) twice a day for 8 weeks. The mRNA expression of PTX3 in the parietal peritoneum was evaluated by RT-PCR, real-time PCR and in situ hybridization, and was compared to the expression level in saline group and normal group without PD (n=6). We also examined PTX3 expression in several types of cultured cell line.

Results: Morphological data revealed that the submesothelial layer of peritoneal membranes in PD rats was markedly thickened with fibrosis and angiogenesis. The expression of PTX3 was detected and enhanced in the peritoneal tissues by continuous exposure to conventional PD fluid. PTX3 was also induced in cultured mesothelial cells as well as macrophage-like cells and fibroblasts.

Conclusion: PTX3 might be a potential biological marker of local micro-inflammation in the peritoneal tissue undergoing PD therapy.

(COI: No)

P2-269

Properties of spontaneous activity in muscularis mucosa of the guinea pig bladder

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Aim: Properties of spontaneous activity in urothelium denuded muscularis mucosa (MM) of the guinea-pig bladder were investigated.

Methods: Effects of ion channel modulators on spontaneous action potentials (SAPs) were investigated using intracellular recording technique, while spontaneous Ca2+ transients were visualized with fluo-4 Ca2+ imaging. Fluorescent immunohistochemistry was also carried out.

Results: The resting membrane potential of MM was -45.3 ± 5.5mV. MM generated SPAs that had the amplitude of 46.7 ± 4.5mV and the frequency of 12.3 ± 9.5/min. Both SAPs and spontaneous Ca2+ transients were abolished by nifedipine. NS 309, a small-conductance Ca2+ activated K+ channel opener, prolonged SAP after-hyperpolarizations and its action was reversed by apamin. NS 1619, a large conductance Ca2+ activated K+ channel opener, caused hyperpolarizations and cessation of SAPs in iberiotoxin-sensitive manner. Y-26763, an ATP-sensitive K+ channel opener, hyperpolarized the membrane and abolished SAPs, and its actions were reversed by glibenclamide. α-smooth muscle actin positive MM bundles forming mesh-like network preferentially run along suburothelial vessels.

Conclusions: SAPs and Ca2+ transients in MM rely on the opening of L-type Ca2+ channels, while the activation of K+ channels results in the suppression of SAPs, and thus properties of spontanepus activity in MM is very similar to that of detrusor smooth muscle. Spontaneous contractions of MM may prevent stretching the vessels in their long axis during urine storage phase.

(COI: No)

P2-270

Regulation of hyperactivated motility by extracellular Na^+ in hamster spermatozoa

Takei, Gen Leon; Fujinoki, Masakatsu; Seo, Yoshiteru (*Dokkyo Medical University Tochigi Japan*)

Mammalian spermatozoa have to undergo "capacitation" to become fertilization-competent. Capacitated spermatozoa exhibit a specialized flagellar movement called "hyperactivation" with increased bend amplitude to penetrate the zona pellucida. In mammals, osmolalities of the fluids from male internal genitalia (seminal vesicle, prostate and epididymis) are higher (<420 mOs*m*) than that of the fluids from female genital tract (approximately 290 mOs*m*). We previously reported that the appearance of hamster sperm hyperactivation was delayed near the osmolality of seminal plasma using mTALP media in which osmolality were adjusted by NaCl. In the present study, we examined whether the delay of hamster sperm hyperactivation was caused by the osmolality or the concentration extracellular Na^+ of the mTALP media. To examine this, spermatozoa were incubated in the mTALP media in which NaCl concentrations were varied from 75 mM to 150 mM, while the osmolalities were fixed at 370 mOs*m* by adding mannitol. The results indicated that the delay of hyperactivation was caused dependently on the concentration of extracellular Na^+, but not the osmolality of the mTALP media. Intracellular Ca^{2+} concentration was decreased as the extracellular Na^+ concentration increased. By contrast, the membrane potential and intracellular pH were not affected by the extracellular Na^+ concentration. SN-6, an inhibitor of Na^+/Ca^{2+} exchanger (NCX), canceled the delay of hyperactivation in the presence of suppressive concentration of Na^+. These data suggest that mammalian sperm hyperactivation is regulated by extracellular Na+ by the action of NCX.

(COI: No)

P2-271

Searching the enzyme responsible for molecular weight reduction of sperm acrosomal protein Equatorin during acrosome reaction

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Equatorin (EQTN) is a heavily glycosylated single transmembrane protein which is localized on the equatorial segment of sperm acrosome. Sperm-egg fusion occurs between the membrane over the equatorial segment and the oolemma only after acrosome reaction. Anti-EQTN antibody MN9 inhibits cortical granule release without inhibiting zona penetration during fertilization. This suggests that EQTN is involved in sperm-egg interaction and early phase of egg activation. Relative molecular weight of EQTN changes during sperm maturation and acrosome reaction. Therefore, it is important to reveal the post-translational modifications of EQTN to understand the molecular mechanism of sperm-egg interaction and egg activation. In this study, we examined the molecular weight shift of EQTN during acrosome reaction. Mild detergent treatments as well as acrosome reaction were able to induce relative molecular weight reduction of EQTN. To detect the enzyme activity responsible for the molecular weight shift, we extracted sperm proteins using mild detergents and mixed with EQTN as substrate. The molecular weight shifts were detected by western blotting. We purified the enzyme responsible for the reduction of EQTN molecular weight to analyze the function during fertilization. Identification of the enzyme will elucidate the function of the fragment cleaved from EQTN which could not be analyzed by EQTN knock out mice.

(COI: No)

P2-272

Basigin interacts with monocarboxylate transporter 2 in the mouse testes and sperm

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Basigin (CD147, EMMPRIN) is a member of the immunoglobulin superfamily and plays various important roles in biological events including spermatogenesis. It is known that basigin is associated with monocarboxylate transporters (MCTs), especially with MCT1 in some cell types. However, the interaction of basigin with MCTs in the testis and sperm has not yet been clarified. Using antibodies against MCT1, MCT2 and basigin, we investigated the localization of the MCTs and basigin in the mouse testis and sperm. During testicular development MCT1 immunoreactivity was localized on the spermatogonia, spermatocytes, and spermatids. Immunostaining for MCT2 was detected in elongate spermatids and the principal piece of the sperm tails in the testis. Basigin immunoreactivity was observed on the spermatocytes, spermatids and principal piece of the testicular sperm. An indirect immunofluorescence study revealed protein localization in sperm from caput and cauda epididymides. Immunoreactivities for basigin and MCT2 were colocalized; in sperm from the caput epididymidis, the reactions occurred on the principal piece of the sperm tail. In contrast, the midpiece of the sperm tail was positive for basigin and MCT2 in sperm from the cauda epididymidis. Furthermore, MCT2 was immunoprecipitated with basigin in mouse testes and sperm. These results indicate that basigin preferably binds with MCT2, not with MCT1, in testicular and epididymal sperm.

(COI: No)

P2-273

Establishment of in vitro differentiation system of male germ stem cells by epigenetic manipulation

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Male germ lineage in mice has an exceptional adult type stem cell system that the stem cells are maintained for years in vitro. Although the long-term cultured germ stem cells, called GS cells, are able to reconstitute all of the testicular germ cells in recipient adult testes, in vitro differentiation system has not been well established. In order to dissect out the molecular mechanisms how stem cells are maintained and differentiate, the establishment of in vitro differentiation system is desired. We previously reported that the stem cells in testes lacked the expression of de novo DNA methyltransferases (Dnmts) and revealed weak global H3K9me2 modification. The protein expression of Dnmts and integration of H3K9me2 modification were induced at the transition from the stem cells to the progenitor cells. We are currently trying to establish in vitro differentiation system by regulating the epigenetic modifications and mimicking the epigenetic status in vivo. We will discuss about whether it is possible to regulate stem cell differentiation by low molecular weight compounds that regulate epigenetic modification enzymes.

(COI: No)

P2-274

Ca^{2+} oscillations of mouse ovarian oocytes can be used as a cytotoxicity assay system for drugs and chemicals

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Background: Cytotoxicity assays for chemicals and drugs are usually carried out by monitoring cell viability or occasionally by checking morphologic or biochemical changes induced in cells. Here we present an assay system which continuously monitors alterations in cell function in response to these substances.
Methods: Ovarian oocytes were obtained from mature ICR mice, loaded with Fura-2/AM and their fluorescence Ca^{2+} images were monitored using an image analyser (ARGUS-50, Hamamatsu Photonics, Japan).
Results: 1) Among medicines, lidocaine (local anesthetic, antiarrhythmic) and phenytoin (antiepileptic) were tested. At therapeutic concentrations, these drugs did not reveal any significant effects on Ca^{2+} oscillations. However, oscillations were reversibly blocked by lidocaine and irreversibly by phenytoin when their concentration exceeded the therapeutic range. 2) Cadmium, an occupational and environmental pollutant, also inhibited Ca^{2+} oscillations reversibly at low concentrations (<0.01 mM) and irreversibly at higher doses (>0.1 mM). 3) Uncouplers of oxidative phosphorylation in mitochondria, e.g. CCCP (100 nM), reversibly inhibited Ca^{2+} oscillations.
Conclusion: The present work shows that Ca^{2+} dynamics of mouse oocytes provides a cytotoxicity assay system for drug screening. This system can also be applied to detect hazardous substances which may cause occupational or environmental diseases.

(COI: No)

P2-275

Kinetics of oocyte elimination by synapsis checkpoint in mice

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Synapsis checkpoint is an essential mechanism to ensure the quality of oocytes. We recently revealed that synapsis checkpoint requires HORMAD2 in mice. However, its mechanism is still poorly understood. The timing of the oocyte elimination is important for the analysis of synapsis checkpoint-associated proteins, but has not been well determined. In the present study, we first examined the kinetics of oocyte cell death during the perinatal period in wild-type, HORMAD1-hetero deficient (having oocytes with mild asynapsis), and SPO11-deficient (having oocytes with extensive asynapsis) ovaries by using cleaved PARP-1 as a marker of apoptosis. The percentage of apoptotic oocytes was similar among all the ovaries examined at 18 dpc (about 2 %), and slightly increased at 0 and 1 dpp in HORMAD1-hetero deficient (about 5 %) and SPO11-deficient (about 5 %) ovaries compared to wild-type (about 2 %) ovaries. However, the detected increase of apoptosis seemed to be relatively small compared to the final rate of oocyte loss in HORMAD1-hetero deficient (about 50 %) and SPO11-deficient (about 90 %) ovaries. We speculate from these data that the oocyte elimination might be dependent on both apoptotic and non-apoptotic mechanisms. Next, we preliminarily examined the possible involvement of autophagic cell death by using LC3 as a marker of the autophagy activation, but detected no increase in the LC3 accumulation in SPO11-deficient oocytes. Although the analysis is still in progress, our analysis will provide fundamental data necessary for the molecular characterization of synapsis checkpoint in mammalian oocytes.

(COI: No)

P2-276

Distribution of prosaposin and its receptors in rat uterus

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Prosaposin (PS) is a trophic factor and activator of sphingolipid hydrolase in lysosomes. G protein-coupled receptor (GPR) 37 and GPR37L1 are receptors for prosaptide and prosaposin. We generated a specific antibody to PS and examined the spatiotemporal distribution of both PS-immunoreactive (PS-IR) cells and prosaposin receptors in Wistar rat uterus. Immunoblotting using uterine tissue showed that the production of PS and its receptors was affected by the estrus cycle. PS-IR cells were distributed in the functional layer of the endometrium. Uterine epithelial and glandular tissues reacted strongly with the anti-PS antibody. To identify PS-IR cells, double and triple immunostaining were performed with antibodies against PS, CD68, and OX62. Large numbers of double- and triple-positive cells were detected, suggesting that antigen-presenting cells in the uterus contain abundant PS. GPR37- and GPR37L1-immunoreactive cells were distributed in the functional layer of the endometrium. Intense expression of PS mRNA, examined using *in situ* hybridization, was observed in rat uterus. In rats, alternative splicing generates two forms of mRNA coding for PS: Pro+9, containing a nine-base insertion, and Pro+0, which lacks the insertion. We examined the expression patterns of both forms of PS mRNA in rat uterus. Both types of mRNA (Pro+9 and Pro+0) were detected, indicating that rat uterus contains various types of PS-producing and/or -secreting cells. These findings suggest diverse pivotal functions for PS in the reproductive system.

(COI: No)

P2-277

Lgr4 is required for endometrial receptivity acquired through progesterone signaling

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LGR4 (leucine-rich repeat-containing G protein-coupled receptor 4) is identified novel family member of glycoprotein type GPCR (G protein coupled receptor) that have LHR and FSHR. We generated *Lgr4* conditional knocked-out mouse ($Lgr4^{K5\ KO}$) using the *Keratin5-Cre* mouse model. These female mice was shown that *Lgr4* exhibited the subfertility with defect of *Lgr4* in the uterine luminal epithelial. There, we focused endometrial receptivity which is the most important event during implantation because the ovarian function is normal in the $Lgr4^{K5\ KO}$ mice. In the early pregnancy hormonal state, the ovarian steroid hormone, progesterone (P4) and estrogen (E2), regulate transition of luminal epithelium to allow blastocysts implantation. Then, to the normal ($Lgr4^{K5\ Ctrl}$) and $Lgr4^{K5\ KO}$ mice, we observed how luminal epithelium would react when both mice was induced receptive stage by administration E2 and P4. Compared with $Lgr4^{K5\ Ctrl}$ mice, proliferative luminal cells were remained in $Lgr4^{K5\ KO}$ undergo P4 stimulation. In addition, the amount of phospho-progesterone receptor in $Lgr4^{K5\ KO}$ was lower and also progesterone receptor target genes, *ihh* and *areg*, was reduced. Together, our result suggested that LGR4 is contributed endometrial receptivity through the P4 signaling and we are continuing further analysis.

(COI: No)

P2-278

Plastic changes of sympathetic vasoconstrictor activity in the ovary by long-term treatment of estrogen

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It has been reported that the reduction in uterine sympathetic innervation is observed, when the serum estrogen level is high during the estrous cycle and pregnancy. In the ovary, the sympathetic innervation may also be suppressed by estrogen to keep good environment for the follicle development. The present study examined the effects of long-term treatment of estrogen on sympathetic vasoconstrictor activity in the ovary. Non pregnant Wister rats received sustained subcutaneous estrogen (water soluble 17-beta estradiol, 5 μg/kg/day) or saline for 2 or 4 weeks. The rats were anesthetized, and artificially ventilated. Respiration, body temperature, and mean arterial blood pressure were maintained at physiological level. The ovarian blood flow was measured using a laser Doppler flowmeter. The ovarian sympathetic nerve (the superior ovarian nerve: SON) was electrically stimulated at the supra-maximal intensity for C-fibers. Electrical stimulation of the SON produced stimulus frequency (1-50 Hz) dependent decrease in the ovarian blood flow in both saline-treated and estrogen-treated rats. However, the attenuated response curves was observed in estradiol-treated rats. This attenuation was more evident especially at low frequency stimulation (2-5 Hz), and also in 4-week treated rats than 2-week treated rats. The results revealed that long-term treatment of estrogen caused plastic changes in sympathetic vasoconstrictor activity in the ovary.

(COI: No)

P2-279

The role of cytokines in the interaction between sperm and cumulus cells

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Eutherian eggs are surrounded by a thick cumulus cell layer. Although cumulus cell-derived factors have been shown to promote fertilization, the molecular mechanism of the promoting effect is unclear. We have shown previously that mouse sperm acrosome contains pituitary adenylate cyclase-activating polypeptide (PACAP) which reacts to PAC1, a PACAP specific receptor, on the plasma membrane of cumulus cells, and stimulates the cells to secrete fertilization-promoting factors. Here, we performed gene expression profiling on cumulus to identify these factors. A total of 166 genes were differentially expressed after 1-h incubation with PACAP; 162 out of 166 genes were upregulated. Among them, we focused on the genes encoding cytokines, including chemokines (CCL2, CCL3, CXCL1, CXCL7), growth factors (HB-EGF, FGF1), and neuropeptides (neurokinin A, substance P). Immunofluorescence analysis showed that CCR1, a chemokine receptor for CCL3, and NK2, a neurokinin A receptor, were both localized on the anterior acrosome and midpiece of flagellum. This result suggests that these cytokines mediate crosstalk between sperm and cumulus cells, and are involved in the processes in fertilization.

(COI: No)

P2-280

ERα Signal Transduction Pathways Controlling Cell Size

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Together with estradiol in human endometrium, estrogen receptor alpha (ER α) plays a critical role through its target molecules in control of proliferation, differentiation, invasion, and migration. Current data support the idea that estrogen receptor alpha (ER α located in cell membrane, cytoplasm, and nucleus collaboratively plays its important roles. The idea suggests that the key functions of ER α might vary in its subcellular locations. To avoid the effects of the subcellular translocations of ER α during analysis, we have allocated ER α to different subcellular locations in ER α-negative Ishikawa cell, using permanent transfection technique. The results from flow cytometry showed that the ER α-negative cells became significantly larger in size following genomic insertion of ER α. While the cytoplasmic ER α has the least impact, the largest cells in size were observed in these carrying the nuclear ER α. Further analysis revealed that the amount of expression was increased in such molecules as pS235/236-S6 Ribosomal Protein and pS2448-mTOR. We also demonstrated that cells with the nuclear ER α showed the best balance between the cell size and its proliferation. Our study indicates that through mTOR pathway, ER α play an important role in regulation of the endometrial cell size.

(COI: No)

P2-281

Sex-related genes' asymmetric expression in early mouse gonads

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Most male mammals have a symmetrical pair of testes and females a symmetrical pair of ovaries. Our transgenic mice (C57BL/6-Ypos) showing sex reversal produced true hermaphrodites, many of which had a testis on the left and an ovary on the right. We suspect that these true hermaphrodites are the result of the asymmetric expression of sex-related genes during a critical window of sex determination, and here we investigated the spatiotemporal changes in the expression of genes *Sry*, *Sox9*, *Fgf9* and *Wnt4* in the XY gonads at embryonic day 11.5 by qRT-PCR and whole-mount immunohistochemistry (Wmt-IHC). qRT-PCR showed that the peak *Sry* expression and increased expression of *Sox9* were earlier in the left gonad than the right. No significant difference was found between the right and left gonads in *Fgf9* and *Wnt4* expression. Wmt-IHC showed that the left gonad's Sox9-positive cells were distributed earlier than the right, at the end of the caudal region. Such minor asymmetric differences do not seem to have much influence on normal testis differentiation. In C57BL/6 mice, whose background is sensitive to genetic disruption, the knock-out of specific genes reportedly leads to XY gonadal sex reversal with true hermaphrodites. These results suggest that the asymmetric differences of sex-related gene expressions contribute to the true hermaphrodites unless some genes are expressed normally.

(COI: No)

P2-282

Selection of stable reference genes for quantitative RT-PCR analyses in developing mouse gonads

Yokoyama, Toshifumi[1]; Hashimoto, Rie[1]; Umemura, Yuria[1]; Omotehara, Takuya[1]; Nagahara, Daichi[1]; Hirano, Tetsushi[1]; Yanai, Shogo[1]; Minami, Kiichi[1]; Kubota, Naoto[1]; Mantani, Youhei[2]; Kitagawa, Hiroshi[2]; Hoshi, Nobuhiko[1] ([1]*Lab. Mol. Morphol., Grad. Sch. Agr. Sci., Kobe Univ., Kobe, Japan;* [2]*Lab. Histophysiol., Grad. Sch. Agr. Sci., Kobe Univ., Kobe, Japan*)

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR) is the most widely used method for studying quantitative gene expression. An important factor when using the qRT-PCR method is the stability of the expression of reference genes; this expression should be stable regardless of changes in the developing stage or the sex. However, it is difficult to know the appropriate normalization gene. Here we report the selection of reference genes for the accurate normalization of quantitative gene expression data in developing mouse gonads from 10.5 days post coitum (dpc) to the adult stage (10.5, 11.5, 12.5 13.5, 15.5, 17.5 dpc, neonatal, 5 and 34 wks). We evaluated the expression stabilities of the genes *Gapdh*, *Hprt1*, *Actb*, *B2m*, *Tbp*, *Ppia*, *Tfrc*, *Gusb*, *Pgk1*, *Ubc*, *Ywhaz*, *Polr2a*, *Rplp0*, *18S* and *Sdha*. To identify the best candidate genes, we combined the scores of three software programs: Bestkeeper, Normfinder, and Genorm. The results suggest *Ppia*, *Polr2a* and *Pplp0* as reference genes to be used in experiments with fetal to adult mouse gonads. In contrast, we identified *Hprt1*, *Tbp* and *Tfrc* as reference genes to be used in experiments with fetal mouse gonads.

(COI: No)

P2-283

Effect of taxol on chromosomal cohesion and blastocyst formation rate in human oocytes

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OBJECTIVE: Premature chromatid separation (PCS), a common condition that results in pre-implantation embryo loss, becomes more prevalent in human oocytes with maternal age. Therefore we attempted to suppress PCS artificially in order to improve post-fertilization development.
METHODS: Chromosome aberrations, oocyte development, and tubulin-associating protein expression were examined in human oocytes that had been produced by in vitro maturation (IVM) either with or without taxol. GV oocytes were exposed to taxol for either 1 or 2 hr and cultured overnight in order to allow them to mature. Chromosome analysis was performed in oocytes treated with 5-100ng/ml taxol for 1 or 2 hr. For blastocyst rates, taxol-treated oocytes were inseminated with the partner's sperm. Immunofluorescent microscopy was used in at least 5 oocytes to detect tubulin and aurora proteins.
RESULTS: Taxol-treated oocytes were associated with a significant reduction in PCS frequency and a significant increase in blastocyst rate. The cortical tubulin network was thicker in taxol-treated GV oocytes than control GV oocytes. Phospho-aurora expression appeared to be up-regulated in the germinal vesicle karyoplasm and chromatin region soon after taxol treatment.
CONCLUSION: Taxol treatment at the germinal vesicle (GV) stage suppressed PCS, resulting in an increased blastocyst formation rate in in-vitro mature oocytes. The result also indicated that tubulin polymerization during prophase may contribute to phospho-aurora kinase localization during meiosis in human oocytes.
(COI: No)

P2-284

Aromatherapy improves sleep quality during the menstrual cycle of healthy women

Fujita, Sayaka (*The University of Shimane*)

The present study aimed to clarify the effects of aromatherapy on sleep quality during the menstrual cycle of healthy women. Sleep quality during the menstrual cycle of 29 women (non-aromatherapy group, n=18; aromatherapy group, n=11) was measured for about 1 month. Basal body temperature during the ovarian follicular, corpus luteum and menses phases was measured while still in bed in the morning, and sleep quality was assessed using a mat-type device. During the study period, the aromatherapy group used lavender or sweet orange aromatherapy throughout the night while sleeping, while the non-aromatherapy group was instructed to not use aromatherapy while sleeping. The total sleeping time and amount of rapid eye movement (REM) sleep differed significantly between the aromatherapy and non-aromatherapy groups ($p<0.01$). Differences were also observed in basal body temperatures during the ovarian follicular, corpus luteum, and menses phases of the menstrual cycle; however changes observed during the menses phase were not significant The amount of time spent in REM sleep in the aromatherapy group differed significantly between the ovarian follicular and corpus luteum phases ($p<0.01$), with the ovarian follicular phase also having an extended total sleep time ($p<0.05$). These results show that the effects of aromatherapy on sleep quality may be different during different phases of the menstrual cycle.
(COI: No)

P2-285

Sperm acrosomal membrane complex analyzed by STED using Equatorin-EGFP transgenic mice

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Background: The acrosome is a sperm organelle enclosed by a continuous acrosomal membrane, which is ultrastructually divided into the outer acrosomal membrane (OAM) and inner acorosomal membrane (IAM). The OAM and IAM make complexes with surrounding matrices containing molecules necessary for fertilization (the complex of acrosomal membranes and associating matrices: CAMAM). The CAMAM has not been visualized in detail under living condition due to the lack of good marker molecules for chasing the subtle changes and the resolution limit of the light microscope.
Purpose: In this study, using Eqtn-EGFP transgenic (Tg) mice and super-resolution stimulated emission depletion (STED) microscopy we analyzed the CAMAM.
Methods and Results: The images of CAMAM of Eqtn-EGFP Tg elongated spermatids taken by high-resolution microscopy, confocal laser scanning microscopy and STED microsocopy, compared with those of immunoelectoron microscopy. The CAMAM, which is generally analyzed at the light microscopy level, was further differenciated into its sub-components corresponding to the OAM-related and IAM-related stuructures (COAMAM and CIAMAM) at the nanometer scale in a whole sperm without sectioning by STED microscopy.
Conclusion: The information in this study will help for understanding the molecular mechanism of fertilization under living condition.
(COI: No)

P2-286

Histological investigation of impaired spermatogenesis in xeroderma pigmentosum group A gene (*Xpa*)-deficient mice

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Xeroderma pigmentosum (XP) has a defect in the initial step of nucleotide excision repair (NER) and consists of seven genetic complementation groups (groups A-G). XP group A patients have a high incidence of UV-induced skin tumors, immature testicular development, and neurological symptome. We reported that xeroderma pigmentosum group A (*Xpa*) gene-knockout mice [*Xpa* (-/-) mice] were deficient in NER and highly sensitive to UV-induced skin tumorigenesis. We found that the testis diminished in an age-dependent manner, and degenerating seminiferous tubules with vacuoles and no sperm were observed in the 24-month-old *Xpa* (-/-) mice. In this study, we investigated degenerating seminiferous tubules of *Xpa* (-/-) mice testis by immunostaining for autophagy-related proteins. We will discuss the implications of autophagy-related proteins immunostaining pattern for vacuole formation in *Xpa* (-/-) mice testis.
(COI: No)

P2-287

Aberrant formation of synaptonemal complex induced by Dnmt1 knockdown with in vivo electroporation of shRNA expression vector in mouse testes

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Mammalian spermatogenesis is an orderly arranged process consisting of spermatogonial proliferation, spermatocytic meiosis and spermiogenesis. Epigenetic factors, such as DNA methylation are thought to be involved in this process and the dynamic changes of methylation levels of CCGG sites were observed during mouse spermatogenesis by HELMET method. Therefore, in the present study, we analyzed the effect of Dnmt1 knockdown in spermatogenesis by in vivo electroporation of shRNA expression vector. LacZ shRNA expression vector was used as a negative control. The expression vectors were electroporated in 15 days-old mouse testes at the condition of 6 square 50 V electric pulses. Mice were sacrificed at 9 days after electroporation and testes were fixed overnight with 4% PFA in 0.01 M PBS (pH 7.4) and embedded in paraffin. The expressions of Dnmt1, 5-methylcytosine (5mC) and synaptonemal complex protein 3 (SCP3) were analyzed by immunohistochemistry. Dnmt1 was strongly expressed in spermatocytes of LacZ shRNA transfected testes and the expression levels of Dnmt1 and 5mC were decreased to 40% and 60% in Dnmt1 shRNA transfected spermatocytes, respectively. Apoptotic cells were increased to 240% and aberrant synaptonemal complex was observed in pachytene spermatocytes. These results suggest that maintenance of 5mC by Dnmt1 plays an essential role in the progression of meiosis through adequate formation of synaptonemal complex.
(COI: No)

P2-288

Goshajinkigan completely recover the severe aspermatogenesis after busulfan treatment in mice

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Busulfan is used as anticancer chemotherapeutic drugs in childhood and adult chronic myelogenous leukemia as well as an immunosuppressive agent before bone marrow transplantation. It is well known that the male infertility including spermatogenesis disturbance is one of side effect. There is little information about therapeutic drugs on male infertility after busulfan treatment. In the present study, we gave goshajinkigan to mice already having severe aspermatogenesis after busulfan treatment to determine whether or not the goshajinkigan can recover the aspermatogenesis. Male C57BL/6j mice were received a single intraperitoneal injection of busulfan at 4-week-old and after 60 days fed on the goshajinkigan-including diet or goshajinkigan-free normal diet for another 60 days. The results showed that after busulfan treatment, the progressively decreases in the weight of the testes (TW) and epididymal sperm count (ESC) in normal diet group from 60 days to 120 days; on the other hand, in goshajinkigan-including diet group, the dramatic recovery of these variables at 120 days, which is similarity to the normal spermatogenesis. These results suggest that busulfan-induced aspermatogenesis was irreversible unless receiving any medication. However, the supplementation of goshajinkigan can completely recover the regeneration of the injured seminiferous epithelium, suggesting that goshajinkigan have a therapeutic effect on busulfan-induced aspermatogenesis.
(COI: No)

P2-289

Identification of 5-bromo-2'-deoxyuridine-labeled cells during mouse spermatogenesis by use of a heat-induced antigen retrieval in lectin- and immunohistochemistry

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DNA replication occurs in the S phase of spermatogonia and preleptotene spermatocytes during spermatogenesis. BrdU is incorporated into synthesized DNA and is detectable in the nucleus by immunohistochemistry. To identify BrdU-labeled spermatogenic cells, the spermatogenic stages must be determined by visualizing acrosomes and the cell type-specific marker molecules must be detected in the seminiferous tubules. However, the antibody reaction with BrdU routinely requires the denaturation of DNA, which is achieved by pretreating tissue sections with hydrochloric acid; however, this commonly interferes with further histochemical approaches. Therefore, we examined optimal methods for pretreating paraffin sections of the mouse testis to detect incorporated BrdU by an antibody and, at the same time, visualize acrosomes with peanut agglutinin (PNA) or detect several marker molecules with antibodies. We found that treatment with heat-induced antigen retrieval (HIAR) consisting of heating at 95C in 20 mM Tris-HCl buffer (pH9.0) for 15 min was superior to that with 2N hydrochloric acid for 90 min at room temperature in the subsequent PNA-lectin histochemistry combined with double IHC for BrdU and one of the marker proteins. With this method, we identified BrdU-labeled spermatogenic cells during mouse spermatogenesis as A1 spermatogonia through to preleptotene spermatocytes.

(COI: No)

P2-290

Age-related changes of the wave of the seminiferous epithelium in rodent testes

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The wave of the seminiferous epithelium (SE) of the seminiferous tubules was assessed using 10-, 30-, 60-, and 90-week-old mice. The PAS-stained serial sections of testes were photographed, 3D-reconstructed in a computer to describe the wave of the SE. The typical complete wave, in which the all stages of the cycle of the SE line sequentially and it repeats along the length of the tubule, was observed only in the 10-week-old mice. In 30-week-old and older animals, the wave frequently reverses at the turning points of the winding tubules and the direction of the wave tends to be same in most tubule fragments. The rate of stage change in the unit length of SE becomes higher in older animals, meaning closer wave of seminiferous epithelium. At 90-week-old mice, the frequency of discontinuous wave significantly increased. Also increased the frequency of single cross sections of the tubule bearing two and more distinct stages of SE. These findings may correlate with decline in efficiency of sperm production in aged animals.

(COI: No)

P2-291

Left-right asymmetry of testicular formation in the chicken embryo

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Although all male vertebrates that use sexual reproduction have testes and the females have ovaries, the mechanism of gonadal formation differs among vertebrates. Most avian species including the chicken develop an ovary only on the female's left side. Testicular development in male chickens has been thought to be symmetric, but here we show that the testicular formation mechanism is asymmetric. We detected DMRT1, the most likely candidate transcription factor as a testicular determinant in the chicken, in not only Sertoli cells in seminiferous cords but also in cells constituting the cortex in the left gonad, whereas AMH, used as a Sertoli cell marker, was not expressed in the cortex. The cortex was gradually degenerated with testicular development, but interestingly, the serial localization of the cells expressing DMRT1 between the cortex and seminiferous cord was observed within a short period after sex determination. The localization of laminin and fibronectin illustrated the attachment of the seminiferous cord to the cortex. Recent studies indicated that Sertoli cells are derived fromnephrogenous mesenchyme in the chicken, but from coelomic epithelium in the mouse. However, the present findings show that only after sex determination and only in the left testis, the cells in the cortex apparently migrate into the seminiferous cord and contribute to Sertoli cells.

(COI: No)

P2-292

Cervical heterotopic transplantation technique of testis and epididymis

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The heterotopic transplantation techniques have been widely used in rat organs, such as kidney and heart, to investigate transplant immunology. On the other hand, the testis and epididymis are known as immunologically privileged organs. To determine the transplant immunology of testis and epididymis, we examined to establish a novel technique of rat testis and epididymis transplantation.

In the present technique, the testis and its artery and vein were isolated with the abdominal aorta and vena cava near the joint of testicular vessels. The anterior ends of removed vessels were ligated and the posterior ends were anastomosed to the common carotid artery and external jugular vein to maintain blood flow. The operations were performed in a syngeneic model, and two allogeneic models (= acute rejection model and chronic rejection model). The testes and epididymises were sampled after 3 or 7 days and the weights, histology and immune reactions of testes in each group were observed.

No weights nor histological changes in donor testes were observed in the syngeneic model and also in chronic rejection model neither at 3 days nor at 7 days. However, in the acute rejection model, spermatogenic disturbances were observed in testes at 3 days and undergoing necrotic changes were detected in testes at 7 days. These results were similar to those reported in heterotopic transplant of kidney and heart.

In conclusion, the newly developed operative procedure appears reliable for investigation of further heterotopic transplantation of the testis.

(COI: No)

P2-293

Quantitative analysis of the cellular composition in seminiferous tubules in normal and genetically modified infertile mice

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The aim of this study was to establish a quantitative standard of the cellular composition in seminiferous tubules at each stage of spermatogenesis in the mouse testis, and thereby evaluate abnormalities in the infertile mouse testis. We applied a combination of lectin histochemistry for acrosomes and immunohistochemistry for various specific cell markers, both visualized with fluorescence, on paraffin sections of the testis. We first examined seminiferous tubules from normal mice and counted the number of each cell type at each stage of spermatogenesis. We then examined seminiferous tubules from genetically modified mice deficient (-/-) for one of the cell adhesion molecules, nectin-2 or nectin-3, which were infertile, and compared the number of each cell type at each stage of spermatogenesis with the corresponding value in normal mice. In both nectin-2-/- and nectin-3-/- mice, despite the apparently normal morphology of the seminiferous epithelia, the later step spermatids were lost progressively, with the numbers of step 11-16 spermatids in nectin-3-/- and step 15-16 spermatids in nectin-2-/- mice being significantly lower than those in normal mice. The present study demonstrated that a quantitative analysis of cellular compositions at different stages in seminiferous tubules was useful for evaluating abnormalities in spermatogenesis.

(COI: No)

P2-294

The 3D-structure analysis of spermatids and the Sertoli cell by serial block face-SEM method

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Cells in biological tissues contact each other 3-dimensionally, and they have various interactions. In the seminiferous epithelium, the spermatogenesis is observed from the spermatogonium to the sperm, and these cells also contact Sertoli cells, suggesting that they have interactions. To understand these interactions, it is important to clarify 3-dimensional connections of the cells. However, for this purpose, TEM level spatial resolution is needed, since the cells are still connected by fine intercellular bridges even after cell division. In addition, analysis of several tens of cells is needed to understand whole 3-dimensional structure, since the spermatogonium undergoes several cell divisions during differentiation into the sperm. Thus, it is difficult to use TEM tomography and confocal laser scanning microscopy for this purpose. To overcome these difficulties, we applied a serial block face-SEM method to analyze the 3-dimensional structure of spermatids and Sertoli cells. In this method, an ultramicrotome is installed in the specimen chamber of a SEM, and the surface of a resin embedded specimen is cut at a predetermined thickness. Then the SEM image of the exposed specimen surface is captured. By repeating this process and stacking the captured SEM images, it is possible to reconstruct the 3-dimensional cell structure in high resolution in a large area. We took about 1000 SEM images of the sliced seminiferous epithelium. We recognized that there were 16 connected spermatids and the spermatid had 4 intercellular bridges. We report the detail in this presentation.

(COI: No)

P2-295

Lectin-binding sites in epithelial cells of the mouse prostate

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Prostate is an exocrine gland in the male reproductive tracts. The prostatic epithelium consists mainly of luminal and basal cells. Although prostate tumors are believed to originate in these cells, the mechanism of normal prostatic epithelial differentiation remains unclear. To understand the cytochemical properties of prostatic epithelial cells, the characteristics of glycoconjugates in the mouse prostate were examined using the technique of lectin histochemistry combined with immunohistochemistry. Characteristic staining patterns depending on the type of lectins were observed in the prostatic epithelium. Luminal cells expressed Mannose in all regions of the prostate, and Galactose, N-acetyl-D-galactosamine (GalNAc), and N-acetyl-D-glucosamine (GlcNAc) in the lateral and ventral regions. Interestingly, luminal cells in the ventral region specifically reacted with Jacalin lectin. Basal cells expressed GlcNac in the apical and dorsal regions of the prostate. These results indicate that the selectivity in lectin reactivity for distinct cell types and segment-dependent staining in the prostate may be related to cellular and regional differences in function. Furthermore, because some lectins stain particular prostatic epithelial cells selectively, these lectins could be useful markers for histopathological evaluation of diseases or diagnosis of male infertility.

(COI: No)

P2-296

Fatty acid binding protein 3 (FABP3) regulates PUFA transfer through trophoblast cells

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OBJECTIVES: Deficiency of polyunsaturated fatty acid (PUFA) transport in prenatal stage has been suggested to result in various adult metabolic diseases. In this study, we examined the localization and functional significance of fatty acid binding proteins (FABPs) in the mouse placenta.
METHODS: Expression of FABPs in the mouse placenta was examined by RT-PCR, western blotting and immunohistochemistry. Radio-labeled FA were administrated into the pregnant mother and its transfer to the fetus was measured by liquid scintillation counter. FA uptake assay was also done on FABP3 knockdowned (KD) BeWo cells. Placental morphology was examined.
RESULTS: In RT-PCR and western blotting, gene and protein expression of FABP3, 4 & 5 were detected in the mouse placenta with temporal differences. In immunohistochemistry, FABP3 was highly localized in the labyrinthine zone of mouse placenta; FABP4 was highly localized in the decidua basalis; FABP5 was weakly and widely distributed in the labyrinthine, decidua and spongiotrophoblast zone. In FABP3KO placenta, transportation of n-3 and n-6 PUFA was significantly decreased compared to wild-type. Consistently, FABP3 KD BeWo cells showed lower PUFAs uptake than control cells.
CONCLUSION: FABPs were expressed in the mouse placenta with spatial differences. Among FABPs, FABP3 may be involved in regulation of cellular transport of PUFAs, possibly being associated with various metabolic/psychiatric diseases caused by the fetal nutritional deficiency.

(COI: No)

P2-297

Different cell death induced in distinct breast cancer subtypes after drug treatment

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Multiple subtypes are including in breast cancer that has different genetic background, drug sensitivity and prognosis. For these features, subtype specific therapy is absolutely required. Recently, we reported that α-Mangostin, one of xthantones isolated from pericarp of mangosteen fruit, can induce apoptosis in highly malignant triple negative(TN) breast cancer cell line (HER2-, ER-, PgR-) and has antitumor activity in a mouse mammary cancer model. In this study, we investigated whether there are significant differences in induced cell death on distinct subtypes of human breast cancer cell lines after α-Mangostin treatment. We used triple negative breast cancer cell line MDA-MB231 and triple positive (TP) cell line MCF7 (HER2+, ER+, PgR+). After treatment, both cells showed significantly decreased viability until 24 hours. DNA damage and apoptosis are induced earlier in TN cell, compared to TP cell. In TN cell, mitochondria-mediated apoptosis was observed in contrast to TP cell. In TP cell, heat shock protein (HSP) are possibly mediate apoptosis because of its increased expression after treatment. To further elucidate the difference, we demonstrate the ultrastructural analysis via transmission electron microscopy.

(COI: No)

P2-298

Epithelium-dependent periodical excitation in response to stretch of guinea pig seminal vesicle

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Seminal vesicle (SV), a male accessory sex gland, is well-known to contract via activation of the sympathetic nerves. In addition to the nervous activity-dependent mechanism, we have found that epithelium of SV generates stretch-sensitive contraction. To explore further mechanisms of the epithelium-dependent contraction, we examined the effects of removal of epithelium from guinea pig SV on isometric contraction and membrane potential of the circular muscles. Epithelium-intact ring preparations contracted periodically at a frequency of 5.3 ± 0.3 /min at 36 ℃. The periodical contractions was abolished by 3μM of nifedipine, suggesting they are associated with periodical activation of L-type Ca^{2+} channels. Removal of epithelium abolished the spontaneous contraction while nerve-evoked contraction was not impaired. To measure the membrane potential from the circular muscle layer, the tissue of SV (5 to 6 × 2 to 3 mm) was stripped of the serosa, longitudinal and outer circular muscle layers by dissection. In the presence of epithelium, the preparations could contract every 10 - 20 s by stretch. Six of nine cells in four epithelium-intact preparations, but none of the 16 cells in 12 epithelium-free preparations, exhibited periodical depolarization at 15.4 ± 0.4 s after the previous depolarization finished. These results suggest that the membrane oscillation in smooth muscles of SV may be produced by cells in the epithelial layer in response to stretch.

(COI: No)

P2-299

Reproductive and behavioral effects of clothianidin in male mice in a chronically stressed condition

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Neonicotinoids, which were developed in the 1990s, have been some of the most widely used pesticides in the world. They act as agonists to the nicotinic acetylcholine receptors (nAChRs) of insects with much higher affinity than to those of mammals, resulting in the death of insects from abnormal excitability in the cholinergic nervous system. However, as honeybee colony collapse disorder is suspected to be caused by neonicotinoids, there is rising concern about other unpredictable adverse effects of neonicotinoids on vertebrates such as birds and mammals. We hypothesized that the effects of neonicotinoids would be clear under chronic stress, which alters the expression of neuronal nAChRs. We performed immunohistochemical and behavioral analyses in male mice actively administered the neonicotinoid clothianidin (CTD) for 4 weeks under an unpredictable chronic stress procedure. We observed vacuolated seminiferous epithelia and decreased antioxidant enzymes, including glutathione peroxidase 4 and manganese superoxide dismutase, in the testes of the CTD-treated mice. In an open field test, locomotor activity and anxiety-like behaviors appeared to increase most in the CTD + stress mice. In summary, CTD and stress may additively affect the reproductive and behavioral functions of mammals.

(COI: No)

P2-300

Electro-acupuncture at sacral region enhances erectile function via central nerves system

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Electro-acupuncture (EA) influences various visceral functions via somato-autonomic reflex. We reported that EA at sacral region occurred increasing intracavernous pressure (ICP) by released nitric oxide from cavernousal nerve. The present study aimed to investigate whether ICP responses by EA at sacral region are mediated via central nervous system. Experiments were performed in isoflurane anesthesia rats. A catheter was inserted into the right carotid artery for blood pressure (BP) monitoring. ICP was measured with probe inserted to corpus cavernosum. EA was delivered by acupuncture needle, which inserted up to periosteal around 3rd foramina sacralia dorsalia, for 60 sec in a pulse width of 0.5 msec at a frequency of 10 Hz with an intensity of 5.0 mA. The spinalization was performed at the 13th thoracic after the EA response obtained, and then EA response was also confirmed at the same electrical condition. ICP was significantly increased by EA with an intensity of 5.0 mA, although BP was decreased. Furthermore, increasing ICP by EA was completely abolished by spinalization. These results demonstrate that EA at sacral region enhances erectile function via central nervous system. In conclusion, EA may be usefulness on the erectile dysfunction via central nerves system.

(COI: No)

P2-301

Partial blockade of Kv2.1 channel potentiates GLP-1's insulinotropic effects in islet β-cells and improves glucose tolerance in type 2 diabetes

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Glucagon-like peptide-1 (GLP-1)-based medicines have been widely used to treat type 2 diabetic patients. Inhibition of voltage-gated Kv2.1 channels in pancreatic β-cells is suggested to contribute to mild depolarization and promotion of insulin release. This study aimed to determine whether blockade of voltage-gated Kv2.1 channels potentiates insulinotropic effect of GLP-1. Kv2.1 channel blocker guangxitoxin-1E (GxTx) and GLP-1 agonist exendin-4 at sub-threshold concentrations, when combined, markedly increased insulin release and cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in a glucose-dependent manner in mouse islets and β-cells. Exendin-4 at sub-threshold concentration alone increased islet insulin release and β-cell $[Ca^{2+}]_i$ in Kv2.1$^{+/-}$ mice. The β-cell $[Ca^{2+}]_i$ response to sub-threshold exendin-4 and GxTx in combination was attenuated by protein kinase-A inhibitor H-89. Kv2.1$^{+/-}$ mice exhibited improved glucose tolerance and increased plasma insulin levels during oral glucose tolerance tests that promote endogenous GLP-1 release, compared to wild-type mice. Furthermore, administration of sub-threshold doses of GxTx and GLP-1 agonist liraglutide in combination markedly increased plasma insulin and improved glucose tolerance in diabetic db/db mice and NSY mice. These results demonstrate that a modest suppression of Kv2.1 channels dramatically raises insulinotropic potency of GLP-1-based drugs, providing a potential therapeutic tool to treat type 2 diabetes.

(COI: No)

P2-302

Differential responses to steroid hormones in fibroblasts from the vocal fold, trachea and esophagus

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Fibroblasts are target cells for steroids such as sex hormones and corticoids. The characteristics of fibroblasts vary among tissues and organs. We compared the action of steroid hormone on cultured fibroblasts from the vocal folds, which are considered to be the primary target of steroid hormones, as well as the trachea and the esophagus in adult male rats. Expression of steroid hormone receptors (androgen receptor (AR), estrogen receptor α, and glucocorticoid receptor) was confirmed by immunofluorescence detection. AR was more frequently expressed in the vocal fold fibroblasts than in the tracheal and esophageal fibroblasts. Cell proliferation analysis exhibited that either administration of testosterone (T), estradiol (E2), or corticosterone (CORT) suppressed cell growth in all three fibroblasts. mRNA expression of extracellular matrix-associated genes represented that the addition of T, but not of E2 or CORT, markedly promoted the expression of procollagen I and III, elastin and hyaluronic acid synthase I only in the vocal fold fibroblasts. These results indicate that each steroid hormone exerts region-specific effects on cervicothoracic fibroblasts with different properties through binding to each receptor. These findings might help clarify the mechanism of voice change and mutational voice disorder during puberty.

(COI: No)

P2-303

Identification of novel estrogen receptor α variants in the human and mechanism of transcriptional activation

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The human ER α gene is composed of eight coding exons, and generates several splice variants. However, it remains to be fully elucidated. Therefore, we decided to identify other human ER α splice variants and to re-examine the genomic organization of the human ER α gene. We cloned novel C-terminally-truncated variants using rapid amplification of cDNA 3'-ends, and identified novel terminal exons and novel alternative splice acceptor sites. Subsequently, we comprehensively analyzed the distribution of human ER α variants in the human peripheral organs using reverse transcription-polymerase chain reaction. The variant mRNAs were detected in a wide range of organs. Subsequently, we constructed expression vectors encoding wild-type, novel C-terminally truncated and artificially truncated ER α proteins, and characterized subcellular localization and transactivation functions of the variants in transfected cells. Moreover, we identified the mechanism of transcriptional activation that is ascribable to the structure of helices 3 to 5 in the ligand binding domain of ER α. In this study, we demonstrated that the ER α gene generates C-terminally truncated variants with distinct localization patterns and functions by alternative usage of intronic exons, and that the helix structure contribute to the transcriptional activation of the ER α protein. These findings provide useful information for further investigation on estrogen related physiological and pathophysiological processes.

(COI: No)

P2-304

Role of IL-6 in metabolic abnormalities of streptozotocin-induced diabetes

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We have found that inhibition of AMPK activity in skeletal muscle in STZ-induced diabetes by preferentially expressing dominant-negative AMPK (DN-AMPK) significantly improved STZ-induced hyperglycemia, and high level of plasma free fatty acids and ketone bodies, although plasma insulin level was low. Moreover, STZ-treated DN-AMPK mice improved atrophy of white adipose tissue (WAT) and skeletal muscle, body weight loss and increased survival rate. Chronic infusion of AMPK inhibitor, compound C, also improved the metabolic abnormities in spontaneously developed non-obese diabetic (NOD) mice as well as STZ-induced diabetes.
In this study, we investigated a role of IL-6 (Interleukin-6), a myokine secreted from skeletal muscle in STZ-induced diabetes. STZ-induced diabetes increased IL-6 protein expression level in skeletal muscle and plasma IL-6 level, and those were returned to the control levels in DN-AMPK mice. IL-6 protein expression was not changed in other tissues, such as liver, adipose tissue or spleen. The downstream factor of IL-6 signal, STAT3, was elevated in soleus muscle, WAT and BAT in STZ-induced diabetes, but this change was abolished in STZ-treated DN-AMPK mice. Infusion of neutral antibody for IL-6 by osmotic minipump improved the metabolic changes and survival rate in STZ-induced diabetes, similar to those in STZ-treated DN-AMPK mice. These results thus unveil a key role for muscle AMPK and IL-6 in metabolic abnormities in STZ-induced diabetes.

(COI: No)

P2-305

E-cadherin mediates Notch signaling in the rat anterior pituitary

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Anterior pituitary of the rat consists of hormone-producing cells and folliculo-stellate (FS) cells. FS cells construct unique microenvironment by homophilic cell adhesion and be assumed to be progenitor cells of hormone-producing cells at least in part. We have shown that Notch signaling plays important roles to regulate proliferation activity and SOX2 expression of FS cells. Notch signaling belongs to the juxtacrine signaling that requires specific cell adhesion. In the present study, we aimed to examine the possibility that E-cadherin, a specific cell adhesion molecule of FS cells establishes the Notch signaling among FS cells. By immunohistochemistry using transgenic rats that express GFP specifically in FS cells, it is shown that Notch2 and jagged1 are major receptor and ligand in FS cells, respectively. They are shown to be expressed specifically in cell clusters with E-cadherin. SOX2 was expressed in these cell clusters. It is also shown that Notch 2 and jagged1 are expressed in the same cells, which strongly suggest that FS cells affect each other to maintain undifferentiated state. Taken together with our previous report that cadherin isoforms switch from E-cadherin to N-cadherin when embryonic progenitor cells of anterior pituitary differentiate into hormone producing cells, results of the present study may suggest that E-cadherin construct a microenvironment for presumptive progenitor cells in the adult anterior pituitary to maintain undifferentiated state by Notch and other signaling.

(COI: No)

P2-306

Extracellular matrix actions in rat anterior pituitary gland: I. Its effect on hormone release from gonadotrophs

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The anterior pituitary gland is composed of five types of hormone-producing cells, folliculostellate cells, endothelial cells, pericytes, and the various extracellular matrixs (ECMs). The ECM supports adhesion of cells and is also important for cell survival, proliferation, differentiation, and migration via its receptors (integrins) in various tissues. Hormone-producing cells and folliculostellate cells form lobules that are surrounded by ECM in rat anterior pituitary gland. In the lobular structure, adhesion of hormone-producing cells and folliculostellate cells to basement membrane is observed and these cells are close to collagen fibril. We previously reported the cells that produce ECM components including collagen, laminin and proteoglycan in rat anterior pituitary gland. However, there is little information about effect of ECM on hormone release from the adenohypophyseal cells. In this study, we investigated whether ECM affect to luteinizing hormone (LH) release using male rat anterior pituitary cells. We compared hormone secretion from cultured cells on ECM-coated plate with that of on non-coated plate in various experimental conditions. LH release level from cultured cells on ECM-coated plate was decreased as compared with cultured cells on non-coated plate. This result suggests that ECM contribute to regulation of LH release from gonadotropes.

(COI: No)

P2-307

Rat uterine oxytocin receptor and estrogen receptor α and β mRNA levels are regulated by estrogen through multiple estrogen receptors

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In the rat uterus, oxytocin receptor (OTR) and estrogen receptor (ER) levels are regulated by estrogen; however, which types of ERs are involved have not been elucidated. This study examined OTR, ER α , and ER β levels in ovariectomized rats treated with: 17 β -estradiol (E2), the ER α agonist (PPT), the ER β agonist (DPN), the GPR30 agonist (G-1), and estren (Es). E2 and PPT increased OTR mRNA levels and decreased ER α and ER β mRNA levels 3 and 6 h post-treatment. DPN decreased ER α and ER β mRNA levels at 3 and 6 h, while OTR mRNA levels increased at 3 h and decreased at 6 h. After Es treatment, OTR mRNA levels increased at 3 h and then declined until 6 h, whereas ER α and ER β mRNA levels decreased by 3 h and remained low until 6 h post-treatment. G-1 had no effect on OTR, ER α , and ER β mRNA levels either at 3 or 6 h. The ER antagonist ICI182,780 (ICI) suppressed Es-induced increases in OTR mRNA levels at 3 h. However, neither ICI nor tamoxifen (Tam) had any significant effect on ER α and ER β mRNA levels in the Es-treated group. In intact rats, proestrus-associated increases in OTR mRNA levels were antagonized by ICI and Tam, but not by the GPR antagonist G15, while decreases in ER α were antagonized by ICI, but not by Tam or G15, and decreases in ER β were antagonized by Tam and G15, but not by ICI. Taken together, these results show that, in the rat uterus, expression of the OTR gene and of ER genes is regulated by estrogen through multiple pathways that involve Es-sensitive ERs and/or GPR30.

(COI: No)

P2-308

The mechanism of intracellular cAMP concentration increase induced by extremely low-frequency magnetic field exposure in mouse adrenal-derived Y-1 cell

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We previously reported that exposure of extremely low-frequency magnetic field (ELF-MF) induces adrenal steroid synthesis in mouse adrenal Y-1 cell line via increase of intracellular cyclic adenosine monophosphate (cAMP) concentration. However, precise mechanism of the cAMP increasing is unknown at present. The cAMP concentration is decided by the ratio of the cAMP synthesis by adenylate cyclase, which activates with Gs family of G-protein, and the degradation by phosphodiesterase (PDE). In this study, we investigates the effect of Gs α -subunit inhibitor NF449 on the increase of cAMP concentration by ELF-MF exposure and PDE activity in ELF-MF exposed cell to clarify the mechanism on the ELF-MF induced adrenal steroid synthesis in Y-1 cell. As the results, NF449 is decreased the cAMP concentration in sham and ELF-MF exposed Y-1 cell, but the cAMP-increasing effect of ELF-MF was preserved. On the other hand, PDE activity was decreased in ELF-MF exposed Y-1 cell. Our results suggest that the increase of intracellular cAMP concentration induced by ELF-MF exposure in Y-1 cell may be involved in the decrease of PDE activity not the activation of G protein-coupled receptors.

(COI: No)

P2-309

Relationship between perinatal hypothyroidism and structure/function of Purkinje cells

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Thyroid hormone (TH) is very important for increasing the basal metabolic rate of almost all cells to achieve the healthy function of organs. It is also important for brain development and maturation during perinatal period. Perinatal hypothyroidism induces anatomical and functional deficiency such as motor discoordination by cerebellum. The most problem thing is that some effects of TH have critical period and deficiency of perinatal short-term hypothyroidism are prolonged until adulthood. However, the relationship between the effect of hypothyroidism on functions of cerebellum and the anatomical and/or electrophysiological changes of cerebellum is remained unclear. In this study, we examine the effect of perinatal hypothyroidism on function and structure of Purkinje cell (PC) in cerebellum by using slice patch clamp technique. We observed the synaptic activity of parallel fiber -PC and climbing fiber-PC synapses and after the experiment, stained the PC with biocytin/streptavidin for anatomical observation. In addition, we investigated the quantity of proteins which are involved in the machinery of synaptic release at presynapses contained soluble N-ethylmaleimide-sensitive factor-attachment protein receptor (SNARE) proteins for hypothyroid mice because we previously reported the possibility of presynaptic dysfunction in congenital hypothyroidism mice (Amano et al., J. Physiol. Sci., S2210, 2014).

(COI: No)

P2-310

Learning deficits in a mouse model of perinatal mild hypothyroidism

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Thyroid hormone (TH) is essential for brain development. It is known that congenital hypothyroidism causes a wide spectrum of severe neurological deficiencies in rodents and human. It is also recently concerned that decrease of taking iodine diet or increase of environmental chemicals cause low level hypothyroidism. However, mild and/or moderate hypothyroidism on brain development is felly studied. Thus, we examined the behavior adult mice which induced mild hypothyroidism during perinatal period by using low dose propylthiouracil (PTU) application. We added the PTU (5 or 50 ppm) in drinking water for the mother mice and their pups from gestational day 14 to postnatal day 21. TH levels of pups were significantly decreased in both 5 and 50 ppm groups compared with those in control pups. However poor bodyweight gain was only shown in 50ppm group. Cognitive performances were assessed using novel object recognition test and novel object location test on postnatal 8 weeks. Clear differences were absent in both short (15 min) and long(24 hr) term memory. To detect discrimination learning, touch-panel discrimination test was performed on postnatal 9-10 weeks. Discrimination levels of 50 ppm group are lower than other groups on day 3-4. However same levels of discrimination index were shown in all groups on day 5-9. These data suggest that mild hypothyroidism partially prevents cognitive function and causes delay of learning according to disorders of neuroplasticity or neuronal circuit function itself.

(COI: No)

P2-311

Mechanism of proliferation of pancreatic beta cells in ventromedial hypothalamus-lesioned rats

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The ventromedial hypothalamus (VMH)-lesions induce hyperplasia of the abdominal organs via hyperactivity of the vagal nerves. Recently betatrophin that is a liver-secreted hormone induces pancreatic β cell proliferation. The aim of this study is to clarify the mechanism of cell proliferation in the pancreas of the VMH-lesioned rats using histological and immunohistochemical techniques and measurement of the gene expression level of betatrophin in the liver. The bilateral VMHs of female Sprague-Dawley rats were electrically lesioned. Specimens were prepared at 5 days after the operations. The expression level of betatrophin mRNA in the liver was measured using a real-time PCR. Mitotic cells were observed in the pancreatic islet of VMH-lesioned but not sham-operated rats. Double immunostaining revealed that Ki-67 was localized in the insulin-immunopositive cells. Mitotic cells consisted mainly mature β cells with small part of undifferentiated cells at electron microscopic level. The gene expression level of betatrophin was significantly higher in VMH-lesioned than in sham-operated rats. These results suggested that proliferation of β cells by VMH lesions was mainly caused by self-replication mechanism. Betatrophin may contribute to self-replication of β cells in VMH-lesioned rats.

(COI: No)

P2-312

Specific localization of zinc transporters and zinc-required proteins in the pancreatic islet of rat

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The islet of rat pancreas is composed by 5 different endocrine cell types (A cell, B cell, D cell, PP cell, and E cell); the B cell occupies the central portion, the others the peripheral portion. By the immunohistochemical studies, two zinc transporters (Znt5, ZIP7) and 3 zinc-required enzymes, Carbonic Anhydrase (CA) II, XII, and Carboxypeptidase (CP) A localized in the peripheral portion of the islet. To identify their origin to the specific endocrine cell type, double immunofluorescence labeling was performed using the antibodies of zinc-related proteins and those of glucagon (GCG), somatostatin (SST) and pancreatic polypeptide (PPY). The frozen sections of normal and zinc-treated rats (ZnSO4 5mg/100g.b.w. i.p. one injection /day for 2days) were subjected to immunofluorescence labeling. The labeling showed, CAII was co-localized with some of PPY-positive cells, but neither with GCG-, nor SST-positive cells. Those of ZnT5, ZIP7, CAXII, CPA were co-localized with GCG-positive cells, but not with SST-positive cells. Although the B cell in the islet is known to contain much zinc for crystallization of insulin molecules in the secretory granules, significance of zinc in the A cell has not been well understood. In this experiment, many zinc-related proteins were specifically localized to the α cell in the islet, but not to the other cell types. The significance of zinc in the A cell will be discussed, considering the specific structural organization of the pancreatic islet.

(COI: No)

P2-313

Continuous GnRH signal affects the ultrastructure of endomembrane systems in male rat pituitary gonadotropes

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Gonadotropin synthesis and secretion are regulated by GnRH signaling. To examine whether continuous GnRH signals affect the morphological characteristics of gonadotropes, we analyzed the ultrastructural and immunocytochemical changes in endomembrane systems of male rat pituitary gonadotropes during sustained treatment with various GnRH agonists. In gonadotropes at 1 day after subcutaneous implantation of osmotic pumps filled with GnRH agonists, leuprorelin or buserelin, patch-like accumulations of chaperons such as calnexin and BiP appeared. By electron microscopy, the ER chaperones were accumulated in the anomalous network of tubuloreticular membranes within the stimulated gonadotropes. To these atypical tubuloreticular membranes, an E3 ligase HRD1 involved in the ER associated degradation (ERAD), was colocalized with the chaperones. Simultaneously, or slightly behind the time, multi-lamellar autophagosome-like structures occasionally appeared in the cytoplasm of gonadotropes containing the ER patches. These findings suggest that continuous GnRH signals induce dynamic reorganization of endomembrane systems related to the intracellular protein degradation.

(COI: No)

P2-314

β-Cell specific *Mafk* Overexpression Impairs Pancreatic Endocrine Cell Development

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Pancreatic β-cells are the only cells that can secrete insulin to maintain normal glycaemia. The MAF transcription factor proteins are homologs of v-MAF, the oncogenic component of the avian retrovirus AS42. We previously found that β-cell specific overexpression of *Mafk* exhibited glucose intolerance in adulthood. The aim of this study is to examine the effect of β-cell specific *Mafk* overexpression in embryonic endocrine pancreas. The developing islets of transgenic embryos appeared disorganized with an inversion of insulin- to glucagon-positive cell ratio at both E15.5 and E18.5. Moreover, the total insulin content significantly decreased in transgenic embryos. Immunohistochemical analysis using Ki67 antibody showed a lower ratio of proliferating β-cells in the transgenic embryos compared to control embryos, suggesting that damaged β-cell proliferation resulted in the abnormal endocrine structure of transgenic islets. The examination of gene expression profiles by Q-PCR revealed that *insulin* genes were significantly decreased accompanied with the reduction of several β-cell related genes including *Slc30A8*/ZnT8, *Npy*, and *G6pc2*. In contrast, both transcription factors essential for β- and α-cell development including *Pax4*, *Nkx2.2*, *Arx*, and *Mafb* were upregulated. Our results suggested that β-cell specific *Mafk* overexpression impaired embryonic endcrine development, and to further examination of this model might be useful for the better understanding of the molecular basis that govern β-cell development.

(COI: No)

P2-315

Calcium imaging of vasopressin neuron in the hypothalamic culture derived from mouse embryonic stem cell

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Recently, mouse embryonic stem cell has been successfully induced to hypothalamic tissue that express variety of hypothalamic neuropeptides. The feederless mES line, EB5, quickly aggregates to form embryoid body in serum-free medium in the phospholipid-coated well (SFEBq). In growth factor-free chemically defined medium (gf-CDM), it differentiates to Rax^+ hypothalamic progenitor cells in SFEBq that further mature to hypothalamic neurons including $vasopressin^+$ neuron. For physiological investigations, we have engineered the vasopressin::GFP cell line ($AVP^{GFP/+}$) from $Rax^{GFP/+}$ mESC using TALEN nuclease system. Hypothalamic progenitor cells expressing Rax were purified by FACS-sorting, and cultured on cover glass for further maturation. A week later, $AVP^{GFP/+}$ cells can be identified by fluorescence microscope and the expression of AVP was identified with immunocytochemistry. On the calcium imaging analysis using Fura2-AM, Glutamate increased $[Ca^{2+}]_i$ and GABA attenuated the glutamate-induced response in the $AVP^{GFP/+}$ cells. In some of the $vasopressin^+$ neuron, glutamate response was suppressed by the addition of NERP-1, which supposed to be a dendritic released transmitter from vasopressin neuron. These findings suggest this system would contribute to elucidate the detailed mechanism of vasopressin release.

(COI: No)

P2-316

Immunoelectron microscopic study on the subcellular localization of kisspeptin, neurokinin B and dynorphin A in KNDy neurons of the female rat

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KNDy neurons in the hypothalamus are coexpressing three kinds of neuropeptide, kisspeptin, neurokinin B (NKB) and dynorphin A (DynA), and are known to be associated with generation of GnRH / luteinizing hormone (LH) pulse to control follicular growth and steroidogenesis. However, the subcellular localization of these neuropeptides in KNDy neurons are not clear. In this study, we analyze the subcellular localization pattern of three neuropeptides using the immunoelectron microscopy. The female rat brain were fixed, dehydrated in ethanol and embedded in epoxy resin. The hypothalamic areas containing KNDy neurons was selected and trimed under stereoscopic microscope, then ultra thin sections were cutted. We performed the post-embedding immunoelectron microscopy with each antibody of kisspeptin, NKB, DynA, and visualized by colloidal gold-2nd antibody complex, and observed them using a transmission electron microscope. Three neuropeptides, kisspeptin, NKB and DynA were observed in each different secretory vesicle in KNDy neurons. We succeeded to reveal that three neuropeptides in KNDy neurons were contained in each different secretory vesicle, suggesting that these neuropeptides in the KNDy neurons were differentially regulated. Our present results will clearly contribute to consider the regulation mechanism of kisspeptin secretion in KNDy neurons, and the generation of GnRH / LH pulse induced by kisspeptin.

(COI: No)

P2-317

Molecular and histochemical analysis on acute modulation of the *Kiss1* expression in the lactating rat hypothalamus mediated by the suckling stimulus

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In female mammals, lactation suppresses GnRH secretion resulting in transient infertility. In rats, GnRH secretion is recovered in 18 h after pup separation (PS) and rapidly re-suppressed by re-exposure of pups. In order to elucidate the mechanisms underlying these modulations, changes in the expression of kisspeptin, a stimulatory modulator for GnRH secretion, were examined. 4h or 18h PS significantly increased *Kiss1* expression in both the anteroventral periventricular nucleus (AVPV) and the arcuate nucleus (ARC), and subsequent 1h exposure of pups re-suppressed *Kiss1* in the AVPV. Change in *Kiss1* expression was observed prior to the changes in GnRH, indicating that the changes in GnRH secretion result from the change of kisspeptin. We further examined the mechanisms of the rapid modulation of *Kiss1* expression. We firstly examined the effect of prolactin. Intravenous administration of prolactin suppressed *Kiss1* expression in the AVPV. We also examined the possibility that the suckling stimulus modulate the *Kiss1* expression through ascending sensory input. Injection of the anterograde tracer to the subparafascicular parvocellular nucleus (SPFpc) in midbrain which relay suckling stimulus revealed direct neuronal connections between the PSFpc and kisspeptin neurons in both the AVPV and ARC. These results indicate that suckling stimulus rapidly modulate *Kiss1* expression directly via neuronal connections, and partially through serum prolactin, resulting in modulation in GnRH secretion.

(COI: No)

P2-318

Morphological analysis of spines of the GnRH neuron through pubertal development

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The onset of puberty is initiated by augmentation of gonadotropin-releasing hormone (GnRH) release from GnRH neuron. However, the precise mechanism which leads the changes in the activity of GnRH neurons at the puberty onset is still unclear. The spine, small protrusions on the surface of neuronal dendrites, normally receives excitatory inputs. In this study, we analyzed the number of spines of GnRH neuron to determine the changes in synaptic inputs through puberty, using 3 and 8 weeks of age GnRH-eGFP transgenic rats. We also measured the diameter of head (DH) of each spine and classified them into small (DH<0.65 μm), large (DH0.65 μm), and giant spine (DH0.9 μm). The greatest number of spines was observed at the proximal dendrite (<50 μm from soma). At the soma and proximal dendrite, the number of spines was greater in adult than in juvenile in both sexes. Classification of spines revealed that the increases in large and giant spines at soma and the proximal dendrite. To further explore the relationship between the spines of GnRH neuron and puberty, we analyzed the adult rats neonatally exposed to estradiol benzoate, in which puberty onset and reproductive functions is disrupted. Neonatal estrogenization resulted in decreases in the number of all types of spines, so that total number of spines, at soma and dendrites in both sexes. These results suggest that GnRH neurons become to receive more and lager excitatory inputs on the soma and the proximal dendrite through puberty, and the changes in the spines play pivotal roles in the normal pubertal development.

(COI: No)

P2-319

Age-related alterations of KNDy neuron and pulsatile LH release in female rats

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After menopause, GnRH/Luteinizing hormone (LH) pulse frequency, which is essential for follicular growth, is decreased. Kisspeptin /Neurokinin B (NKB) /Dynorphin (KNDy) neuron in hypothalamic arcuate nucleus is known to be associated with GnRH/LH pulse generation, but the age-related changes in the expression of KNDy neuron and pulsatile LH release in female rats has not been examined.
In this study, we studied on this unsettled issue by using 8-16 week-old (young), 13, 20, and 24-month-old (13M, 20M, 24M) female rats. Blood samples were collected every 6 minutes for 3 hours to measure LH concentration by RIA. After fixation of rat brains, the coronal cryosections were prepared for in situ hybridization of *Kiss1* (Kisspeptin gene), *Tac2* (NKB gene), and *Pdyn* (Dynorphin gene) mRNA.
Plasma LH concentration was reduced during aging as previously reported. The expression of *Kiss1* was significantly decreased in 20M and 24M compared to young and 13M. Although both *Tac2* and *Pdyn* expression were also significantly decreased in aged animals compared to young, *Tac2* expression was maintained at relatively high level compared to *Pdyn* in aged animals.
These results suggest that each gene in KNDy neuron may be controlled by different signal pathway, and the reduction of KNDy expression may cause altered pulsatile LH secretion in perimenopausal period.

(COI: No)

P2-320

Functional significance of angiogenesis-associated vascular plasticity in neurosecretion of the neurohypophysis

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Hypothalamo-neurohypophysial system releases arginine vasopressin (AVP) and oxytocin (OXT) from axonal terminals of the neurohypophysis (NH) into blood circulation for controlling body fluid homeostasis and lactation. Chronic osmotic and suckling stimulations have been shown to cause neurovascular and neuroglial reconstruction in the NH of adult mammals and no study has been reported for vascular dynamics. The aim of this study was to elucidate the occurrence of continuous angiogenesis and growth factor-dependent neurovascular reconstruction in the NH of adult mice. Active proliferation of endothelial cells was observed using the immunohistochemistry of bromodeoxyuridine and Ki-67. Vascular endothelial growth factor A (VEGFA) and VEGF receptor 2 (VEGFR2) were highly expressed at pituicytes and endothelial cells respectively. Administration of the selective tyrosine kinase inhibitor AZD2171 for VEGFRs significantly decreased proliferation of endothelial cells. Moreover, AZD2171 treatment decreased vascular density by facilitating apoptosis of endothelial cells and the withdrawal of its treatment led to remarkable rebound proliferation of endothelial cells. AZD2171 decreased the density of both AVP- and OXT-containing axonal terminals. Thus, this study demonstrates that the signaling pathways of VEGF are crucial mediators for determining proliferation of endothelial cells and the density of AVP- and OXT-containing axonal terminals in the NH.

(COI: No)

P2-321

Identification of transcriptional and posttranscriptional regulation of human estrogen receptor expression in the testis

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Expression of estrogen receptors (ERs) are subject to complicated regulation. Transcription of the ER genes is initiated by multiple promoters. Furthermore, alternative promoter usage and alternative inclusion of untranslated internal exons yield multiple variants with distinct 5'-untranslated regions (5'-UTRs), which influence translational efficiency and mRNA turnover. Estrogens exert their effects via activation of two types of ERs (ER α and ER β), and have pivotal roles in the testis. However, several previous reports are inconsistent in expression patterns of ER α and ER β in the human testis. Therefore, we examined the expression and regulation of the human ER genes in the testis. RT-PCR analysis revealed that both ER α and ER β mRNAs are expressed in the testis. Then, we analyzed expression of promoter-specific ER isoforms to evaluate which promoters are selectively utilized in the testis. The expression of ER α mRNAs was initiated mainly by activation of F and T promoters. All promoter-specific ER β isoforms (0K, 0N, and E1 isoforms) were highly expressed. Subsequently, we assessed posttranscriptional regulation of the isoforms using luciferase reporter assays. The analysis demonstrated that the translation of ER α T isoforms was severely suppressed. These results indicate that expression of human ER α is repressed posttranscriptionally in the testis and that transcription of testicular ER β mRNA is initiated by strong activation of three promoters.

(COI: No)

P2-322

IL-1β produced by Microglia/macrophage in the organum vasculosum of the lamina terminalis is involved in the suppression by lipopolysaccharide of steroid-induced luteinizing hormone surge in ovariectomized rats

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Inflammatory/immune challenge is known to suppress luteinizing hormone (LH) secretion. We previously reported that pretreatment with minocycline (Mino), a potent inhibitor of microglial activation, significantly alleviate the suppression by lipopolysaccharide (LPS) of ovarian steroid-induced LH surge, but did not affect the increment of serum cytokines, treatment in ovariectomized (OVX) rats. In this study, we examined the effect of Mino on cytokines induction by LPS in the preoptic area of ovarian steroid-primed OVX rats. Mino or saline was administered intraperitoneally once a day for four consecutive days. LPS or saline was injected intravenously at noon. Brain fragments including the preoptic area were collected at 14.00 h, and gene expressions of IL-1β, IL-2 and IL-6 were measured by real-time PCR. Cytokine was also examined localization. IL-1β and IL-6, but not IL-2, gene expressions were increased by LPS treatment. Mino pretreatment significantly attenuated the induction of cytokines by LPS. IL-1β immunoreactivity was found only in Iba1 (a marker of microglia/macrophage) immunoreactive cell in the organum vasculosum of the lamina terminalis (OVLT) in LPS treated rats. These results suggest that IL-1β produced by microglia/macrophage in the OVLT might be involved in the inflammatory/immune challenge induced suppression of LH secretion.

(COI: No)

P2-323

Postnatal Changes of Distribution of S-100 Protein Positive Cells, Connexin 43 and LH-RH Positive Sites in the Pars Tuberalis of the Rat Pituitary Gland

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Folliculo-stellate cells are characterized by their star-like morphological features and specific ability to form follicles found within the adenohypophysis of rats. The architecture of luteinizing hormone-releasing hormone (LH-RH) nerve ends and the S-100 protein containing folliculo-stellate cells forming gap junctions in the pars tuberalis is basically important in understanding the regulation of the hormone producing mechanism of anterior pituitary glands. In this study, we investigated the sexual maturation of the anterior pituitary glands through the postnatal development of S-100 positive cells, connexin 43 and LH-RH nerves. Intact male rats 5 to 60 days old were prepared for immunohistochemistry, the S-100 containing cells in pars tuberalis were first detected on day 30 and increased in number to day 60; this was parallel to the immunohistochemical staining of gap junction protein, connexin 43. LH-RH positive sites were clearly observed on just behind the optic chiasm and on the root of pituitary stalk on day 30. On day 60, the width of layer increased, while follicles and gap junctions were frequently observed between agranular cells of pars tuberalis. It is suggested that the folliculo-stellate cell system including the LH-RH neurons in the pars tuberalis participates in the control of LH secretion along with the portal vein system.

(COI: No)

P2-324

Expression of vasopressin in rat peripheral tissues

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Vasopressin and oxytocin, neurohypophysial hormones, are nine-amino acid peptides which are best known for their classical abilities to increase peripheral vascular resistance by water reabsorption in the collecting ducts of the kidney nephron and by vasoconstriction, and contract smooth muscle of uterine and mammary gland, respectively. In addition, they seem to work in the central nervous system and other peripheral organs than described above. We have showed oxytocin distribution in the peripheral organs (2014, The 119th Annual Meeting of the Japanese Association of Anatomists). Then, we performed an analysis for vasopressin distribution in peripheral organs. Using real time RT-PCR technique, in addition to hypothalamus and posterior pituitary gland, vasopressin mRNA was expressed widely in anterior pituitary gland, pancreas, parotid gland, submandibular gland, heart and kidney. At the cellular expression level by immunohistochemistry, vasopressin immunoreactivity was seen in secretary duct epithelial cells in pancreas and salivary glands, in secreting cells of anterior pituitary gland and parotid gland, and in uriniferous tubules and podocyte. Majority of those cells also shows oxytocin immunoreactivity. However, cells in islets of Langerhans, which synthesize oxytocin, showed no vasopressin immunoreactivity. Results suggest that some peripheral tissues synthesize both vasopressin and oxytocin, and that vasopressin and oxytocin may interact together to control cells in a paracrine manner.

(COI: No)

P2-325

Effects of proton pump inhibitor on organs synthesizing estrogen in male rats

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Proton pump inhibitor (PPI) suppresses a gastric acid secretion of parietal cells of the stomach. It has been known that the parietal cells in rats synthesize a large amount of 17 β-estradiol. However, effects of PPI on their estrogen synthesis are unclear. Furthermore, the effects on the synthesis in the testis and adrenal gland, which are known as the main organs of the 17 β-estradiol synthesis in male, is also unclear. Twelve adult male Wistar rats were separated into the following three groups; LPZ1d: oral administration (OA) of lansoprazole (30 mg/kg) in 0.5% carboxymethylcellulose (CMC) solution for 1 day, LPZ3d: OA of lansoprazole in 0.5% CMC solution for 3 day, control: OA of 0.5% CMC solution. The 17 β-estradiol level of the blood in the portal vein and abdominal aorta was examined by ELISA. The aromatase mRNA and protein level of the gastric mucosa, testis, and adrenal gland were examined by real-time PCR, and by immunohistochemistry and Western blotting, respectively. The 17 β-estradiol level in the portal vein and abdominal aorta in LPZ3d was higher than that in the control. The aromatase mRNA and protein in the gastric mucosa were much enhanced in LPZ3d. The aromatase in the testis and adrenal gland was much lower than that of the gastric mucosa. These results suggest that PPI facilitates the estrogen synthesis in not the testis and adrenal gland but the parietal cells. The facilitation should cause an increment of the 17 β-estradiol level in the blood.

(COI: No)

P2-326

Analysis of the effect of retinoic acid on anterior pituitary cell functions in adult rat

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Retinoic acid (RA) is a lipid-soluble molecule, which is derived from vitamin A. This molecule serves as a ligand for two families of nuclear receptors that directly regulate gene expression, retinoic acid receptors (RARs) and retinoid X receptors (RXRs). RA is also one of the regulating molecules affecting pituitary cell functions. It activates growth hormone (GH) gene transcription. Moreover, RA stimulates the expression of D2R in lactotroph and suppresses TSH beta subunit expression in thyrotroph. However, the functions of RA on pituitary cells have not been well understood. The purpose of this study is to analyze the effect of RA on gene expression in primary pituitary cells in culture. The cells were isolated from anterior pituitary of adult Wistar rats. The cells were exposed to a graded concentration of all-trans RA (ATRA). In time-course experiments, the cells were cultured in the medium containing ATRA for 24 to 72 h. By using real-time PCR, we measured the expression level of GH releasing hormone receptor (GHRH-R) and GHS-R and SST-R in anterior pituitary cells. The treatment of ATRA (10^{-6} M, 24h) increased the expression of GHRHR and GHSR by 2 times. The stimulatory effect of ATRA on GHRH-R and GHS-R expressions were dose-dependent and time-dependent manner. These results suggest that RA controls the expression of receptors of stimulating factors and then induces the release of GH from somatotroph. In addition to GHRH-R and GHS-R gene expression, we have analyzed novel RA-induced or -suppressed genes in anterior pituitary cells by means of DNA microarray analysis.

(COI: No)

P2-327

Analysis of collagen producing cells in human anterior pituitary gland; normal pituitary and pituitary adenoma

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Extracellular matrix (ECM), especially collagen, is essential in the physiology of normal pituitary and in tumorigenesis, However the role of ECM and their changes in pituitary adenomas have not been well studied. To clarify this, we first identify the collagen producing cells in control tissue and pituitary adenomas by means of in situ hybridization and immunohistochemistry. Human pituitary adenomas with various type of clinical consistencies were obtained during surgery at Toranomon Hospital. We performed in situ hybridization for collagen I and III, and double stained by α SMA as a pericyte marker, and cytokeratin as an epithelial cells marker. In addition, we performed in situ hybridization for RGS5 which is also a pericyte marker. In pituitary adenomas, there are 4 types of collagen-producing cells, 1) myoepithelial like cell, 2) pericyte, 3) myofibroblast, 4) fibroblast. In hard elastic consistency typed tumors collagen producing cells are predominantly the myoepithelial like cell and myofibroblast. In soft consistency typed tumors, the majority of collagen producing cell is pericyte. We cathegorize and analyze the proportion of collagen producing cells type in each type of tumor. This study shows that there are alterations in collagen producing cells in normal pituitary and pituitary adenomas.

(COI: No)

P2-328

Expression of heparin-binding growth factor midkine/pleiotrophin family in the estrogen induced prolactinoma of rat

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Midkine (MK) and pleiotrophin (PTN) belong to a family of secreted heparin-binding growth factors. These growth factors have multiple functions, such as regulation of cell proliferation, migration, survival, differentiation, and tumorigenesis. We recently reported that MK and PTN mRNA is expressed in the anterior and posterior lobes of pituitary in adult rat. In the anterior pituitary gland, these mRNAs were expressed in the folliculostellate cells, which do not produce classical anterior pituitary hormones. It is suggested that MK and PTN play a role as paracrine signaling molecules in the pituitary gland. However, the presence of these growth factors in pituitary tumors has not been demonstrated. In this study, we examined the expression of MK and PTN in diethylstilbestrol-induced prolactinoma of LEXF RI rats. Adult male rats were subcutaneously implanted with a silastic tube containing diethylstilbestrol (DES) for 1 month, 2 months, and 3 months. Using in situ hybridization with digoxigenin-labeled cRNA probes, we detected cells expressing MK and PTN in prolactinomas. MK expression gradually decreased in the gland during tumorgenesis. On the other hand, PTN-expressing cells were increased in prolactinoma. Double-staining revealed that PTN mRNA was only expressed in folliculostellate cells in the adenoma. These results suggest that PTN relates to cell proliferation and tumorigensesis of lactotrophs.

(COI: No)

P2-329

Effect of human mesenchymal stem cells (hMSCs) administrated into pancreases on diabetic mice

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Diabetes is a chronic life-style disease which is characterized by hyperglycemia and destruction of insulin producing beta cells in the pancreatic islet. Although intravenous (iv) transplantation of human mesenchymal stem cells (hMSCs) temporary ameliorated the hyperglycemia in rodent model, stable and sustainable effect has not obtained yet. We examined here effect of local administration of hMSCs into pancreas (intrapancreas; ipan) on streptozotocin (STZ)-induced diabetic mice. C57/BL6 mice were injected STZ (115mg/kg, intraperitoneally) at day 0 and were transplanted hMSCs (10^6) either iv or ipan at day 7. Another set of the diabetic animals were transplanted hMSCs twice at 1 and 4 weeks, collected blood and pancreas at 8 weeks, and were measured insulin level in blood and pancreas. All animals were measured blood glucose during the experimental periods. STZ injection increased blood glucose within 1 week. hMSCs decreased the blood glucose levels both in iv and ipan groups, and ipan one was greater effect than iv one. Twice hMSCs injections lowered blood glucose to diabetic border line. The animals increased significantly body and pancreatic weight, and plasma insulin level. The animals also increased insulin-positive islet size, number and density compared with vehicle one. We demonstrated that ipan hMSCs were much effective strategy compared with iv one to ameliorate diabetic symptoms.

(COI: No)

P2-330

A new function of Neuromedin U/Neuromedin S system in the CNS

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Neuromedin U (NMU) is a neuropeptide which was isolated from porcine spinal cord in 1985. In 2000, its specific receptors, NMUR1 and NMUR2, were identified, then the physiological functions have been examined. We have reported that this NMU system has important roles in central regulation of food intake, energy expenditure, stress responses, and circadian rhythmicity by using gene genetically modified mice. Another endogenous ligand, Neuromedin S (NMS), was identified in 2005, and its physiological roles were reported as regulation of circadian rhythm and food intake. However, NMU/NMS system seems to have other unknown physiological functions. It was reported that NMUR2 was expressed in the nucleus accumbance (NAc) in the brain where is related with reward system (Brain Res Gene Expr Patterns, 2001, 1:1-4), and some other reports suggested that central Neuromedin U mRNA was up-regulated in addiction models related with reward systems (PLoS One, 2010, 5:e15643). Recently, the central regulation of food intake is suggested to be deeply related with "higher brain functions" including reward system, preference, and recognition. Based on these evidences, we are focusing on examining a higher brain function of NMU/NMS system related with energy homeostasis. Here, we have performed a series of behavioral test by using newly established NMU/NMS double knockout mice and found this double KO mice shows some abnormal behavior response, such as anti-anxiety behavior and will figure out the molecular mechanism of this new physiological functions of the central NMU/NMS system.

(COI: No)

P2-331

COA-Cl-induced adipogenesis is associated with an increased cell cycle progression and down-regulation of $p27^{Kip1}$ in mouse pre-adipocyte 3T3-L1 cells

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Mouse pre-adipocytes 3T3-L1 cells undergo mitotic clonal expansion upon induction of adipogenesis. This clonal expansion is associated with down-regulation of cell cycle inhibitors, $p27^{Kip1}$ and/or $p21^{Cip1}$. We found that COA-Cl, a synthesized adenosine analogue with pro-angiogenic activity, enhances lipid accumulation and adipocyte marker gene expression 8 days after treatment with 1 μg/mL insulin (Ins), 1 μmol/L dexamethasone (Dex), and 500 μmol/L 1-methyl 3-isobutylxanthine (IBMX) in 3T3-L1 cells. Here we examined the effect of COA-Cl on the cell cycle in 3T3-L1 cells after induction of adipogenesis. The cell cycle distribution was evaluated with a flow cytometric analysis of propidium iodide fluorescence. When 3T3-L1 cells were stimulated with Ins, Dex and IBMX in the presence of COA-Cl, the cell cycle distribution in G0/G1-phase decreased from 86 to 83% and that in S/G2-phase increased from 14 to 17% ($p<0.05$). The treatment with COA-Cl down-regulated the expression of $p27^{Kip1}$. These results suggest that COA-Cl-mediated enhancement of adipogenesis is associated with an increased cell-cycle progression and down-regulation of $p27^{Kip1}$.

(COI: No)

P2-332

Expression analyses of stress-related factors in single prolonged stress rats

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Post-traumatic stress disorder (PTSD) is a stress-related anxiety syndrome that develops after exposure to traumatic experience. Many individuals with PTSD remain chronically symptomatic, implying that sustained structural and functional changes in brain is predisposing factors of PTSD. However, until today, biological basis of PTSD is almost unknown. Single prolonged stress (SPS) is an established animal model proposed for PTSD, and mimic the phathophysiological and behavioral characteristics of PTSD. In this study, by using SPS paradigm, we investigated the expressions of corticotropin-releasing hormone (CRH) and glucocorticoid receptor (GR) in the brain of adult male rats. SD rats at 8 weeks, were subjected to a single session of prolonged stress consisting of immobilization, forced swimming, and exposure to ether vapor. Seven days after SPS treatment, mRNA and protein expression levels of CRH and GR were examined by real-time PCR and immunohistochemistry. In SPS rats, no change was detected in expression of GR protein in hippocampus. However, CRH mRNA and proteins showed significant increase in the central nucleus of the amygdala which is known to be involved in the expression of emotion such as anxiety and fear. These results suggest that SPS paradigm alters stress-related factors in mammalian brains, and may provide the physiological and behavioral basis of PTSD.

(COI: No)

P2-333

Effect of dominant negative thyroid hormone receptor in Purkinje cells to the cerebellar development

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Thyroid hormone (TH) deficiency during the fetal and early postnatal periods results in severe mental and physical retardation, known as cretinism in humans. We have generated a transgenic mouse line expressing a dominant-negative TH receptor (TR) in cerebellar Purkinje cells to study the role of the TH in cerebellar development. A mutant human TRβ1 (G345R) was subcloned into full-length L7/ Pcp-2 gene, which is specifically expressed in Purkinje and retinal rod bipolar cells. We confirmed the transgene localization specifically in Purkinje cells by immunohistochemistry. By Western blot, we detected the expression of the transgene from postnatal day (P)2. It was kept increasing during development. There is no significant retardation in general growth or cerebellar weight in Tg/Tg mice, which is compatible to no difference of plasma free T3 and free T4 levels. However, the motor coordination of Tg/Tg mice was significantly disrupted at P15 and P30 by Rotarod test. To elucidate the reason why cerebellar development was markedly delayed in the Tg/Tg mice, we examined the changes in the expression levels of TH-responsive genes using realtime quantitative RT-PCR. To our surprise, not only Purkinje cell-expressed genes, but also the other cell type-expressed genes were suppressed by mutant TRβ1 in Purkinje cells. These results indicate that TH action through TR in Purkinje cells is also important for development of other subsets of cerebellar cells such as granule cells.

(COI: No)

P2-334

Molecular mechanism of Neuromedin U system in NAFLD/NASH

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Neuromedin U (NmU), which is neuronal peptide isolated from porcine spinal cord, has multiple physiological functions including appetite suppression, stress response and so on. Some groups have reported the relationship between NmU and inflammation, however, its precise function has not been elucidated yet. NmU reacts with NmUR1 and NmUR2, known as specific receptors for NmU, and NmUR1 is mainly expressed in the peripheral tissues and NmUR2 in the central nervous systems. With that in mind, we are attempting to verify the participation of NmU system in obesity related inflammation disease such as nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH). First of all, we have examined NmU and its receptors expression levels in liver tissue of high fat diet (HFD) mouse model. Normally, it is not detected NmU mRNA in liver tissue without any pathological change, however, NmUR1 but not NmU expression level has some tendency to increase at HFD mouse liver. Next, we have examined diet induced NASH model and found that NmU and NmUR1 mRNAs are markedly increased in NASH liver. To assess what kind of cell type is expressed NmU in the liver tissue, we have performed to do immunohistochemistry and observed that NmU was co-expressed with macrophage specific marker, F4/80. More than this, NMU expression in NASH liver is detected not only in the mouse model but also in human NASH patient. These data suggests that NmU/NmUR1 pathway has some roles in NASH pathophysiology, and also NmU has a possibility to be a biomarker of NASH.

(COI: No)

P2-335

Trasncription factor MafA is critical for maintenance of the mature β-cell phenotype

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Pancreatic β-cells are highly differentiated and regulate blood glucose levels by secreting insulin in response to glucose stimulation. Insulin gene transcription factor MafA is expressed in mature β-cells, while MafB, another Maf family transcription factor, is expressed in immature β-cells. Pancreatic islets in MafA knockout (KO) mice were normal at birth, but lost insulin expression in β-cells with increased population of the glucagon-expressing α-cells over time. Analysis of mRNA expression in MafA KO islets at 7 weeks of age showed reduced expression of selective genes important for β-cell function. In parallel, the upregulation of genes that are normally "disallowed" in mature β-cells, such as Mct1, or transcription factors transiently expressed in endocrine progenitors was identified in MafA KO islets as a hallmark of dedifferentiation. By the lineage tracing analyses of β-cells in MafA KO mice using the Cre-LoxP system, the conversion of β-cells to the glucagon-expressing cells with reduced/lost expression of insulin was observed in MafA KO mice. TEM of MafA KO islets demonstrated numerous "empty" vesicles without insulin granules in β-cells. These results suggested that the maturation factor MafA is critical for the homeostasis of mature β-cells and regulates cell plasticity. The loss of MafA in β-cells leads to a deeper loss of cell identity, which is implicated in the pathology of diabetes.

(COI: No)

P2-336

Adrenaline modulates glucagon-like peptide-1 secretion from enteroendocrine L cells

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Adrenaline is known to induce diverse physiological responses in many tissues and cells through adrenergic receptors. Although enteroendocrine L cells secrete glucagon-like peptide-1 (GLP-1) in response to the variety of nutrients, neurotransmitters, and hormones including adrenaline, the precise molecular mechanism by which adrenaline-induced GLP-1 secretion have been poorly understood. To clarify the molecular mechanism of adrenaline-induced GLP-1 secretion, we used enteroendocrine L cell line, GLUTag cells. RT-PCR analysis showed that all types (α_1, α_2, and β) of adrenergic receptors were expressed in the cells. Application of adrenaline to the cells induced a marked increase of the intracellular calcium concentration ($[Ca^{2+}]_i$) and GLP-1 secretion. Inhibition of Gq signaling pathway significantly inhibited the increase of $[Ca^{2+}]_i$ and the adrenaline-induced GLP-1 secretion. Although the intracellular cAMP concentration ($[cAMP]_i$) was little changed by the application of adrenaline, overexpression of α_2 adrenergic receptors induced the decrease of $[cAMP]_i$ by application of adrenaline. These results suggest that expression spectrum of α_1, α_2, and β adrenergic receptors in the cells would modulate the amount of GLP-1 release.

(COI: No)

P2-337

Comprehensive expression pattern analysis of a tumor suppressor gene, *REIC/Dkk3* in the mouse

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In recent tumor suppressor and therapeutic gene research, the reduced expression in immortalized cells (REIC)/Dickkopf (Dkk)3 gene has attracted many researchers. Previous studies demonstrated that the intratumoral introduction of *REIC/Dkk3* gene suppresses tumor growth in mouse models of prostate, breast and testicular cancer and malignant mesothelioma, suggesting that *REIC/Dkk3* is a tumor suppresser gene. However, the functions of *REIC/Dkk3* in vivo are widely unknown. Here, we investigated the comprehensive expression pattern of *REIC/Dkk3* in adult mice by in situ hybridization and immunofluorescence analyses. Firstly we made frozen sections of major mouse organs and performed in situ hybridization. *REIC/Dkk3* mRNA was strongly expressed in many organs, such as the brain, eye, heart, thymus, adrenal gland, testis, ovary and gastrointestinal tracts. In contrast, *REIC/Dkk3* was weakly expressed in the spleen and pancreas. We further performed immunofluorescence analysis for REIC/Dkk3 protein on the tissues in which intense *REIC/Dkk3* mRNA expression was observed. We found that REIC/Dkk3 protein was specifically present in certain cells of these organs. Unexpectedly, REIC/Dkk3 protein was detected intensely in the lumen of digestive organs. In summary, the central nervous system, digestive system and genital organs had strong *REIC/Dkk3* expression. In addition, REIC/Dkk3 protein appeared to be secreted to the lumen of digestive organs.

(COI: No)

P2-338

Investigation of FABP7 expression in mouse Kupffer cells

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Fatty acid binding proteins (FABPs) are intracellular carriers of long chain fatty acids. They have 12 different subtypes with spatial and temporal differences in their tissue expression patterns. We previously reported that FABP7 is involved in cytokine production upon LPS stimulation and phagocytosis against apoptotic cells in liver Kupffer cells (KCs). However, the mechanism by which FABP7 expression is regulated in the liver environment is still unknown. In this study, we first examined the detailed localization of FABP7 by immunohistochemistry in the mouse liver during early postnatal period. Next, in order to see how bone-marrow derived cells acquired FABP7 expression, we examined the occurrence of FABP7+ macrophages in the liver using mouse models treated with clodronate-liposome and of bone marrow transplant. In immunohistochemistry, KCs started to show FABP7 expression from postnatal day 4 (P4), while F4/80+ cells were constantly detected in the liver from P0 to P10. Although FABP7 was not detected in the blood monocytes, it was expressed in the newly derived macrophages in the liver after KC-depletion by clodronate-liposome treatment or in the bone marrow transplant model. The results strongly indicate that the environment provided by a mature liver are required for FABP7 expression in KCs.

(COI: No)

P2-339

The three-dimensional reconstruction of serial sections for an analysis of the microvasculature of the human spleen

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The spleen acts as a blood filter and is responsible for the immune response against blood-borne antigens. Although a number of papers have been published on the splenic microcirculation by light and/or scanning electron microscopy, some controversies still remain on the vascular arrangement, especially in the human spleen. Thus, the aim of the present is to reexamine the microvasculature of the human spleen by using a three-dimensional (3D) reconstruction technique of immunohistochemically stained tissue sections. Human spleens (obtained from patients with gastric cancer) were perfused with warmed physiological saline via the splenic artery, followed by fixation with 4% paraformaldehyde. Serial sections were made from the paraffin or Epon-embedded tissue blocks, immunostained for CD34 (a marker for blood vessel), and observed by light microscopy. The 3D reconstruction was made from the serial section images using a computer program (Avizo, VSG inc., France). Our findings revealed that the splenic follicle is surrounded by anastomosed capillaries, which is elaborately developed in the marginal zone. Most of these capillaries are branches of the penicilar arterioles coming apart from the central artery in the different white pulp system. Further details will be demonstrated especially on the spatial relationship between blood vessels and parenchymal tissues.

(COI: No)

P2-340

Diversity of the peritoneal mesothelial cells: Germinal epithelial cells of the ovary in mice

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Benign lymphangiomas can be easily induced by Freund's incomplete adjuvant (FIA), but the tumors reveal some diversity in their components. Our previous studies demonstrated that a single intraperitoneal injection of FIA into young adult mice induced the phenotypic transformation of peritoneal mesothelial cells to form lymphangiomas. They become tall as early as 5-7 days after the injection and lost their polarity by detaching each other within 4 weeks after injection. The tumor cells increased and uptook a lot of FIA as cytoplasmic vacuoles of various sizes, resulting in typical lymphangiomas with honey-comb like features. As they grew, the positivity of podoplanin (Pdp), one of lymphatic endothelial markers, became much more evident in lymphangioma cells than in the normal mesothelial cells. On the other hand, surface (or germinal) epithelial cells in the ovary did not form any tumor mass, although they become strongly positive for Pdp similarly to other peritoneal mesothelial cells forming lymphangiomas at different sites in the peritoneal cavity. These results suggest that the peritoneal mesothelial cells may have a diverse potentiality for their phenotypic transformation depending on the site of peritoneal cavity.

(COI: No)

P2-341

Transforming growth factor beta 2 from folliculostellate cells induces collagen synthesis in pericytes of rat anterior pituitary gland

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Anterior pituitary gland consists of endocrine cells and non-endocrine cells. These cells are distributed in an appropriate manner within lobules, which are surrounded by extracellular matrix such as collagens. It is considered that the structural properties are important in the maintenance of anterior pituitary functions. Our recent study showed that folliculostellate (FS) cells are required for collagen synthesis of pericytes. However, a factor that induces collagen synthesis in pericytes has not been identified. In the present study, we investigated whether FS cells express a major fibrogenic cytokines, transforming growth factor beta family (TGFb1-3). We performed RT-PCR, in situ hybridization, and immunohistochemistry to detect TGFb and their receptors (TGFBRI-III), and utilized primary cell culture of rat anterior pituitary to examine the effects of TGFb on collagen synthesis. RT-PCR and in situ hybridization revealed that TGFb2 was expressed in the FS cells and the TGFBR-II was expressed in the pericytes. TGFb2 induced Smad2 nuclear translocation in the pericytes and increased collagen synthesis dose dependently. These results suggested that TGFb2 secreted from FS cells affects on pericytes to induce collagen synthesis. Currently, we are investigating the effects of TGFBR inhibitor on collagen synthesis to confirm the action of endogenous TGFb2 actions.

(COI: No)

P2-342

KGF/KGFR control on the epithelial cell proliferation of mouse ear skin

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Keratinocyte growth factor (KGF) is a mesenchymal-cell-derived paracrine growth factor that specifically stimulates epithelial cell growth. In this study, we investigated the effects of over-expressed KGF during epithelial cell proliferation by using a cell labeling system. After anesthetized ICR mice Flag-hKGF cDNA (provided by Dr Jeffrey S. Rubin, the National Cancer Institute/CCR/LCMB) was transfected into ear skin with electroporation. KGFR selective inhibitor (SU5402) was administered in some ears after vector transfection. At 1, 4 and 7 days after transfection, 9 mice at each time-point were sacrificed. For direct assessment of DNA synthesis activity, 5-bromo-2'-deoxyuridine (BrdU) was injected 24 h prior to vector transfection and 5-ethynyl-2'-deoxyuridine (EdU) was injected 2 h prior to each sacrifice. Immunohistochemistry for Flag, KGF, KGFR, BrdU and keratin (K)14 was performed. EdU was detected by manufacturer's protocol. Each plasmid was transfected into the epithelial and subepithelial cells, successfully. After KGF transfection, keratin accumulations were observed at DAY 4. Moreover, increased number of BrdU(-)EdU(+) cells were detected at DAY 1 and BrdU(+)EdU(+) cells were detected in the upper layer of thickened K14 positive epithelium at DAY 4 after KGF transfection. The treatment with SU5402 prevented BrdU(-)EdU(+) or BrdU(+)EdU(+) cell proliferation completely. These findings indicated that KGF may possibly induce a progenitor cell proliferation of epithelium and KGFR inhibitor may be a possible drug for proliferative dermatitis.

(COI: No)

P2-343

Ontogeny of localization of epithelial sodium channel (ENaC) in bullfrog ectoderm

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An ENaC is an ion channel (consisting of three subunits: α-, β-, and γ-ENaC) that is concerned with Na^+ absorption across epithelia. Adult bullfrog skin is an osmoregulatory organ and it absorbs Na^+ ions from its apical to basolateral side via ENaC. Application of amiloride (Am), a specific inhibitor of the α-ENaC subunit, to the adult skin's apical side inhibits such absorption. Amphibia change habitat from aquatic to terrestrial during their developmental stages. The functions of the skin, such as Na^+ absorption, are directly affected by environmental conditions, and the role of the α-ENaC in such functions must change during development. We generated an anti-α-fENaC (anti-α-ENaC against bullfrog) and by immunohistochemistry used it to investigate when α-ENaC develops in bullfrog skin and the related organs. An α-fENaC signal was not detected in stage (St.) 13 embryos, but α-fENaC was expressed in cement glands in Sts. 18, 19, and 21 embryos. The signal was also detected in gills in Sts. 21 and 23-24 embryos. In the skin, α-fENaC was expressed in St. 21 embryos and thereafter (i.e., in larval skin and adult skin), but its activity (Am-blockable response) was not detected in larval skin. The reason for this discrepancy was that α-fENaC was not localized in the apical plasma membrane of such larval skin.

(COI: No)

P2-344

Live-imaging of the basement membranes in mammalian systems

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Basement membranes (BMs) are thin sheet-like extracellular matrix found beneath epithelial cell layers of multicellular organisms. The epithelial cells adhere to and receive signals from BMs through cell surface receptors. Vice versa, the formation of BMs are tightly regulated by neighboring cells. The cell-BM interactions play significant roles in many biological processes including epithelial morphogenesis during embryonic development. Several studies using hydra, worm, and insects demonstrated that the BMs are dynamic structures. However, the dynamics of BMs in mammalian development are largely unknown. Here we developed a novel probe to visualize the BM dynamics in mammalian systems. Nidogen-1, a ubiquitous BM protein was applied to label BMs. Recombinant human Nidogen-1 fused with green fluorescent protein (nid1-EGFP) was added to the culture medium of embryoid bodies (EBs), a 3D model of BM formation, derived from mouse embryonic stem cells. The nid1-EGFP was successfully incorporated into the BMs formed in EBs. Continuous observation revealed that the incorporation of nid1-EGFP was observed not only at the beginning of the EB-differentiation but also in the later stages, suggesting that the BMs in EBs were formed and maintained under continuous turnover. The probe we developed for live-imaging of BMs will provide a powerful tool to study the mechanisms of morphogenesis.

(COI: No)

P2-345

Immunohistochemistry of adult rat dorsal root ganglion neurons

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Adult rodent dorsal root ganglion (DRG) neurons can be divided into three principal subgroups by their soma size and characteristic markers; large neurons (immunoreactive for 200 kDa neurofilaments (NF200), small peptidergic neurons (immunoreactive for calcitonin gene-related peptide (CGRP) and high-affinity nerve growth factor (NGF) receptor trkA), and small non-peptidergic neurons (immunoreactive for glial cell line-derived neurotrophic factor (GDNF) family receptor components (RET, GFR α1) and binding to isolectin B4 (IB4)). By employing double immunofluorescent staining with the markers for the respective subgroups (i.e. anti-NF200 and anti-CGRP antibodies, and Alexa Fluor dye-conjugated IB4), we investigated the precise localization of galectin-1 (GAL-1), galectin-3 (GAL-3), and glucagon-like peptide 1 receptor (GLP-1R) in the sections of 3-month-old Wistar rat DRG. Both GAL-1 and GAL-3 were predominantly localized at IB4-binding small non-peptidergic neurons, whereas GLP-1R was predominantly localized at NF200-immunoreactive large neurons and CGRP-immunoreactive small peptidergic neurons. Consistent with such distribution patterns, GAL-1 and GAL-3 are involved in the neurotrophic activities of GDNF on cultured DRG neurons, and exendin-4, a GLP-1R agonist, restores pyridoxine-induced large fiber neuropathy and diabetes-induced large and small fiber dysfunction. These findings illustrate the correlation of immunohistochemical localization of the individual molecule with its functional roles in the sensory nervous system.

(COI: No)

P2-346

Characterization of the GFAP-like gene which is expressed in the brain of the Japanese newt *Cynops pyrrhogaster*

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In our study we have observed the Japanese newt medulla oblongata by immunohistochemistry with the anti-glial fibrillary acidic protein (GFAP) antibody. GFAP is an intermediate filament specific to astrocytes. Ependymal cells lining the fourth cerebral ventricle showed GFAP immunoreactivity, and their processes which ran through the gray and white matter also showed positive reactivity. The white matter contained a few neuroglial cells and a few positive cells. To clarify the expression of the GFAP gene in the newt's brain, we attempted to isolate the GFAP cDNA clones by RT-PCR method. Total RNA was isolated from brain tissues of the adult *Cynops pyrrhogaster* under anesthesia. The primers were designed based on the known GFAP sequence of vertebrates. We used 5' RACE method to obtain the 5' end of the provided cDNA by RT-PCR. As a result, about a 1kbp partial sequence was obtained. This sequence showed partial similarity with the GFAP of other vertebrates. Because the provided sequence could be read in a continuous amino acid sequence, it has the potential to be in the inside of an ORF. The amino acid had a sequence similar to GFAP, lamin and neurofilament. We made artificial polypeptides from two regions of unique amino acid sequences. Polyclonal antibodies were raised against these polypeptides. Proteins extracted from the newt's brain were separated by SDS-PAGE and analyzed by the immunoblotting procedure. We recognized a single-labeled band of about 60kD. This data suggest that we have cloned the partial cDNA sequence which encodes one of the intermediate filaments.

(COI: No)

P2-347

Localization of ionotropic glutamate receptor mRNAs in the pigeon retina

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Organization of the retina conserves well in the vertebrate. In mammals, main cells in the retina consist of glutamatergic neurons (photoreceptor cell, bipolar cell, ganglion cell) and GABAergic neurons (horizontal cell and amacrine cell). In birds, it is known that bipolar cells and ganglion cells are glutamatergic and amacrine cells are GABAergic. However, cell types receiving glutamate from glutamatergic cells remains unknown well. In the present study, distribution of mRNAs for ionotropic glutamate receptor subunits was examined to detect receptor cells in the pigeon retina by in situ hybridization. Adult pigeons were perfused with 4% paraformaldehyde under anesthesia and frozen sections of eye balls were cut on a cryostat. Several gene probes for in situ hybridization were used: glutamate receptors for AMPA (GluA1, 2, 3, 4), kainate (GluK1, 2, 4) and NMDA (GluN1, N2A) types, vesicular glutamate transporter 2 (vGluT2) and glutamic acid decarboxylase 65 (GAD65). VGluT2 mRNA was expressed in bipolar cells, amacrine cells, and ganglion cells. GluA1, 2, 3, 4 were expressed in bipolar cells, amacrine cells and/or ganglion cells. GluK1, 2, 4 were localized in bipolar cells and ganglion cells. GluN1 and N2A were expressed in an inner part of the inner nuclear layer and ganglion cells. GAD65 was strongly expressed in an inner part of the inner nuclear layer. The present study showed that bipolar cells and ganglion cells are glutamatergic and they express several ionotropic glutamate receptors in the pigeon retina.

(COI: No)

P2-348

Melanin pigmentation and Pacinian corpuscles in human juxta-oral organ

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The juxta-oral organ (JOO), which is first reported in 1885 by the Danish anatomist J. H. Chievitz, exists bilaterally in the bucca of all mammals and some reptiles. It consists of a central epithelial cord, embedded in connective tissue rich in nerve fibers and sensory receptors. In spite of over 100 years of studies, little is known about its precise structure and function, probably because of the difficulty of macroscopical dissection of human JOO. According to Zenker (1982), human JOO is 7-17mm in length and 1-2mm in diameter, and is possible to be dissected under a surgical microscope. However, there have been no reports that Japanese cadaveric JOO was macroscopically dissected, but only two autopsy case reports in which existence of melanin pigmentation and Pacinian corpuscles were pointed out. But their incidences are unknown. In this study, existence and incidences of melanin pigmentation and Pacinian corpuscles were studied by analyzing cadaveric JOOs. Serial sections of eighteen blocks of buccal tissues obtained from fourteen Japanese cadavers were made and HE staining was performed on them. After confirming the location of JOO, existence of melanin pigmentation and Pacinian corpuscles in the organ were observed. Among eighteen specimens, JOO was confirmed in fifteen cases, in which melanin pigmentation was observed in ten cases and Pacinian corpuscles in none. These results suggest that the existence of melanin pigmentation is common while the existence of Pacinian corpuscles is rare case in Japanese JOO.

(COI: No)

P2-349

Age- and sex-dependent changes in prosaposin and its receptors in the lacrimal glands of rats

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Saposin-deficient patients have various ophthalmic disorders indicating a relationship between ocular diseases and prosaposin (PS). Although the lacrimal glands are responsible for maintaining normal ocular health, and dysfunction of these glands causes many ophthalmic disorders, there is a paucity of information regarding PS and the lacrimal glands. In this study, we investigated the changes in PS and its receptors, GPR37 and GPR37L1, in the lacrimal and extraorbital lacrimal glands of rats using immunohistochemistry. We used young (1 month old), growing (2 months old), and adult (18 months old) male and female rats. A histological analysis revealed that PS immunoreactivity in the lacrimal glands decreased with age in male rats, while the opposite result was observed in female rats. On the other hand, a higher level of PS was observed in the extraorbital lacrimal glands of young and adult rats of both sexes. GPR37L1 was more highly expressed than GPR37, and showed decreasing trend with age in both glands of male rats while very low levels were detected in female rats. In fact, the female rats showed a lower level of PS and its receptors than male rats at all ages examined. In conclusion, we found age and sex differences in the levels of PS and its receptors in rat lacrimal gland, suggesting different roles for PS in the maintenance of ocular health in male and female rats at different ages.

(COI: No)

P2-350

Alternative reaction of Harderian gland after lacrimal gland removal in mice

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Harderian gland is an exocrine gland present in most groups of vertebrates. Harderian gland locates within the eye orbit, and secretes lipids (oil fluid) on surface of eyes for protection of eye. We recently established mouse dry eye model by removal the lacrimal glands. The dry-eye mouse caused corneal damage at 1 week after removal operation, but the corneal damage was gradually recovered and looks normal at 8 weeks. We check the tear volume level, but it keeps less than 20% before operation for 8 weeks. Interestingly, weight of the harderian glands significantly increased from 2 weeks after lacrimal gland removal. The number of Ki67, proliferation marker, immunopositive cells in harderian gland significantly increased and peaked at 1 week after lacrimal gland operation. Then we checked the lipid metabolism genes mRNA levels by real-time PCR analysis. In the results, lipoprotein lipase, which relating promotion of the cellular uptake of lipoproteins and free fatty acids, level significantly increased at 3 days. Conversely, monoacylglycerol lipase, relating hydrolysis of monoglycerides to lipoprotein triglycerides, level significantly decreased at 1 and 2 weeks after removal operation. These results suggesting that harderian gland alternatively caused hypertrophy and hyperfunction for protecting the cornea after lacrimal gland removal.

(COI: No)

P2-351

α1-Adrenoceptors relate Ca^{2+} modulation and mucin secretion in rat lacrimal grand

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The lacrimal gland is responsible for secretion of electrolytes, water and proteins, collectively known as lacrimal gland fluid, into the tear film. Cellular secretory activities are enhanced by noradrenaline (NA) as well as by cholinergic stimuli. Here, lacrimal gland acinar cells response to adrenoceptors activation were examined, with special reference to intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) dynamics. Detection of mRNA of acinar cells specific to adrenoceptor subtypes was determined by RT-PCR. All kinds of adrenoceptors were detected except α2c and β1 in lacrimal glands. NA induced an increase in $[Ca^{2+}]_i$ in acinar cells, and these $[Ca^{2+}]_i$ changes showed a biphasic behavior. The removal of extracellular Ca^{2+} and the use of Ca^{2+} channel blockers did not inhibit the NA-induced $[Ca^{2+}]_i$ increases. In addition U73122 did not completely block the NA-induced $[Ca^{2+}]_i$ increase. Phenylephrine induced a atrong increase in $[Ca^{2+}]_i$. However, clonidine and isoproterenol failed to induce a [Ca2+]i increase. Mucin secretion was quantified by the peroxidase activity in rat lacrimal glands. Ca^{2+}-dependent exocytotic secretion of peroxidase was detected in rat lacrimal glands. RT-PCR results showed that Muc1, Muc5AC, Muc5B, and Muc16 mRNA were expressed in acinar cells. These findings indicated that NA activates α1 adrenoceptors which cause an increase in $[Ca^{2+}]_i$ by production of IP_3 and these receptors were main receptors in calcium-related cell homeostasis and protein (mucin) secretions in lacrimal glands.

(COI: No)

P2-352

The distribution of PACAP receptor on the sweat glands

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Pituitary adenylate-cyclase activating polypeptide (PACAP) has pleiotropic functions that contribute to neurotransmission, neuroprotection and vasodilatation. In addition, PACAP has been shown to influence the activity of several exocrine glands. Recently, we reported that PACAP and its receptor, PAC1R are localized to the parasympathetic nerve cells in the mouse lacrimal gland. Furthermore, PACAP instillation induced tear secretion. As structure of sweat glands and lacrimal glands structure are similar, we assumed that PACAP has a capacity to induce sweating in the sweat glands. However, its effects on the composition of the sweat glands are not known yet. To investigate the localization of PACAP and PAC1R in mouse sweat glands, we performed immunohistochemistry and RT-PCR. In this study, we examined PACAP, PAC1R, VPAC1, VPAC2 and VIP expression in mouse footpad by RT-PCR. The expressions of all these genes were detected in mouse footpad. In addition, we demonstrated that Cytokeratin (CK)19 was expressed in both ductal and secretory cells of sweat glands in mouse foot pad. By immunohistochemical staining, we found that PAC1R was expressed in the mice footpad consistent with the position of the sweat glands which were identified by CK19. Abundant PAC1R immunoreactivity was observed in secretory cells and in the excretory duct of sweat glands. The present results suggest that PACAP has an important role in secreting sweat in the sweat glands. For farther study, we are now examining the sweat secretion after PACAP administration in vivo.

(COI: No)

P2-353

Possible participation of synovial lining cells in vascularization in the rat temporomandibular joint

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The lining layer of the synovial membrane in the temporomandibular joint (TMJ) contains two types of synovial lining cells (SLC): a macrophage-like type A cell and fibroblast-like type B cell. We previously demonstrated that the desmin-positive B cell resembles an activated pericyte during angiogenesis in both immunoreactivities and ultrastructure in rat TMJ. The present study investigated the immunocytochemical characteristics of the SLC, focusing on their possible participation in the synovial vasculature. Adult male Wistar rats were used. Some animals were intravenously injected with FITC-labeled tomato lectin before fixation. Decalcified cryostat sections were processed for immunohistochemistry using antibodies to desmin, RECA-1 (endothelial marker), and ninein (sprouting tip cell marker). Ultrastructurally, RECA-1-positive SLC--not luminal in shape--were type A cells. Lectin-perfusion enabled the representation of functional vessels in vivo in the TMJ. Arterioles and venules in the synovial folds with RECA-1-positive endothelial cells and desmin-positive pericytes gave rise to numerous capillaries which were distributed densely in the synovial lining layer. Some capillaries with RECA-1-reaction lacked lectin-staining in the distal portion, indicating a loss of blood-circulation due to vessel sprouting or obliteration. RECA-1 and desmin-positive SLC often attached to this portion. A few capillaries also expressed ninein. These findings suggest that SLC might contribute to angiogenesis in the synovial membrane.

(COI: No)

P2-354

Lymphangiogenesis and NOS localization in the healing process after tooth extraction in a mouse diabetes model

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Objective: Type I diabetes causes the dysfunction of vascular endothelial cells comprising blood and lymph vessels, and arteriosclerosis progresses due to reduced nitric oxide (NO) production, showing a close association between NO and diabetes. In this study, we immunohistochemically investigated lymphangiogenesis and NOS expression in the healing process after tooth extraction using the Akita mouse diabetes model.
Materials and Methods: The lower first molar was extracted in C57L/6J and Akita mice at 12 weeks after birth. The mandible was excised and decalcified with 10% EDTA. After embedding in paraffin, immunohistochemical staining with antibodies against nNOS, iNOS, eNOS, VEGF-C, VEGFR-3、and vWF was performed employing the standard procedure, and the preparations were observed under a light microscope.
Results: Among the time-points: 1, 4, and 10 days after tooth extraction, the strongest reactions of NOS, VEGF-C and R-3, and vWF were noted at 4 days. At 10 days, osteoblasts in formed bone were positive.
Discussion: It was suggested that the host defense mechanism and vasodilatory action became active 4 days after tooth extraction, leading to lymphangiogenesis. Osteoblasts were activated at 10 days, suggesting active osteogenesis in Akita mice.

(COI: No)

P2-355

A histopathological study of possible visceral mycosis in Medaka(*Oryzias latipes*)

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Background: Medaka (*Oryzias latipes*) is widely used as a vertebrate model animal in various research fields. We have used medaka populations as an analogy of human populations, in order to understand the relationship between genetic polymorphisms and phenotypes, including genetic and or inflectional disease. The problem is however, there are little pathological data. To provide substantial information on histological and pathological aspects of medaka, we have prepared the whole-body serial tissue sections of the medaka with aberrant formation, and performed histopathological observations.
Materials and Methods: An individual medaka in a laboratory closed colony derived from a wild population caught in Kunming, China, showed serious abdominal swelling and examined. It was fixed for 10 days in Davidson solution, and then the conventional method was used for preparing the whole-body serial tissue paraffin sections at intervals of 5 μm. After the deparaffinization, the sections stained by HE and PAS and Grocott were investigated under an optical microscope.
Results and Discussion: Through three types of staining, we observed visceral mycosis like- diagnoses were widely observed in the organs including kidney and ovary. Since such an infection of fungus in organs in medaka has not been reported so far, it must be valuable finding if the diagnoses are truly visceral mycosis.

(COI: No)

P2-356

Effect of quadriceps femoris muscle contraction by electrical stimulation before bicycle ergometer exercise on physical energy metabolism

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This study was performed to clarify the effect of quadriceps femoris muscle contraction (MC) by low-intensity transcutaneous electrical nerve stimulation (TENS) before bicycle ergometer exercise on physical energy metabolism. All healthy male volunteers (n = 6) were participated two experiment groups. In MC group, quadriceps femoris muscles of subject were passively contracted by low-intensity TENS for 30 min before bicycle ergometer exercise. In rest group, subject was rested on the bed for 30 min before the bicycle ergometer exercise. In both groups of MC and rest, the subject performed ramp incremental exercise to exhaustion on bicycle ergometer. Plasma free fatty acid (FFA) concentration before bicycle ergometer exercise in MC group has tended to be higher than that in rest group, but there was no significant difference between the two groups. The ventilation threshold (VT) shown by the exercise load during bicycle ergometer exercise in MC group was significantly higher than that in rest group ($p < 0.05$). There was no significant difference of blood lactate concentrations after bicycle ergometer exercise between the two groups. These results give a hypothesis that the localized muscle contraction by TENS before bicycle ergometer exercise might increase recruitment of FFA from adipose tissue, and might change the proportion of the substrate of ATP synthesis during bicycle ergometer exercise.

(COI: No)

P2-357

A possibility to promote the recovery from fatigue after submaximal pedaling exercise by bathing with high concentration CO_2-water in healthy subjecs

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We investigated an influence of immersion into bathtub water containing comparable amount of CO_2 to CO_2-hot spring to recovery from fatigue caused by submaximal exercise. Six male subjects (Age; 21-22 yrs) performed 10 min pedaling exercise at 60% HRmax were given one of the following 3 treatments after the exercise in a different day; immersion into tap-water (CO_2p<20 ppm) or artificial CO_2-water (CO_2>1000 ppm) (30℃, 10 min), or dry bathtub sitting rest (Air). Blood flow in the immersed skin (BF) and ECG were recorded continuously throughout the experiment. Cardiac autonomic nerve activity was evaluated by R-R interval fluctuation power spectrum analysis (PSA). Muscles stiffness (MS), salivary cortisol (SCo), VAS were evaluated at pre-exercise, immediately after exercise, during immersion and at 10 min after the end of immersion. At 10 min after immersion, MS in CO_2-water treatment was significantly small (22.2± 1.2 tone, $p<0.01$) compared with air (28.0± 2.0 tone) and tap-water treatment (31.8± 2.2tone). A LF/HF ratio in PSA was smaller in CO_2-water treatment than in tap-water treatment. Compared with the air, SCo was significantly decreased in tap (24%) and CO_2-water immersion (48%). Results suggested that the recovery from enhanced sympathetic nerve activity and MS caused by submaximal exercise was facilitated by following CO_2-rich water immersion.

(COI: No)

P2-358

Inhibition of fibrinolytic activity in overweight young men after acute strenuous exercise

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Introduction: Some studies have reported an increase in fibrinolytic activity after acute strenuous exercise. Conversely, fibrinolytic activity is inhibited in overweight person. In this study, our aim was to evaluate whether being overweight affects fibrinolytic activity after acute strenuous exercise. Being overweight was defined using body mass index (BMI) as a measure of the degree of obesity.
Subjects and Methods: Twelve healthy young men aged 19 to 23 years old who engaged in daily exercise participated in this study. Seven of these men were categorized in the BMI < 25 group, and five were in the BMI > 25 group. Venous blood samples were collected from the subjects pre- and post-performance of the Cooper test. This test involved running as far as possible within a 12-minute period. α 2-plasmin inhibitor / plasmin complex (PIC, as a marker of fibrinolytic activity) levels were measured using the collected blood samples.
Results: The PIC levels increased significantly in the BMI < 25 group (pre: 0.5 ± 0.02 μg/mL, post: 1.9 ± 0.3 μg/mL, $p < 0.05$), but these were not significantly increased in the BMI > 25 group (pre: 0.5 ± 0.08 μg/mL, post: 1.0 ± 0.1 μg/mL, $p > 0.05$).
Conclusions: Using BMI as an index for evaluation, this study showed that fibrinolytic activity is inhibited in overweight young men after acute strenuous exercise.

(COI: No)

P2-359

An influence of local bathing on recovery of performance after muscle fatigue: a pilot study comparing the effect of CO_2 enriched- and normal-tap water

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Soaking the body into CO_2 hot spring evokes prominent vascular dilation in skin and skeletal muscle of underwater portion of the body, and the skin reddens within several minutes, even in relatively low water temperature. Hence, we hypothesized that muscular fatigue may recover effectively due to facilitation of the local blood flow. To investigate the hypothesis, 9 healthy subjects (5 male, 4 female) immersed forearms into water (35 ℃) for 5min resting between sets. A set consists of a series of continual 30-times grip measurements for producing muscle fatigue. The grip measurements were carried out in both hands simultaneously. Forearms treatments during resting between sets were as follows: 1) without immersion, 2) immersion of one side into CO_2-water (CO_2>1000ppm) and another side into general tap water. Because sexual difference in muscle fatigue was not observed so far, results were analyzed without distinguishing the sex. The grip decreased by the 30th measurement by approximately 40% in each set, but a significant difference caused by treatment of the forearm during resting between sets was not observed, changes in muscle blood flow as well. The first grip of each set was significantly decreased as a set advances. Though a significant difference between forearm treatments with two kinds water was not detected under the present experimental conditions, recovery of muscle performance from fatigue was significantly promoted by water immersion after the exercise.

(COI: No)

P2-360

Eccentric muscle contraction stimulates mTOR signaling in human skeletal muscle

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The mammalian target of rapamycin (mTOR) signaling pathway has a key role in stimulating muscle protein synthesis and muscle hypertrophy. An acute bout of resistance exercise is well known to increase mTOR signaling in human skeletal muscle. However, whether concentric or eccentric muscle contraction stimulates mTOR signaling in human skeletal muscle remains unclear. The purpose of the present study was to investigate the effect of concentric and eccentric muscle contraction on mTOR signaling in human skeletal muscle. Sixteen young males performed 10 unilateral isokinetic concentric or eccentric knee extensions (90 deg/s) × 4 sets. Muscle biopsies (~15 mg) were obtained from the vastus lateralis 40 min before and 1 h after the resistance exercise and analyzed using the immunoblotting. The average peak torque for each of the four sets of 10 maximal eccentric contractions was significantly higher than that in concentric contractions. The blood lactate concentration immediately after eccentric contractions was significantly lower than that in concentric contractions. Eccentric muscle contractions significantly increased mTOR(Ser2448), S6K1 (Thr421/424), S6(Ser235/236), eIF4E(Ser209), p38(Thr180/Tyr182) and ERK1/2(Thr202/Tyr204) phosphorylation compared with those of concentric contractions. These results suggest that eccentric muscle contraction increases mTOR signaling in human skeletal muscle, which may be leading to muscle hypertrophy.

(COI: No)

P2-361

The forced respiration during the deeper upright water immersion causes the greater inspiratory muscle fatigue in healthy young men

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The purpose of this study was to evaluate the influence of the inspiratory load breathing (ILB) with various depths of water immersion on inspiratory muscle fatigue.
Methods: Eight healthy men (21.3 ± 0.5(SD) years) participated in three trials in random order, i.e., the subjects performed ILB during upright water immersion up to the umbilicus, 4th-rib, or clavicles. Maximum inspiratory pressure (PImax) was assessed in a sitting position with water immersion up to umbilical level before (baseline, BL) and immediately after the ILB. The 15-min ILB was performed with a respiratory frequency of 15 breaths/min and with a load of 30% PImax at BL. The percent changes in PImax following the ILB (δ %PImax) were calculated.
Results: The PImaxs were significantly decreased following the ILB in all trials ($p<0.05$). The δ %PImax was significantly greater in the clavicle-trial than those in the other trials (umbilicus, -7.0 ± 1.5(SD) %; 4th-rib, -6.7 ± 4.8%; clavicle, -20.1 ± 4.1%; $p<0.05$).
Conclusion: Our results suggested that the forced respiration during the upright water immersion up to the clavicular level resulted in the greater inspiratory muscle fatigue than those in the shallower levels, probably due to the increased demand on the intense inspiratory muscle strength to expand the chest against the greater hydrostatic pressure.

(COI: No)

P2-362

The effect of postural change on the distribution of cerebral blood flow during passive heating in elderly

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PORPOSE: The distribution of blood flow to cerebral tissues may be limited by heat stress and further by postural change from supine to upright. The purpose of this study was to assess the responses in the internal carotid artery (ICA), external carotid artery (ECA) and vertebral artery (VA) to postural changes during normothermia (NT) and mild-hyperthermia (HT).
METHODS: Ten elderly healthy men (72 ± 2.1 yrs, mean ± SD) underwent measurement of blood flow in the common carotid artery (CCA), ICA, ECA and VA by ultrasonography in a sitting (SIT) and supine (SUP) during NT and HT. Subjects were passively heated by lower legs immersion in 42 ℃ water, and esophageal temperature (Tes) increased from NT (36.4 ± 0.2 ℃, mean ± SE) to HT (37.4 ± 0.2 ℃). Tes, and skin temperature (Tsk) were measured continuously.
RESULT: Blood flow in ECA was significantly increased during both HT-SIT and HT-SUP compare with NT-SIT by 68 ± 35 and 104 ± 54%, respectively ($P < 0.05$), however there were no effects of postural change and HT on blood flow in CCA, ICA and VA.
CONCLUSION: These data indicated that intracranial blood flow is well maintained during mild-hyperthermia regardless of postural changes.

(COI: No)

P2-363

Experimental validation of teleological hypothesis of cardiolocomotor coupling: effects on gas exchange and muscle deoxygenation during treadmill walking

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The aim of this study was to determine whether cardiolocomotor synchronization (CLS) affects gas exchange efficiency and muscle O_2 utilization during exercise. A healthy elderly subject repeated graded treadmill walking test at constant speed for 20 min. The initial speed and grade were set such that the heart rate intersected the cadence rate. After 3 min warm up the grade was increased 0.5% every 1 min. Minute ventilation (V_E)and O_2 uptake were measured and the ventilatory equivalent for O_2 (V_E/Vo_2) was determined as a measure of gas exchange efficiency. Change in deoxyhemoglobin (Δ[HHb]) at the soleus muscle was sampled by NIRS. CLS was defined as being present when the phase difference between the onset of heartbeat and cadence was fixed over 20 s with the SDs of phase differences being below 0.1. The changes in gas exchange parameters and muscle deoxygenation indices during CLS were evaluated as the differences between the observed and predicted values which were obtained by fitting least squares regression to the desynchronized data. Decrease in V_E/Vo_2 and relative increase in Δ[HHb] were observed during CLS. The reduced V_E/Vo_2 could be accounted for by the reduction of V_E and slight increase in Vo_2. We assume that CLS might decrease the accumulation of ischemic metabolites that are produced during muscle contraction by impeding intramuscular arteries, which would, in turn, act to reduce ventilation. The observation of a relative increase in Δ[HHb] during CLS suggests the increased muscle microvascular O_2 extraction.

(COI: No)

P2-364

Site-specific differential effects of nutrition and exercise on rat musculoskeletal system

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Purpose: Both nutrition and exercise are necessary to maintain musculoskeletal system. This probably is a condition imposed by evolutional necessity for the survival of animals. With this in mind, we studied site-specific effects of nutrition and exercise on the musculoskeletal systems of rats.
Methods: F344 female rats (6 weeks old) were divided into three groups of control (n=10), exercise with dietary restriction (n=6), and exercise without dietary restriction (n=7). Rats of the exercise groups voluntarily ran on a rotary wheel ergometer with a load of 30% of body mass for 8 weeks. The control and exercise without dietary restriction groups were allowed to take diet freely during the experiment period. The exercise with dietary restriction group was allowed to take diet of an amount similar to that of the control group.
Result: Dietary restriction suppressed exercise-induced down-regulation of myostatin with a corresponding increase in muscle mass in plantaris muscle, but not in soleus muscle. Exercise selectively increased the bone volume and mineral density of trabecula in metaphysis. This increase was suppressed by the dietary restriction.
Conclusion: Dietary restriction suppressed exercise-induced growth of musculoskeletal system differentially in a site specific manner. Exercise effects on musculoskeletal system of static function seemed to be more resistant against dietary restriction.

(COI: No)

P2-365

Contribution of EP2 receptor to generation of delayed onset muscle soreness

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We previously demonstrated that nerve growth factor (NGF) and glial cell line-derived neurotrophic factor (GDNF) were up-regulated in exercised muscle through activation of B2 bradykinin receptor and up-regulation of cyclooxygenase (COX)-2, respectively, and they sensitized nociceptors resulting in mechanical hyperalgesia (delayed-onset muscle soreness, DOMS). However, the receptor subtypes that bind prostaglandins produced by up-regulated COX-2, has not been identified. Here we examined which prostaglandin receptor subtype was involved in generation of DOMS.
Wild type (WT), EP2 deficient (EP2-/-) and IP deficient (IP-/-) mice were subjected to lengthening contraction (LC). Another group of mice received injection of an EP2 agonist ONO-AE1-259-01 to the lateral gastrocnemius (LGC) muscle. Before and after these treatments the mechanical withdrawal threshold of LGC was evaluated. The change in expression of NGF, GDNF and COX-2 mRNA in the muscle was examined using real-time RT-PCR.
LC induced mechanical hyperalgesia 6-24 h after LC in WT and IP-/- mice, but not in EP2-/- mice. Upregulation of NGF, GDNF and COX-2 mRNA was observed 3 h after LC in WT mice but not in EP2-/- mice. Injecting ONO-AE1-259-01 to the muscle increased expression of COX-2 mRNA.
These results suggest that EP2 contributes to generation of DOMS and EP2 also induces the up-regulation of COX-2 expression.

(COI: No)

P2-366

Regulatory role of VMH-specific Ad4BP neurons in glucose metabolism

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The ventromedial hypothalamus (VMH) plays a crucial role in the regulation of whole body energy homeostasis. Adrenal 4 binding protein (Ad4BP) expressing neurons are limited to the VMH in the hypothalamus. To explore the role of VMH specific Ad4BP neurons on glucose metabolism, we are using Designer Receptors Exclusively Activated by Designer Drugs (DREADD) technology. DREADD is a pharmacogenetic method to activate or inhibit specific neurons in the brain. Upon expression of the stimulatory DREADD 'hM3Dq' or inhibitory DREADD 'hM4Di', a small drug like molecule CNO (clozapine-N-oxide) is used to activate or inhibit neuronal activity, respectively. We implanted VMH targeting bilateral steel cannulas in Ad4BP-cre recombinase transgenic mice and infected double floxed DREADD AAV vectors through it. Brain sections showed expression of DREADD-mCherry only in the VMH. Activation or inhibition of Ad4BP neurons using DREADD system was verified by electrophysiology. We found that Ad4BP-hM3Dq infected mice showed improved glucose clearance during glucose tolerance test (GTT). It also showed increased insulin sensitivity in insulin tolerance test (ITT). In contrast, Ad4BP-hM4Di infected mice had impaired glucose tolerance and decreased insulin sensitivity. Overall, selective activation of VMH Ad4BP neurons improves whole body glucose homeostasis by regulating glucose turnover and increasing insulin sensitivity. Thus, our results elucidate the role of VMH Ad4BP neurons and its importance in regulating whole body glucose metabolism.

(COI: No)

P2-367

Change of sudomotor function evaluated by quantitative direct and axon reflex test with age and sex

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The aim of this study was to quantitatively investigate peripheral sudomotor function through axon reflex-mediated (AXR) and directly activated (DIR) sweating in healthy male (n = 46) and female subjects (n = 48) in the 30-70s groups and to evaluate the correlation between age and sweating function. Quantitative sudomotor axon reflex testing (QSART) with iontophoresis (2 mA for 5 min) and 10% acetylcholine (ACh) were performed to determine AXR and DIR sweating. All experiments were conducted in a thermoneutral condition (temperature, 24.0+/-0.5C; relative humidity, 40+/-3%). The onset time of AXR (r2=0.567) was positively correlated with advancing age, whereas sweat rates of AXR (1) (with iontophoresis) and AXR (2) (without iontophoresis) (r2=0.571, r2=0.486, respectively), DIR (r2=0.594), activated sweat gland density (r2=0.496) and sweat gland output (r2=0.551) were negatively correlated in the two genders with advancing age. Differences between males and females were observed in all age groups. These observations suggest that an attenuation of sudomotor function occurs with ageing due to neurodegeneration resulting from nerve fiber demyelination. Variation in sweating between sexes exists in all age groups

(COI: No)

P2-368

Oligonol supplementation affects sudomotor activity and serum orexin level after heat load in humans

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Oligonol is a low-molecular form of polyphenol, which possesses anti-oxidant and anti-inflammatory activities, and is a potential candidate for an anti-pyretics. This study investigated the effects of Oligonol supplementation on sudomotor activity and serum orexin level, which is related with thermoregulation and energy metabolism, after heat load in 19 healthy male volunteers. We conducted placebo-controlled cross-over trials. Participants took a daily dose of Oligonol 200 mg or placebo for one week. After a 2 week washout period, the subjects were switched to the other study arm. Half-body immersion into hot water (42+/-0.5 C for 30 min), as a heat load, was performed in an automated climate chamber after each supplementation. Tympanic temperature was measured and sudomotor activity was tested in four areas of skin. Serum concentration of orexin (hypocretin) was analyzed immediately after the immersion. Oligonol intake attenuated the increases in tympanic temperature and serum orexn level after heat load, compared with placebo. In addition, Oligonol contributed to the reduction in sweat rate and volume, activated sweat gland density and sweat gland output, but onset time of sweating was the opposite. This study demonstrates that Oligonol supplementation for one week may help human avoid excessive water loss and elevation of body temperature under the heat load.

(COI: No)

P2-369

Required ATP/NAD concentration for the constant ATP production by glycolysis

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Required ATP/NAD concentration for the constant ATP production by glycolysisManami Fuchigami, Akira AmanoDepartment of Life Science, Ritsumeikan UniversityConstruction of a mathematical model for cardiac energy production system could help in understanding the cardiac energy production and consumption balance. In this study, we focused on investigating how the glycolysis system works under energy deficiency conditions such as low ATP or high NAD concentrations. We used the glycolysis model proposed by Lambeth et al., which analyzed steady states in two conditions: normal glucose condition (glucose mode) and the condition without glucose (glycogen mode). Result showed that the ATP and NAD concentrations act as ATP production rate controlling factors to glycolysis. The Glycogen mode is superior to the glucose mode. When the ATP concentration is high, the PFK reaction is rate-limiting step. As a result, FBP production comes to stop and glycolysis system doesn't work. In the same way, when the ATP concentration is low, the PK reaction is rate-limited step. To keep high ATP production rate, ATP concentration must be high. To keep high ATP production rate under low glucose or glycogen concentration, a high ATP and NAD concentrations were necessary. When the oxygen level decreases, LDH is extremely important to keep glycolysis activities.

(COI: No)

P2-370

The effect of difference in cooling regions between two exercises on rectal temperature and endurance exercise capacity in the hot environment

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The purpose of this study was to investigate whether difference in cooling region between two exercises affect core temperature and endurance exercise capacity in the hot environment.
Eight adult men volunteered for this study. As a preliminary test, participants were measured in maximal oxygen uptake (VO_2max) by an incremental ramp test with bicycle ergometer. Initially, participants performed a bicycle exercise for 20 min at 60% VO_2max (EX1) and then rested for 15 min on the bicycle ergometer (REST). Subsequently, they performed an incremental ramp test to exhaustion (EX2). They participated in three experiments having three different REST conditions which were non-cooling (CONT), cooling of quadriceps region (QUAD) and cooling of neck region (NECK). Each experiment was separated by at least seven days. All experiments were conducted in hot environment (room temperature: 28.8 ± 0.6 deg C, relative humidity: 60.7 ± 2.0%).
As a result, rectal temperature was no significant difference between those conditions. Subjective parameter by Borg scale was no significant difference between those conditions. Although local cooling did not affect rectal temperature and subjective parameters, exercise time at EX2 in NECK was significantly longer than that in CONT ($p<0.05$). VO_2peak at EX2 in NECK was significantly higher than that in CONT ($p<0.01$). These results suggested that cooling of neck region between two exercise might increase exercise time and VO_2 peak.

(COI: No)

P2-371

Adipose tissue malfunction in prenatally arsenic-exposed mice

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The adverse effects of contaminated arsenic in drinking water have become a concern in developing countries. A possible association between developmental exposure to arsenic and the higher incidence of diabetes has been reported in several epidemiological studies. In our previous study, we have already clarified that the administration of sodium arsenite (85 ppm) in drinking water to pregnant mice from gestational day 8 to 18 leads to impaired energy metabolism in male offspring at 60 weeks of age, such as hyperglycemia, glucose intolerance and insulin insensitivity. In this study, we aimed to test whether impaired energy metabolism in prenatally arsenic-exposed mice is mediated by the malfunction of adipose tissue which contributes to energy metabolism. Firstly, we examined the weight and histology of perirenal fat pad and found an increase of weight with adipocyte hypertrophy in prenatally arsenic-exposed males. According to the increase of fat pad, plasma leptin level was significantly increased. Secondly, we analyzed several gene expressions related to energy metabolism and insulin sensitivity in the fat pad by real-time PCR. Among genes we analyzed, the expression of TNF-α, a cytokine linked to insulin resistance, was significantly up-regulated, indicating the possibility that higher expression of TNF-α impairs insulin action in prenatally arsenic-exposed male. These results suggest that the adverse effect by developmental exposure of arsenic could be mediated by the malfunction of adipose tissue.

(COI: No)

P2-372

Effect of cellular retinol-binding protein II on the enzymatic activity of lecithin:retinol acyltransferase

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Vitamin A is a fat-soluble vitamin needed for many physiological activities. Retinyl esters (REs), animal-derived forms of vitamin A, are hydrolyzed to retinol before entering the absorptive epithelial cells. The retinol thus produced binds to cellular retinol-binding protein (CRBP) II in absorptive epithelial cells, forming a retinol-CRBP II complex. This complex is esterified to REs by lecithin:retinol acyltransferase (LRAT). We asked whether CRBP II affected the amount of REs produced by LRAT. We quantified REs in HEK293T cells overexpressing CRBP II and/or LRAT. Overexpression of LRAT in HEK293T cells led to the accumulation of a large amount of REs in the cells within ten min of addition of retinol to the medium. CRBP II overexpression enhanced the accumulation of REs in LRAT-transfected HEK293T cells.

(COI: No)

P2-373

High-fat diet feeding impaired platelet-derived growth factor receptor alpha (PDGFRα)-mediated up-regulation induced by fasting in NG2-positive oligodendrocyte precursor cells of the hypothalamus in mice

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NG2 cells are thought to differentiate into oligodendrocytes which have ability to maintain myelin structure. Therefore, they are known as oligodendrocyte precursor cells. However, their functions are no only limited as oligodendrocytes: it is suggested that they develop into neurons. It was also discovered that NG2 cells express PDGFR α for their survival. In the present study, we examined whether high-fat diet induced PDGFR α expression in the male mice. The hypothalamus including the ventromedial hypothalamus, the arcuate nucleus, the dorsomedial hypothalamus, and the lateral hypothalamus was dissected as a block. RNA and proteins were extracted. In immunohistochemical study, mice were perfused with 4% paraformaldehyde. Firstly, we confirmed NG2 cells also expressed PDGFR α. Microglia (CD11b, Iba 1), astrocyte (GFAP), and oligodendrocyte (NS, O4) did not expressed PDGFR α. This indicated that PDGFR α-positive cells were NG2 cells and therefore oligodendrocyte precursor cells. Fasting for overnight in the chow diet (control diet) group significantly increased PDGFR α mRNA and its protein. High-fat diet for 10 days also increased PDGFR α mRNA and its protein. However, we found that high-fat diet for 5 weeks did not affect the expression of PDGFR α and β proteins. We suggest from the present study that a high-fat induces inflammation which resulted in dysregulation of PDGFR signals.

(COI: No)

P2-374

Effects of green tea beverage on the spontaneous locomotor activity and intakes of food and water in juvenile mice loaded with isolation stress

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Juvenile stage is susceptible to influences from physical and/or mental stress significantly, irrespective of human and animals. In this study, therefore, we examined whether administered green tea beverage influences the spontaneous locomotor activity of juvenile mice loaded with isolation as mental stress or not. The findings were compared with those obtained from mice bred in a group. Five weeks old-mice were divided into two groups; singly breeding group (three week-isolation stress group; S) and group breeding group (no-isolation stress group). Both group mice were further divided into two groups, respectively; freely water-intake group (W) and freely intake of marketing green tea beverage group (T). Spontaneous locomotor activity of mice was measured as wheel running, fasting blood glucose, oxidative stress, and antioxidant capacity also were measured. Three weeks isolation stress significantly decreased the increasing rate of body weight in W+S group, compared with W group, but not in T and T+S groups. Intake volumes of water and food as well as body weight were decreased with isolation stress. Spontaneous locomotor activity in W+S was decreased, suggesting that isolation influences them. However, no change occurred in the activities in T+S, strongly suggesting that green tea improves lowered locomotion due to the isolation stress, although green tea used in this study significantly increased serum oxidative stress.

(COI: No)

P2-375

The role of TRP channel related to thermal effect during carbon dioxide-rich water immersion

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Carbon dioxide-rich water (CO_2) bathing have been used for balneotherapy of patients with hypertension or peripheral occlusive arterial disease. We have previously reported that CO_2 bathing exerts more thermal effects than fresh water (FR) bathing. Current concepts on modified skin thermoreceptor activities by CO_2 has revealed that the mechanism of lowered a neutral sensation by 2° during CO_2 bathing. Transient receptor potential vanilloid 1 (TRPV1), a member of non-selective cationic channel family expressed in the human sensory neuron, and it has been reported that activation of TRPV1 induces an influx of cations (i.e, Na^+, Ca^{2+}) which are activated by heat above 43° and various chemical agonists such as capsaicin or H^+. Our previous study observed that thermal sensation caused by capsaicin application to the skin is increased by immersion in CO_2 at 33°, suggesting that percutaneously absorbed CO_2 activated TPRV1. However, the mechanism of CO_2 action on TRPV1 has only been partially clarified in humans. The present study examined the role of TRPV1 related to an increase in thermal sensation when immersed into CO_2 of FR at 33° after capsaicin application to the skin. Furthermore, we suggested changes in thermal sensation of immersion into theCO_2 when Capsazepin application to the skin blocked the activity of TRPV1 channels.

(COI: No)

P2-376

Association between energy consumption and behavioral thermoregulation assessed by selection of alternative floor temperatures using mice

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We developed a new experimental apparatus to assess behavioral thermoregulation, involving the selection of two different floor plate temperatures by mice. We used eight ovariectomized adult mice. The experimental box was with two lined hollow stainless floor plates (pl. 1 and 2) of 10 x 20 x 1 cm. The plates were connected to water baths (temperatures, Tpl-1 and -2). The ambient temperature (Ta) was 25℃. We set Tpl-1 and -2 to 30 and 35℃, respectively. We placed a mouse with an implanted temperature telemeter (Tc) in the box and alternated between Tpl-1 and -2 every 5 min and recorded Tc, Ta, and the time staying on the plates once a min for 60 min. We measured oxygen consumption (Vo2) by sampling air in the box. After the measurement on the first day, we fasted the mice and performed measurement once a day on the 2nd and 3rd days. Although the mice stayed on the plate at 35℃ for 58.3 ± 2.3% (mean° SE) of the 60-min experimental duration on the 1st day, the duration increased to 69.0 ± 3.3% on the 3rd day. Tc decreased significantly ($p<0.05$) after fasting. Although Vo2 on the 1st day was 0.7 ± 0.1 L/g (body weight)/min, Vo2 on the 3rd day decreased to 0.6 ± 0.1 L/g/min. At Ta of 25℃, mice stayed on the warmer plates accompanied with decreases of Tc and Vo2, which suggest that the fasted mice decreased energy consumption by activating behavioral thermoregulation. We also performed the same measurements for Ta at 15 and 35℃ (the details were omitted).

(COI: No)

P2-377

β-adrenergic stimulation induces nuclear accumulation of phospho-nuclear kappa B in brown adipocytes of rodents

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Brown adipose tissue (BAT) generates heat in response to sympathetic activation, and is involved in body temperature regulation and body weight control. Previous studies showed that BAT of mice activated by cold exposure or menthol stimulus accumulated phospho-nuclear factor kappa B (pNFκB) in their nucleus. The purpose of this study is to clarify the mechanism of this response. pNFκB was detected by immunohistochemistry (IHC) and by western blot analysis (WB) using anti-pNFκB phosphorylated at Ser276. Mice were kept at 30℃ for 24 h. Half of them were exposed to cold (410℃) for 1h to activate BAT. IHC showed nuclear accumulation of pNFκB in the BAT of mice exposed to cold, but not in the BAT of mice kept at 30℃. WB analysis of whole cell lysates showed a band near 65 kD, corresponding to the known molecular weight of NFκB p65. To clarify the mechanism, experiments were done in primary culture of brown adipocytes. Isoproterenol (Iso), a β-adrenergic agonist, induced nuclear accumulation of pNFκB in cultured brown adipocytes at a concentration range from 10^{-8}M to 10^{-6}M. Notably, this response was evident only in UCP1-positive cells. The response started within 5 min after the Iso application and reached to the maximum level around 10 min. This response was mimicked by forskolin, an activator of adenylate cyclase, and inhibited by H89, an inhibitor of A kinase. These results indicate that nuclear accumulation of pNFκB in BAT occurs through β-adrenergic stimulation followed by the cAMP-A kinase signaling pathway.

(COI: No)

P2-378

Influence of Hepatectomy on Body Temperature Changes in Rats

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The abdominal surgical operation, especially liver resection and transplantation, is known to increase body temperature (BT) during and after surgery, but the precise mechanism(s) are not well understood. Therefore, the aim of the present study was to identify possible mechanisms by which the abdominal surgical operation increases BT using an experimental rat model. Specific pathogen-free male Sprague-Dawley rats, 4 weeks of age, underwent two-thirds partial hepatectomy (PH), one-third splenectomy (PS), or left kidney resection (KR) and rectal temperature (RT) was measured for 5 consecutive days after surgery. RT in PH rats increased and peaked on day 4. However, there was no increase in RT 4 days after PS or KR. In the second part of experiments, we examined the influence gadrinium chloride (GC) and interleukin-1β monoclonal antibody (IL-1β mAb) on the increase in RT following 2/3 PH. Treatment of rats with 20 mg/kg GC or 200 μg IL-1β mAb inhibited the increase in RT induced by PH along with the decrease in IL-1β and prostaglandin E2, which act as pyrogens that change the thermoregulatory set point in the hypothalamus. These results suggest that the abdominal surgical operation, especially liver resection, caused an increase in endogenous pyrogen production, resulting in increased BT.

(COI: No)

P2-379

Application of practical pre-cooling to alleviate thermal strain

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Pre-cooling (i.e., removal of heat from the body immediately prior to exercise) is a popular strategy for alleviating thermal strain and improving exercise performance in hot conditions. Whole body water immersion is the procedure most commonly used to pre-cool in experimental studies. However, the supply of a large volume of water and ice in all field settings is not always possible, or practical. In the present study, we examined the effectiveness of hands and foot water immersion and wearing a cool-vest as practical pre-cooling method on heat strain while wearing protective clothing. Eight males engaged in 60 min of walking at a moderate speed (2.5 km/h) in a hot environment (37℃, 50%relative humidity). Before walking, they immersed hands and foot in water at 18℃ or 28℃ and wore a cool-vest for 30 min. The water was wiped off and the vest was put off, then they wore protective clothings. Rectal temperature increased by 1.0 ± 0.1℃ at the end of the walking in the control trial (without the pre-cooling). The pre-cooling inhibited the increases (18℃, 0.5 ± 0.1℃; 28℃, 0.6 ± 0.1℃, $p<0.05$). In addition, sweat rate, thermal unpleasantness, physical and psychological fatigues were significantly lower in the pre-cooling than in the control trial ($p<0.05$), regardless of water temperature. In 18℃ pre-cooling trial, the changes in heart rate, thermal sensation, and damp sensation were also attenuated ($p<0.05$). Hands and foot water immersion and wearing a cool-vest could be an alternative pre-cooling method alleviating heat strains.

(COI: No)

P2-380

Impairment of cognitive function during passive heat stress

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Excessive elevation of internal body temperature causes a significant strain on either the brain function or the locomotive system. Although hyperthermia impairs psychological and working memory performances, the effect of hyperthermia on cognitive processing remains unknown. We hypothesized that a passive heat stress impairs the cognitive function when the internal temperature was excessively increased. Thirteen healthy males performed an auditory oddball paradigm before and after heat stress (Pre and Post) and when esophageal temperature was increased by 0.8 ℃ and 2.0 ℃ (Mild and Severe). The reaction time and event-related potentials (ERPs) were recorded in these four sessions. As a time control, subjects performed the same sessions without heat stress. The reaction time was shortened while esophageal temperature was elevated relative to the Pre but did not change in the time control trial. However the peak latency and amplitude of N100 did not change throughout the experiment. Although the latency of P300 was unaffected due to heat stress, the amplitude of P300 was significantly reduced at the Severe ($10.5 \pm 5.9\,\mu$V) and Post ($11.1 \pm 5.5\,\mu$V) relative to at the Pre ($16.3 \pm 4.7\,\mu$V). These results suggest that excessive elevation of internal temperature impairs cognitive processing but not auditory processing.

(COI: No)

P2-381

Role of the prostaglandin system in fever following intracranial hemorrhage in mice

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Fever is common after intracerebral hemorrhage (ICH) though its molecular mechanism is unclear. In this study, we established a mouse model of ICH-fever and analyzed the molecular mechanism. To induce ICH, collagenase (Type VII) was injected into the brain under isoflurane anesthesia. When collagenase was injected into the preoptic area, the body temperature started to elevate 30 min after the injection. It then reached the maximum level of 3℃ higher than the baseline between 4 h and 6 h after the injection. Rise in body temperature was positively correlated with the ICH volume. Heat-inactivated collagenase neither induced ICH nor elevated the body temperature. When collagenase was injected into the striatum or pons, ICH was developed but the body temperature did not elevate. Intraperitoneal injection of diclofenac, a non-selective inhibitor of cyclooxygenase (COX), suppressed the elevation of body temperature. On the other hand, NS398, a COX-2 selective inhibitor, did not suppress it. RT-PCR and immunohistochemistry revealed the presence of COX-1, COX-2 and mPGES-1 in the preoptic area after ICH. The present study established a mouse model of ICH-fever, in which COX-1-mediated elevation of prostaglandin (PG), possibly PGE_2, seems to be essential. Although COX-2 is upregulated by ICH, its role in ICH-fever is unclear.

(COI: No)

P2-382

Skin warm/cold threshold during passive heating are attenuated in elderly men

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Autonomic heat dissipative responses are known to be deteriorated with aging, while little is known whether the perception of the increases in skin and esophageal temperatures (T_{sk} and T_{es}) is also deteriorated with aging.

Methods: Seventeen young (21 ± 2 yrs, mean ± SD) and nine elderly (72 ± 3 yrs) healthy men underwent measurements of noticeable increase and decrease (±0.1℃/sec) of skin temperature (warm and cold threshold, respectively) at forearm by using a thermode (6.25 cm^2), and of whole body thermal sensation (VAS) in normothermia (NT; T_{es}, 36.6 ± 0.2℃ in young and 36.5 ± 0.2℃ in elderly, mean ± SE) and mild-hyperthermia (HT; T_{es}, 37.3 ± 0.1℃ in young and 37.5 ± 0.2℃ in elderly; after 40 min of lower legs immersion in 42℃ water). T_{es} and T_{sk} were measured continuously.

Results: Skin warm and cold threshold were blunted in elderly than young men in both NT and HT (all, $P<0.05$). During HT, cold threshold were blunted from NT in young men ($P<0.05$) while it remained unchanged in elderly men ($P=0.97$). Whole body thermal sensation increased during HT from NT in both groups ($P<0.05$), while it showed lower values in elderly than young men during both conditions with a significant difference during NT ($P<0.05$).

Conclusion: Skin warm and cold threshold and also whole body thermal sensation were blunted with aging.

(COI: No)

P2-383

Detection of dynamical human brain temperature changes during tasks and anesthesia

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Human brain temperatures have been measured noninvasively by magnetic resonance spectroscopy (MRS). However, it is not clear whether the brain temperature changes or not during brain activations. We have tried to monitor brain temperature changes precisely during exercises, tongue stimulations and anesthesia (sedation level). The exercises were the lower leg flexion and hand grasp at the rate of about 1 Hz. A capsaicin solution (0.1 %, 20 μL) was used for the stimulation of tongue. The sedation was induced by a single shot intra venous injection of midazolam. Brain temperatures rose monotonously about 0.5 ℃ by 30 min during knee flexion and returned gradually after the end of exercise. The esophagus temperature rose about 0.2 ℃. The net brain temperature change was estimated as 0.01 ℃/min. This temperature rise was found in relatively large regions of the brain. We detected the transient brain temperature falls during light tasks such as hand grasp and tongue stimulation. The brain temperature also fell during sedation. We could estimate the brain energy decrease during sedation as 0.2 W with brain temperature changes. The energy difference between arousal and sedation in our case was about 1 % of the energy that brain needs. Dynamical brain temperature changes could be detected by MRS, and its direction depends on the tasks and maneuvers.

(COI: No)

P2-384

A new portable device to measure sweat rate in hyperthermia for field test

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Sweat rate (SR) in hyperthermia has been measured by a ventilated capsule method. However, this technique is only limited to an experimental chamber. Here, we have developed a portable device to measure SR for the field test. 7 males and 3 females (21-45 yrs) performed cycling exercise for 20-30 min at ~65% peak oxygen consumption rate [30℃ Ta; 50% RH]. SR was measured with a ventilated capsule perfused with dry air at 1.5 l/min on the left chest (SR_{vent}; 12.6 cm^2 area) and a portable device in which 7.5 g of silica gel was contained to absorb water vapor from sweat on the right chest (SR_{pd}; 10 cm^3 volume), while monitoring esophageal temperature (T_{es}). We determined the T_{es} threshold for increasing SR_{pd} (TH_{pd}) and the sensitivity of SR_{pd} in response to increased T_{es} ($\Delta SR_{ad}/\Delta T_{es}$) and those for SR_{vent} (TH_{vent} and $\Delta SR_{vent}/\Delta T_{es}$). T_{es} at rest was ~36.5℃ and increased by 1.4℃ by the end of exercise when SR_{vent} and SR_{pd} increased to ~1.0 mg/min/cm^2 and ~100 μg/cm^2, respectively. Although TH_{pd} was 36.7 ± 0.1℃ significantly lower than TH_{vent} of 37.0 ± 0.1℃, they were significantly correlated ($y = 0.92x + 3.10$, $r = 0.89$, $P = 0.0006$). $\Delta SR_{pd}/\Delta T_{es}$ was $101.5 \pm 23.8\,\mu g/cm^2/℃$, significantly correlated with $\Delta SR_{vent}/\Delta T_{es}$ of 2.0 ± 0.4 mg/min/cm^2/℃ ($y = 0.01x + 0.74$, $r = 0.85$, $P = 0.0017$). Thus, the portable device can be used to measure SR in hyperthermia, suitable for the field test.

(COI: No)

P2-385

Effects of β-amyloid-infusion on behavioral thermoregulation and acquired heat tolerance in rats

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We investigated behavioral thermoregulatory function and ability for acclimating to heat of β-amyloid (Aβ)-infused rats. Male Wistar rats were anesthetized with pentobarbital sodium (50 mg/kg, i.p.) and implanted in the intraperitoneal cavity with a temperature transmitter. A solvent of 35% acetonitrile and 0.1% trifluoroacetic acid (pH 2.0) was used as the vehicle for Aβ peptide (4.9-5.5 nmol). An osmotic pump contained 234 ± 13.9 μl of Aβ solution was subcutaneously implanted in the back and was cannulated into the left cerebral ventricle. Moreover, 0.5 μg of $AlCl_3$ was injected into the right cerebral ventricle with a micro syringe pump. Vehicle-infused rats were used as controls. After 2 weeks, rats were placed in a thermal gradient and their intra-abdominal temperature (T_{ab}) and their ambient temperatures (T_a) selected (T_s) were measured for 3 consecutive days. After the measurements, rats were kept at a T_a of 32°C for 4 weeks to attain heat acclimation. Then rats were subjected to a heat tolerance test, i.e. they were exposed to a T_a of 36°C for 3 h. Although there were clear day-night variations of T_s and T_{ab} in all rats, their patterns were significantly altered by Aβ infusion. Moreover, heat acclimation obtained by heat acclimation was attenuated by Aβ infusion. These results suggest that Aβ-infusion in the lateral ventricle modifies behavioral thermoregulation and lowers an ability to acclimate to in rats.

(COI: No)

P2-386

Serum IL-6 and sweating rate responses to passive heating dependent on age

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It is known that sweating responses to heat decrease with age. It is possible that inflammatory cytokines have an effect on sweating responses. We investigated the changes in the serum interleukin 6 (IL-6), the eardrum temperature (Te), and the sweating rate (SR) to the passive heating stress for 60 minutes in younger and elderly men. Three elderly (75.7 ± 3.5yrs) and three younger (23.3 ± 0.6yrs) healthy men participated as volunteers. Changes in the SR of younger men, in response to >0.3 degrees Celsius (°C) of difference between the base line and the Te (DTe), were greater than those of elderly men in response to >0.5°C in the DTe. The slope of SR against the DTe in the younger men was steeper than that of the elderly men. The concentrations of IL-6 in the younger men were significantly greater than those in the elderly men during the heating stress ($p<0.05$). The concentrations of IL-6 showed little change during the 60 min passive heating in younger men. In contrast they displayed an increasing trend in the elderly. It is possible that the concentrations of IL-6 in the elderly men are higher than those in younger men, and have an effect on the different sweating responses between the two populations.

(COI: No)

P2-387

Reduction of plasma estradiol level affects daily rhythms of body core and tail skin temperature in female rats

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Purpose: We assessed the effect of plasma estradiol (E_2) on daily changes of thermoregulatory responses of the tail vasculature by simultaneous measurements of body core (T_{core}) and tail skin temperature (T_{skin}).
Methods: Female Wistar rats (n=10, age of 8-12 wk) were bilaterally ovariectomized, and placed a telemetry device for the measurements of T_{core} and spontaneous activity (ACT) in the abdominal cavity. T_{skin} was estimated by thermography. Two silicon tubes containing 17-beta estradiol were s.c. placed in one group (n=5, E_2 (+)), and empty tubes for the other (n=5, E_2 (-)). The tubes were removed 10 days after the surgery. T_{core}, ACT, and T_{skin} were measured a day before the tubes removal (PRE), and14 days after (Day 14).
Results: On PRE, T_{core} was higher ($P<0.05$) in the E_2 (-) group than in the E_2 (+) group at 12:30-17:00 (37.2 ± 0.2℃ and 36.5 ± 0.2℃, respectively). T_{skin} was higher ($P<0.05$) in the E_2 (-) group than the E_2 (+) group at 20:00-0:30 (33.4 ± 0.3℃ and 31.2 ± 0.6℃, respectively). On Day 14, both T_{core} and T_{skin} in the E_2 (-) group remained unchanged from the levels of PRE. On the other hand, in the E_2 (+) group, T_{core} became higher than PRE level at 14:30-18:30. T_{skin} was higher than PRE level throughout the day.
Conclusion: A reduction of plasma estradiol may attenuate thermoregulatory response of the tail vasculature to maintain circadian T_{core} rhythm in female rats.

(COI: No)

P2-388

Search of the limbic and cortical regions involved in psychological stress-induced hyperthermia

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Psychological stress-induced hyperthermia is a fundamental autonomic response in mammals. Recently, we have reported that stress induces thermogenesis in brown adipose tissues (BAT), hyperthermia and tachycardia by activating a neural pathway from the dorsomedial hypothalamus (DMH) to the medullary raphe. However, the upper neurons that provide the DMH with stress signals to drive the stress responses are unknown. To identify such upper neurons, in this study, we performed *in vivo* electrophysiological experiments using anesthetized rats, in which neurons in several forebrain regions implicated in emotion were stimulated with nanoinjections with bicuculline, a $GABA_A$ receptor antagonist, and the effects on sympathetic effectors were examined. Nanoinjection of bicuculline into either the medial prefrontal cortex (mPFC) or the lateral septal nucleus (LS) increased BAT sympathetic nerve activity, BAT thermogenesis and heart rate. These evoked sympathetic responses were diminished by inhibition of bilateral DMH neurons with muscimol nanoinjections. Inhibition of bilateral mPFC neurons with muscimol injections in free-moving rats strongly reduced BAT thermogenesis and hyperthermia induced by exposure of the rats to social defeat stress, a sociopsychological stress model. In contrast, inhibition of LS neurons exhibited a weaker inhibitory effect on these stress responses. These results suggest that the mPFC is involved in psychological stress-induced BAT thermogenesis and hyperthermia through activation of DMH neurons.

(COI: No)

P2-389

Pleasantness induced by local thermal stimulus may be related to the activity of the frontal cortex: assessment by near-infrared spectroscopy

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Purpose: Thermal stimuli to body core and surface can evoke pleasantness, although the neural mechanisms are not fully understood. In the present study, we assessed blood oxygenation level dependent (BOLD) signals of the frontal cortex by NIRS, when human subjects had local skin heating or cooling during whole-body warming or cooling.
Methods: Ten healthy subjects had local thermal stimulus of 41℃ or 17℃ during whole-body stimulus of 45℃, 33℃, or 24℃. The local stimuli were delivered on the left forearm with the Peltier device, and the whole-body stimuli were conducted by wearing a water-perfusion suit. A subject sat on chair at least until the skin temperature became stable, the local skin stimulation with either temperature was conducted four times. During the period, the BOLD signals were obtained by NIRS, and the subjects were asked to report thermal sensation and pleasantness using visual analogue scale (VAS) in the last session.
Results: There were no differences in local thermal sensation among the three whole body temperatures, although the local thermal pleasantness was different. When the thermal unpleasantness in the forearm was augmented, the BOLD signal decreased in the frontal cortex.
Conclusion: NIRS could be a tool to assess thermal pleasantness induced by local thermal stimuli.

(COI: No)

P2-390

Effect of systemic estradiol administration on tail-hiding behavior and cFos expression of brain areas in female rats in the cold

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INTRODUCTION: Rats place their tails underneath their body trunks in the cold (tail-hiding behavior), which may be a thermoregulatory behavior. The aim of the present study was to test the effect of estradiol (E_2) on tail-hiding behavior and neural activity in brain areas in the cold.
METHODS: Ovariectomized rats were implanted a silastic tube with or without E_2 (22.3mg) underneath the dorsal skin (E_2(-) and E_2(+) groups), and exposed to 27℃ or 10 or 16℃ for 2-h with continuous body temperature (T_b), tail skin temperature (T_{tail}), and tail-hiding behavior measurements. cFos immunoreactive (cFos-IR) cells in the insula, secondary somatosensory cortex, medial preoptic nucleus, parastrial nucleus, amygdala, lateral parabrachial nucleus were counted in four segments: seg1, 2, 3, and 4 (bregma -0.36, -1.44, -2.64, and -9.00 mm), respectively.
RESULTS: T_b and T_{tail} were not different between the E_2(-) and E_2(+) groups. Only at 16℃, the duration and the onset of tail-hiding behavior in the E_2(+) group were higher than that in the E_2(-) group. Only at the insula in seg2 at 16℃, cFos-IR cells in the E_2(-) group were greater than that in the E_2(+) group.
CONCLUSION: E_2 might modulate a tail-hiding behavior of female rats in a mildly cold, and the insula may relate to the response.

(COI: No)

P2-391

Possible role of hypothalamic FABP7 in the control of glial cell proliferation and food intake

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Introduction: A growing evidence suggests that hypothalamic fatty acid sensing is associated with regulation of systemic energy balance, including insulin secretion, adipose deposition and food intake. We have so far reported that fatty acid binding protein 7 (FABP7) is highly expressed in astrocytes and oligodendrocyte precursor cells (OPCs) and functionally involved in their proliferation. In this study, we examined the detailed localization of FABP7 in the hypothalamus including the arcuate nucleus (ARC) and median eminence (ME). Furthermore, we sought to explore the role of FABP7 in the hypothalamus through the phenotypic analysis of FABP7 KO mice under high fat diet food feeding.
Method and Results: In immunohistochemistry, 70% of FABP7+ cells in the ARC and ME were revealed to be NG2+ cells, and 30% of FABP7+ cells were GFAP+ cells. When the hypothalamic cells were labeled with bromodeoxyuridine (BrdU) by drinking water or interventricular injection, approximately 80% of BrdU+ cells in the ARC of wild-type (WT) mice were FABP7+ cells, but the significant decrease in the density of BrdU+ cells was detected in the ARC of FABP7 KO mice. In addition, FABP7 KO mice fed with high fat diet (HFD) showed the significant decrease in their HFD food intake as well as their body weight gain compared with WT mice.
Conclusion: Fabp7 regulates proliferation of NG2+ cells in hypothalamic ARC and ME. The role of FABP7 in the control of neuronal activity in hypothalamus and its possible involvement in the regulation of HFD food intake were highly suggested.
(COI: No)

P2-392

DGKε deletion induces lipid metabolism impairment and adipose tissue insulin insensitivity

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Triglyceride (TG) synthesis and breakdown are closely related to glucose homeostasis, which is regulated by insulin. TG is synthesized from diacylglycerol (DG) by the action of DG acyltransferase. DG is also known to activate novel PKC (nPKC) leading to interruption of appropriate insulin signaling, i.e. insulin resistance. These facts suggest that DG metabolism is critical in insulin signaling and TG synthesis. DG kinase (DGK) is an enzyme that converts DG to phosphatidic acid. Previously, we demonstrated that high fat diet (HFD) induces lipid accumulation and glucose intolerance in DGK ε -KO mice. In this study, we investigated detailed mechanisms for these phenotypes. To this end, HFD-fed DGK ε -KO mice were examined by glucose and insulin tolerance tests. We also measured serum insulin, TG and free fatty acid levels, and performed histological and immunoblot analyses. In DGK ε -deficient adipose tissue, phosphorylation level of PKC θ was increased whereas that of Akt was attenuated. These results suggest that DGK ε depletion results in PKC θ activation and Akt inactivation under HFD feeding, which may lead to impaired insulin signaling. Furthermore, we found that protein levels of adipose triglyceride lipase (ATGL) and its transcription factor FOXO1 were also attenuated, suggesting that DGK ε deficiency downregulates lipolysis in adipose tissue. On the other hand, insulin signaling was kept intact in other organs, such as liver and skeletal muscle. These results suggest that DGK ε -KO mice show dysregulation of lipid metabolism and impaired insulin signaling in adipose tissue.
(COI: No)

P2-393

Global gene expression profiling of the inhibitory effect of estrogen on the cell proliferation of MDA-MB-231 breast cancer cells stably transfected with estrogen receptor

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Treatment with estrogen leads to the inhibition of proliferation of estrogen receptor (ER) negative breast cancer cells stably transfected with ER α cDNA. The elucidation of the ER-mediated inhibitory mechanism will provide a novel strategy for the down regulation of breast cancer growth. In this study, the ER negative MDA-MB-231 breast cancer cells were stably transfected with ER α cDNA and several clones (MDA-ERs) were established. Although the cell proliferation of all the clones was inhibited by the treatment with estrogen for longer times, 24 h estrogen treatment inhibited cell proliferation of MDA-ER#3 but not of MDA-ER#1 clone, despite the same genetic background of the two clones. To compare the gene expression levels of MDA-ER#3 with MDA-ER#1, we applied cDNA microarray analysis to those two clones treated with or without estrogen for 24 h. In each clone, the genes which expressions were altered more than twice by treatment with estrogen were identified as up-regulated or down-regulated genes. Among the up-regulated genes, 119 genes exhibited 142-465% higher estrogen-responsiveness in MDA-ER#3 than that in MDA-ER#1. Among the down-regulated genes, 14 genes exhibited 40-69% lower estrogen-responsiveness in MDA-ER#3 than that in MDA-ER#1. These differentially regulated, estrogen-responsive genes included proliferation-related genes, which may play an important role in the antiproliferative effect of estrogen.
(COI: No)

P2-394

wx/ae rice has effects of improving hyperlipemia and fatty liver induced by high-fat diet

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wx/ae rice includes more resistant starch and γ -Oryzanol than Koshihikari which have effects of reducing the lipid in the blood. In this study, male C57BL/6J mice aged 8 weeks were fed of chow diet (10% kcal), high-fat diet (45% kcal), high-fat diet + wx/ae brown rice, a high-fat diet + Koshihikari brown rice, for 12 weeks. The powder of brown rice was contained 30%. In the high-fat diet + wx/ae brown rice group, feces amount is increased to 1.5 times, triglycerides content of feces per 1g was more than double compared to the high-fat diet group. The high-fat diet + wx/ae brown rice group, triglycerides and cholesterol in the blood are reduced to 50% compared to high-fat diet group, and became a concentration similar to the normal diet group. In addition, after feeding high-fat diet for 8 weeks, these mice divided 3 groups as follows: chow diet, high-fat diet + Koshihikari brown rice, high-fat diet + wx/ae brown rice were switched for 4 weeks. The fatty liver is improved by switching to high-fat diet + wx/ae, not Koshihikari brown rice. These results suggested that wx/ae brown rice has the effect of improving hyperlipemia and fatty liver by egestion of lipid to feces.
(COI: No)

P2-395

Expression of purinergic receptors on mouse brown adipocytes

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Neurotransmitter receptors on brown adipocytes and sympathetic nerve fibers contribute to thermogenesis by mediating Ca^{2+} dynamics among brown adipocytes. We investigated the functional expression of purinergic receptor subtypes on brown adipocytes of mouse interscapular fat. Ca^{2+} imaging showed that applied 10 μM ATP, 10 μM BzATP (a $P2X_1$, $P2X_7$ and $P2Y_1$ agonist), 1 μM 2MeSATP (a $P2Y_1$ and $P2Y_{11}$ agonist) or 100 μM UTP (a P2Y agonist) increased intracellular Ca^{2+} concentration. RT-PCR suggested the expression of $P2X_1$, $P2X_3$, $P2X_4$, $P2X_5$, $P2X_7$, $P2Y_1$, $P2Y_2$, $P2Y_6$, $P2Y_{13}$ and $P2Y_{14}$ among the seven P2X subtypes and seven P2Y subtypes examined. Immunoblotting confirmed the expression of $P2X_1$ and $P2X_7$. These results showed the functionally expression of $P2X_1$ and $P2X_7$ on mouse brown adipocytes. The roles of purinergic receptor subtypes in the thermogenesis are discussed.
(COI: No)

P2-396

Gene Expression Analysis of immune cells in response to Nucleoprotein

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Salmon soft roe (Shirako) contains in plenty nucleic acids and protamin protein, termed as Nucleoprotein (NP). Previously we showed that crude NP derived from food and also purified DNA components affect on immune cells to express some IL-8 and induce differentiation into mature immune cells. We did further analysis focus on the stimulation mechanism of immune cells in response to purified DNA components by Micro Array Analysis and subsequently IPA Analysis. As a result, 62 and 109 genes were up and down-regulated, respectively. Here, we suggest that purified DNA component taken from food affect on immune cells to induce some cytokines and increase mucosal immunity which contribute to our health and anti-aging process.
(COI: No)

P2-397

Effect of intermittent sucrose intake for several weeks on central glucose-sensing system in mice

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The central glucose-sensing system has neurons to respond to glycemic levels and regulates feeding behavior. Intermittent sucrose intake under food deprivation induces overconsumption of sucrose. We previously reported that the feeding pattern enhances glucose tolerance and attenuates the anorectic effect of systemic glucose injection in mice. However, it remains unclear whether the intermittent sucrose intake changes the glucose-sensing system. Here, we investigated the effect of intermittent sucrose intake on neuronal responses to systemic glucose injection. Mice under 20-h food deprivation received 4-h access to chow with or without sucrose solution (FD/Suc and FD mice, respectively) for 24 days. Sucrose intake of FD/Suc mice was greater than water intake of FD mice after Day 2. After Day24, the mice were food deprived overnight followed by an intraperitoneal glucose injection. Then, the mice were perfused 90 min after the injection and the brains were processed for Fos-immunoreactivity. The number of Fos positive cells in the lateral hypothalamus and perifornical area (LH/PFA) in FD/Suc mice were smaller than those in FD mice. This finding demonstrates that the intermittent sucrose intake under food deprivation attenuates the neuronal responses to systemic glucose injection in the LH/PFA. It is suggested that the change in the central glucose-sensing system contributes to overconsumption of sucrose.

(COI: No)

P2-398

Effect of postprandial chewing gum on diet-induced thermogenesis

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The present study was to examine the effect of postprandial chewing gum on diet-induced thermogenesis (DIT). After baseline measurements for 20 min in the overnight fasting state, twelve healthy normal-weight males fractured a 620-kcal test meal for as fast as they could. In the chewing gum (RG) trial, they started chewing a 3-kcal gum immediately after meal, and chewed the gum for 15 min. In the no-chewing gum (RN) trial, they ingested 3 kcal of sugar with test meal instead of the gum. DIT was calculated from oxygen uptake and body mass, and was recorded until 180 min after meal. Duration and the number of chews during test meal showed no significant differences between the RN trial and the RG trial (304 ± 32 vs. 298 ± 26 s, 238 ± 20 vs. 237 ± 24 times, RN vs. RG, respectively, $p>0.05$). The number of chews for 15-min chewing gum (RG trial) was 836 ± 51 times. DIT was significantly greater in the RG trial than in the RN trial (14 ± 4 vs. 20 ± 5 kcal / 180 min, RN vs. RG, $p<0.05$). Effect of postprandial chewing gum on DIT was observed until 45 min after meal. Previous study reported that energy expenditure by solely chewing gum for 12 min (chewing frequency; 100 times / min) was 11 kcal, and increased energy expenditure returned to baseline levels immediately after cessation of chewing (Levine 1999). In the present study, postprandial chewing gum had an influence on DIT until 45 min even after chewing gum. These results suggest that 15-min chewing gum immediately after meal increases DIT.

(COI: No)

P2-399

SIRT1 in the central nervous system regulates food preference

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In order to address the role of SIRT1 in the food preference regulation, we either over-expressed or knocked-out SIRT1 in all neurons by crossing Tau-Cre mice with either Rosa26-Sirt1 mice or Sirt1-flox mice, and measured their food preference. SIRT1 overexpression increased preference for high-fat diet, whereas SIRT1 knock-out increased preference for high-sucrose diet. Expression analysis of hypothalamus, ventral tegmental area, nucleus accumbens, and prefrontal cortex of these mice fed normal chow or high-fat diet revealed that SIRT1 overexpression suppressed the expressions of tyrosine hydroxylase (Th) and dopamine transporter (Dat) and increased the expression of oxytocin (Oxt) in the hypothalamus. Next, we either overexpressed or knocked-down Sirt1 in hypothalamic N38 and N41 cells and checked the effect on the expressions of Th, Dat, and Oxt. We found that SIRT1 regulates the expression of these genes accordingly. Dopamine system is known to promote fat preference, whereas oxytocin is known to suppress carbohydrate preference. Therefore, Sirt1 in the central nervous system may regulate food preference through the modulation of dopamine and oxytocin system.

(COI: No)

P2-400

Glucagon directly activates vagal afferent neurons: possible role in feeding regulation

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Background and Aim: It has been reported that glucagon is transiently secreted immediately after meals and implicated in meal-evoked satiety. Intraperitoneal injection of glucagon reduces feeding, and this effect is attenuated by subdiaphragmatic vagotomy, suggesting the involvement of the vagal afferent nerves. However, the mechanism by which glucagon influences vagal afferents is less defined. In this study, we investigate the direct action of glucagon on vagal afferent nodose ganglion (NG) neurons.
Results: Glucagon receptor mRNA was detected in mice NG using by RT-PCR. Glucagon at 10^{-9}-10^{-7} M, but not 10^{-10} M, increased cytosolic Ca^{2+} ($[Ca^{2+}]_i$) in isolated single NG neurons. Glucagon at 10-8 M exerted a maximal effect, inducing $[Ca^{2+}]_i$ increases in approximately 8% of NGNs. Glucagon-induced $[Ca^{2+}]_i$ increases were attenuated by a glucagon receptor antagonist. All of the glucagon-responsive NG neurons exhibited $[Ca^{2+}]_i$ responses to cholecystokinin-8 (CCK-8), a hormone known to reduce food intake via direct interaction with vagal afferents.
Conclusion: These results demonstrate that glucagon directly interacts with the subpopulation of vagal afferent neurons that respond to CCK-8. This interaction may underlie the production of satiety after meals. This study also suggests that glucagon and CCK-8 share a common vagal afferent-mediated pathway that inhibits feeding.

(COI: No)

P2-401

Peripheral oxytocin directly activates vagal afferents to decrease food intake

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Oxytocin (Oxt), produced in the paraventricular nucleus and supraoptic nucleus of hypothalamus, regulates feeding. Peripheral administration of Oxt suppresses feeding and ameliorates obesity. However, the route through which peripheral Oxt informs the brain is unclear. We investigated whether vagal afferents mediate the sensing and anorexigenic effect of peripherally injected Oxt in mice. Oxt evoked action potential firings and increased cytosolic Ca^{2+} concentration ($[Ca^{2+}]_i$) in single vagal afferent neurons. The Oxt-induced $[Ca^{2+}]_i$ increases were inhibited by Oxt receptor antagonist. Intraperitoneal injection of Oxt decreased feeding and increased c-Fos expression in the nucleus tractus solitarius (NTS) of medulla, to which vagal afferents project. This feeding suppression and c-Fos expression in NTS were blunted by subdiaphragmatic vagotomy. In obese diabetic *db/db* mice, leptin failed to but Oxt increased $[Ca^{2+}]_i$ in vagal afferent neurons, and single injection or sub-chronic infusion of Oxt decreased feeding and body weight gain. These results demonstrate that peripheral Oxt injection suppresses feeding by activating vagal afferents, and that this "peripheral Oxt-vagal afferents-brain" axis is effective for treating hyperphagia and obesity.

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P2-402

Accumulation of vitamin A and radiocontamination of arctic animals

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We have performed a systematic characterization of the hepatic vitamin A storage in mammals and birds of the Svalbard archipelago and Greenland. The top predators including polar bear, arctic fox, bearded seal and glaucous gull contained about 10-20 times more vitamin A than all other arctic animals studied as well as their genetically related continental top predators. This massive amount of hepatic vitamin A was located in large lipid droplets in hepatic stellate cells (HSCs). The droplets made up most of the cells' cytoplasm. The development of such an efficient vitamin A-storing mechanism in HSCs may have contributed to the survival of top predators in the extreme environment of the arctic. The HSC that has capacity of taking up and storing a large amount of vitamin A plays pivotal roles in maintenance of food web, food chain, biodiversity, and eventually ecology of the arctic. In March 2011, the big earthquake and tsunami attacked the east coast of Northern Japan. Radiocontamination was caused by the damage of Fukushima power plant. We visited Svalbard in August-September 2011 and August-September 2013 and examined radiocontamination in fauna and flora of the arctic. We would like to discuss based upon the data obtained.

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P2-403

Change metabolic enzymes activity and effects of chaga mushroom (*Inonotus obliquus*) in diabetes mellitus

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Introduction: It was thought that type II diabetes mellitus was induced by impaired insulin secretion and through inhibiting effects of insulin by glucose tolerance factor. But, it was not known very well how various metabolic enzymes were affected by diabetes mellitus (DM).
Objectives: The changes of metabolic enzymes activities caused by DM are analyzed. Then, effects of chaga mushroom well known as antioxidant factors are estimated to make clear the anti-diabetes activity.
Materials and methods: Streptozotocin (STG) sol was administrated by intra-peritoneal injection into SD rat induce DM. Then, various metabolic enzymes activities were analyzed by apiRZYM system (SYSMEX bioMeneux Co. Ltd, Tokyo) in DM rats. Still more, the metabolic enzyme activities of chaga administrated rats were similarly analyzed to clarify the anti-diabetes activity.
Results and discussion: High blood-sugar and cholesterol levels and low levels of lipid metabolic enzymes were detected were detected in DB rats induced by STG. Especially, it was made clear that β-galactosidase activity as an aging factor increased in DB rats. Still more, It was seemed that these changes were inhibited by chaga extracts.
Conclusion: High blood-sugar and cholesterol levels and β-galactosidase activity increased in DB rats induced by STG. It was made clear that these changes were inhibited by chaga as amti-diabetes.
(COI: No)

P2-404

Global gene expression analysis to identify molecular mechanisms that enable hibernation in mammals

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Hibernation is a state during which drastic metabolic suppression occurs to survive severe winter condition with little or no food. Mammalian hibernators must achieve adaptive remodeling in tissues and cells to hibernate without severe tissue injuries or massive cell death. Interestingly, several reports suggested that the adaptive remodeling is not observed in non-hibernating seasons but is induced in the pre-hibernation periods. However, the molecular and cellular mechanisms for the adaptive remodeling remain to be poorly understood. To reveal this, we have conducted global gene expression analysis with RNAseq by using syrian golden hamster (Mesocricetus auratus), which initiates hibernation under short day and cold acclimation condition after prolonged periods (about 4~12 weeks). We have examined several organs, including liver, skeletal muscles, white adipose tissues and brown adipose tissues, that are involved in energy homeostasis at the systemic level. This analysis have identified genes that are specifically up-regulated or down-regulated during hibernating periods. Possible contribution of those genes to the establishment of adaptive mechanisms for hibernation will be discussed.
(COI: No)

P2-405

Exploring adaptive mechanism in adipose tissues that enables animals to survive hibernation periods

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Hibernation is an energy-saving behavior to survive during winter with little or no food. During hibernation, animals experience dramatic decreases in core body temperature, heart rate and oxygen consumption, which require adaptive remodeling in tissues and cells for the animals to survive without tissue injuries or cell death. Interestingly, the adaptive remodeling seems to be mainly induced before the entrance to hibernation, because it is not observed in non-hibernating season. However, molecular mechanisms responsible for the adaptive remodeling in tissues and cells remain largely unclear. In this study, we investigate the remodeling mechanism of brown and white adipose tissues by utilizing syrian golden hamster (Mesocricetus auratus), which initiates hibernation under short day and cold acclimation condition after prolonged periods (about 4~12 weeks). To reveal molecular and cellular mechanisms for adaptive remodeling for hibernation, we have conducted global gene expression analysis with RNAseq and revealed that expression of several genes related to development of adipose tissues, lipid metabolism and synthesis, and non-shivering thermogenesis, changes during hibernation periods, which may contribute to the establishment of adaptive energy metabolism and thermogenesis for hibernation.
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