Symposia

Symposium 01
Regulation of the cardiac gap junction and its pathological aspects

(March 16, 9:00–11:00, RoomA)

1S1A-1

Downward remodeling of cardiac gap junction connexin 43 and arrhythmogenesis

Imanaga, Issei(*Fukuoka University, Hara-Doi Hospital*)

Dysfunction of the cardiac gap junction which fundamentally contributes to electrical cell-to-cell coupling is regarded as one of essential factors generating arrhythmias. The function of the gap junction depends on the upward or downward regulation of connexin(Cx) which composes the gap junction channel. In cardiac muscle tissue,Cx30.2, Cx40, Cx43 and Cx45 are expressed showing regional specificity. Cx43 is predominantly expressed in the ventricular tissue. A suppression of the PKA-mediated phosphorylation of Cx43 induced by hypoxia which leads to intracellular Ca overload and acidosis causes scant ~ heterogenous expression of Cx43 at the gap junction area in the intercalated disk and a decrease in electrical resistance of the gap junction. An activation of PKA prevents deteriorative effects of hypoxia on Cx43. An augmentation of the PKC-mediated phosphorylation of Cx43 induced by PKCε activator such as Angiotensin II makes sparse expression of Cx 43 at the gap junction area, lateralization, annural profile and internalization of the gap junction, a decrease in amount of Cx43 protein and an increase electrical resistance of the gap junction. Proteolytic degradation of Cx43 is accelerated when the PKCε-mediated phosphorylation of Cx43 is augmented. The downward remodeling of Cx43 make the heart more susceptible to the ventricular tachyarrhythmia. On the contrary,an augmentation of the PKA-nediated phosphorylation of Cx43 induced by PKA activators or cyclic AMP analogs is followed by an increment of expression and amount of Cx43. The upward remodeling of Cx43 makes the heart low susceptibility to arrhythmias. No COI.

1S1A-2

A Connexin40 Mutation Associated with a Malignant Variant of Familial Cardiac Conduction Defect

Makita, Naomasa[1]; Seki, Akiko[2]([1]*Department of Molecular Physiology, Nagasaki University,* [2]*Department of Cardiology, Tokyo Women's Medical University*)

Familial cardiac conduction defect (CCD) is a hereditary arrhythmia characterized by progressive conduction disturbances in the His-Purkinje system. CCD has been linked to genes such as SCN5A that influence cardiac excitability but not to genes that influence cell-to-cell communication. In order to explore whether mutations in connexin genes would associate with clinical cases of CCD, we screened 156 probands with CCD. In addition to 12 sodium channel mutations, we found a germ line GJA5 (connexin40 [Cx40]) mutation (Q58L) in 1 family. Heterologous expression of Cx40-Q58L in connexin-deficient neuroblastoma cells resulted in marked reduction of junctional conductance (Cx40-wild type [WT], 22.2 ± 1.7 nS, n=14; Cx40-Q58L, 0.56 ± 0.34 nS, n=14; $P<0.001$) and diffuse localization of immunoreactive proteins in the vicinity of the plasma membrane without formation of gap junctions. Heteromeric cotransfection of Cx40-WT and Cx40-Q58L resulted in homogenous distribution of proteins in the plasma membrane rather than in membrane plaques in 50% of cells; well-defined gap junctions were observed in other cells. Junctional conductance values correlated with the distribution of gap junction plaques. Mutation Cx40-Q58L impairs gap junction formation at cell-cell interfaces. This is the first demonstration of a germ line mutation in a connexin gene that associates with inherited ventricular arrhythmias and emphasizes the importance of Cx40 in normal propagation in the specialized conduction system. No COI.

1S1A-3

Alterations of connexin expression and cardiac arrhythmogenesis

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Gap junction (GJ) channels provide a pathway for intercellular communication between adjacent cells. Coordinated propagation of electrical impulse in the heart depends on current flow through GJ channels. Alterations in the expression and organization of GJ subunits, connexins (Cxs), may lead to cardiac arrhythmias through cell-to-cell uncoupling and modification of impulse propagation. We demonstrated that cardiac hypertrophy induced by pressure overload in rats is associated with dislocation of immunelabeled Cx43 GJs from the intercalated disc at the site of end-to-end contact to the lateral border of ventricular myocytes. Some of the displaced Cx43 GJs appear as intracellular annular profiles in electron microscopy, probably reflecting GJ degradation during rapid remodeling. In addition, a hamster model of cardiomyopathy, UM-X7.1, shows a reduction of ventricular Cx43 expression and immunolabeling, at the stage of heart failure, which is mediated by a decrease in nuclear β-catenin, a transcriptional activator of Cx43. Optical mapping in perfused hearts reveals an increase in spatial heterogeneity of the ventricular action potential and slowing of impulse propagation with marked distortion of the wavefront. UM-X7.1 hamsters show an increased vulnerability to sustained ventricular tachycardia and fibrillation, which are characterized by multiple rotors and wavebreaks. In conclusion, alterations of Cx expression associated with cardiac hypertrophy and heart failure may provide structural substrates for tachyarrhythmias. No COI.

1S1A-4

Role of connexins on cardiac arrhythmia

Seki, Akiko[1]; Nishii, Kiyomasa[2]; Shibata, Yosaburo[3]; Kobayashi, Yasushi[2]; Hagiwara, Nobuhisa[1]([1]*Department of Cardiology, Tokyo Women's Medical University,* [2]*Department of Anatomy and Neurobiology, National defence medical college,* [3]*Fukuoka Prefectural University*)

Compared to the major cardiac gap junction protein Cx43,relatively little studies have been carried out on Cx45. To clarify the functional importance of Cx45 in the heart, we investigated electrophysiological characteristics of Cx43 / Cx45 mutant hearts with targeted disruption in Cx43 or Cx45 or both, together with region specific deletion of Cx45 in the myocardium and in the endothelium. In adult, a tamoxifen-inducible Cre-Lox system was used to circumvent embryonic lethality of the $Cx45^{-/-}$ mice. In this presentation, we will discuss the role of Cx45 in cardiac development and in the conduction system. No COI.

Symposium 02
Regional variation and functional overlapping of taste sensor molecules expressed in the oral cavity, gut and brain

(March 16, 10:05–12:05, Room D1)

1S2D1-1

Development of the basal cells in taste buds and taste cell differentiation

Miura, Hirohito[1]; Nakayama, Ayumi[1]; Scott, Jennifer K.[2]; Tomonari, Hiroshi[1]; Ooki, Makoto[1]; Barlow, Linda A.[2]; Harada, Shuitsu[1]([1]*Department of Oral Physiology, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan,* [2]*Department of Cell and Developmental Biology, School of Medicine, University of Colorado, Aurora, CO, USA.*)

In mammals, taste buds consist of 50–100 elongated cells and a small number of basal cells, and are maintained by continuous cell renewal throughout life. The round-shaped basal cells of taste buds are assumed to be in a premature state, while the elongated cells include functional taste cells that are classified into three cell types, I, II and III originally based on their morphological characteristics. We have previously reported Sonic hedgehog (Shh), an evolutionally conserved morphogen, is specifically expressed in the basal cells in taste buds. BrdU incorporation studies showed that Shh (+) basal cells are in a postmitotic state and have a short lifetime, leading the hypothesis that Shh (+) basal cells are postmitotic immediate precursors of other cells in taste buds. Recently, we showed Shh(+) basal cells give rise to all elongated cell types, type I, II and III, using Cre/loxP system. Here, we will discuss the development and cell fate of the basal cells in taste buds. Also, regional differences in taste cell differentiation within the oral cavity will be discussed. No COI.

1S2D1-2

Sweet taste signaling in the oral cavity and the gut: functional roles of leptin, endocannabinoids and GLP-1

Yoshida, Ryusuke; Jyotaki, Masafumi; Takai, Shingo; Shigemura, Noriatsu; Ninomiya, Yuzo(*Section of Oral Neuroscience, Graduate School of Dental Science, Kyushu University, Fukuoka, Japan*)

Both taste cells in the oral cavity and enteroendocrine cells in the gut detect taste substances in foods and drinks and their signals play important roles in the regulation of food intake and nutrient absorption. Recent studies have demonstrated that they share a variety of molecules involved in taste signal detection, hormonal secretion and nutrient absorption. For example, enteroendocrine cells express sweet taste receptor T1R2+T1R3, which is originally found in taste cells. Activation of T1R2+T1R3 is shown to lead to facilitation of glucose absorption in the gut. In constant, taste cells express glucose sensors, SGLT1 and GLUTs, and peptide hormones, GLP-1 and CCK, which are known to function in the gut. However it remains unclear whether these molecules would be involved in taste signal detection and transmission in the oral taste system. Regarding sweet taste, we recently found that sweet taste sensitivities of taste cells are modulated by orexigenic and anorexigenic mediators such as leptin and endocannabinoids, which regulate food intake by acting on hypothalamic receptors. But functional roles of leptin and endocannabinoids in the gut taste system still remain unclear. In this talk, we will focus on leptin, endocannabinoids and GLP-1 and will report their functional roles in sweet taste signaling in the oral and gut taste systems. No COI.

1S2D1-3

Characterization of taste-like cells in the gastrointestinal tract

Iwatsuki, Ken(*Institute of Molecular and Cellular Biosciences, The University of Tokyo, Japan*)

Recent studies have demonstrated that taste signaling molecules are distributed not only in the gustatory epithelium but also in other tissues including the gastrointestinal (GI) tract, airways, testes and brain. These signaling molecules are thought to participate in detecting sweet, umami and bitter compounds. In order to identify T1R2-expressing cells in vivo, T1R2-LacZ knock-in mouse was generated and examined for LacZ expression in the GI tract. We found that LacZ expression was seen in the oral epithelium, duodenum, small intestine, large intestine but not in the stomach. Most of LacZ positive cells expressed chromogranin A which is a marker of enteroendocrine cells. We have further identified that these cells also express proteins necessary for taste signal transduction such as PLCbeta2 and IP3R3. Some of LacZ positive cells also expressed glucagon-like peptide 1 (GLP-1). Interestingly, unlike taste cells in the oral cavity, enteroendocrine cells that express T1R2 rarely express alpha-gustducin. Furthermore, there was a clear morphological difference between enteroendocrine cells and alpha-gustudicin positive cells. Therefore, one assumption could be made that there are at least two subsets of taste-like cells in the GI tract; enteroendocrine cells that express sweet taste receptors and brush cells that express alpha-gustducin. In summary, we have identified two separate populations of cells that seem to have distinct function in detecting nutrients in the GI tract. No COI.

Symposium 03
New aspects of molecular mechanisms of ENaC-mediated Na+ homeostasis and body fluid regulation

(March 16, 8:40–10:20, Room E)

1S2D1-4

GLP-1 may be involved in sweet specific taste transmission from taste cells to gustatory nerve fibers

Takai, Shingo[1]; Yasumatsu, Keiko[1]; Inoue, Mayuko[1]; Iwata, Shusuke[1]; Yoshida, Ryusuke[1]; Shigemura, Noriatsu[1]; Drucker, Daniel J.[2]; Margolskee, Robert F.[3]; Ninomiya, Yuzo[1](*[1]Sect. Oral Neurosci., Grad. Sch. Dental Sci., Kyushu Univ., Fukuoka, Japan, [2]Dept. Med., Mt. Sinai Hosp., Lunenfeld Tanenbaum Res. Inst., Univ. Toronto, Toronto, Canada, [3]Monell Chemical Senses Center, Philadelphia, USA*)

Recent studies demonstrated that taste bud cells express several gut peptides, such as GLP-1, NPY, and glucagon, and secret these peptides in response to various taste stimuli. Interestingly, the secretion patterns of peptides are correlated with taste qualities, suggesting the possibility that these gut peptides would contribute to taste quality coding. In this study, we report that mice genetically lacking of GLP-1 receptor showed decreased behavioral responses to sweet compounds in short-time lick test and reduced sweet taste responses in chorda tympani nerve recordings. Additionally, we measured GLP-1 concentrations released from single taste buds, and cells in response to various taste stimuli by using ELISA method. As a result, we found that GLP-1 is secreted from a subset of sweet responsive cells by sweet taste stimulation in a concentration dependent manner. Furthermore, it is found that i.v. injection of GLP-1 produced transient increase of neural activities in a subset of sweet specific single nerve fibers without affecting those of other taste fibers. Moreover, we found that human sweet taste sensitivity is associated with two SNP sites existing in GLP-1 receptor gene. All these findings suggest that GLP-1 may be involved in normal sweet taste signal transmission in mice and human. No COI.

1S3E-1

Hormonal modulation of salty and sweet taste sensitivities

Shigemura, Noriatsu; Yoshida, Ryusuke; Yasumatsu, Keiko; Ohkuri, Tadahiro; Iwata, Shusuke; Takai, Shingo; Jyotaki, Masafumi; Niki, Mayu; Sanematsu, Keisuke; Ninomiya, Yuzo(*Section of Oral Neuroscience, Graduate School of Dental Sciences, Kyushu University, Fukuoka, Japan.*)

Information derived from taste receptor cells, such as sweet, bitter, salty, sour and umami is important for evaluating the quality of food components. Modulation of the gustatory information influences food preference and intake. Even so, the molecular mechanisms for modulating taste sensitivity are poorly understood. Our recent studies have demonstrated that salty and sweet taste sensitivities are affected by hormones. Angiotensin II (AngII), which plays important roles in the maintenance of sodium homeostasis in various organs including brain and kidney, modulates peripheral salt and sweet taste sensitivities by acting on AngII type 1 receptor co-expressed with αENaC (an amiloride-sensitive salt taste receptor) or T1r3 (a sweet taste receptor component) in taste cells, and this modulation influences ingestive behavior in mice. Leptin (an anorexigenic mediator) and endocannabinoids (orexigenic mediators), which reciprocally regulate food intake via central nervous systems, modulate palatability of foods by altering sweet taste responses. Thus peripheral modulations of taste information by these hormones critically influence food intake, and may play important roles in regulating sodium and energy homeostasis. Understanding basic principles about these taste modulations may lead us to design novel strategies to maintain and improve the quality of life in human. No COI.

1S3E-2

An essential role of p38 on ENaC trafficking in aldosterone-stimulated Na+ reabsorption in renal epithelial A6 cells

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Aldosterone is an important hormone to regulate epithelial Na+ channel (ENaC)-mediated Na+ reabsorption in the distal nephron for controlling ECF volume and blood pressure. In our previous study, we found p38 plays an essential role on the regulatory mechanism of ENaC-mediated Na+ reabsorption by aldosterone. Inhibition of p38 by SB202190 (a specific p38 inhibitor: SB) abolished the aldosterone-stimulated Na+ reabsorption (INa) with remarkable reduction of apical ENaC conductance. In this study, we investigate a role of p38 on the surface expression of ENaC through exocytic and endocytic pathways. Pretreatment with MG132 (a proteasome inhibitor) mostly recovered the SB-induced reduction of ENaC protein content, ENaC surface expression and INa, although MG132 slightly increased them in the absence of SB. On the other hand, we detected that p38 regulated Akt/PKB activation, which was correlated to ENaC ubiquitylation through the SGK1-Nedd4-2-dependent pathway. Indeed, suppression of p38 and Akt/PKB decreased Nedd4-2 phosphorylation and ENaC protein abundance. These suggest that the p38-mediated signal pathway is essential to suppress ubiquitylation-dependent ENaC degradation contributing to the stimulation of Na+ reabsorption by aldosteone. Supported by JSPS (19590212, 20390060), SSRF (0837, 1035) and FFPR. No COI.

1S3E-3

Regulations of ENaC Expressions by Nedd4L and tubular Renin-angiotensin SystemsImplications for sodium sensitivity and hypertension

Ishigami, Tomoaki[1]; Umemura, Masanari[2]; Araki, Naomi[1]; Minegishi, Shintaro[1]; Yamana, Hisako[1]([1]*Yokohama City University Graduate School of Medicine Department of Cardiorenal Medicine and Medical Science, Yokohama, Japan ,* [2]*Yokohama City University Graduate School of Medicine Cardiovascular Research Institute*)

It was suggested that primary molecular abnormalities existed in tubular sodium transports by molecular genetical analyses for human hereditary and familial hypertension by R.C..Lifton and J.M.Lalouel. We have analyzed molecular pathophysiological dissections for sodium sensitivity and hypertension focusing on Nedd4L which is ubiquitin ligase for epithelial sodium channels located in distal nephron and tubular renin-angiotensin systems (RAS) consisted of angiotensinogen in proximal tubules and renin in connecting tubules. By detailed genetical analyses for human and rodents, we discovered molecular diversities of Nedd4L gene (JHG 2002, BBRC 2006) and different Nedd4L isoforms with C2 domain regulating ENaC expressions in vivo (Hypertension 2008). Using Nedd4L C2 domain knock-out mice, we also discovered oral sodium sensitive enhancements of sodium reabsorption via ENaC in the distal nephron (ISHR, 2013). Tubular RAS are regulated independently from systemic RAS and enhanced by sodium dependent manner in mice sodium sensitive model of hypertension (Nephron, 2012). Taken together, we proposed that comprehensive understanding for integrated tubular endocrinogolical models of both tubular RA systems and ENaC-Nedd4L systems is important to regulate pathophysiological basis of sodium sensitivity and hypertension. No COI.

1S3E-4

ENaC-dependent and -independent roles of serine proteases in the aldosterone-induced hypertension and renal fibrosis

Kakizoe, Yutaka; Kitamura, Kenichiro(*Department of Nephrology, Kumamoto University Graduate School of Medical Sciences*)

Aldosterone has an important role in the regulation of blood pressure by modulating the activity of epithelial sodium channel (ENaC) in the kidney. It induces a molecular weight shift of γENaC from 85 to 70 kDa that is necessary for the channel activation, and serine protease prostasin plays a critical role in this shift. A synthetic serine protease inhibitor camostat mesilate (CM), which suppresses the prostasin activity, blocks the proteolytic cleavage of γENaC induced by aldosterone and concomitantly it increases urinary sodium excretion, indicating a natriuretic effect of serine protease inhibitors.On the other hand, recent studies suggested that aldosterone causes kidney injury independent of its hemodynamic effect. However, the precise mechanisms of these direct effects remain to be elucidated. We isolated a serine protease from rat kidney that was activated by co-administration of aldosterone and a high-salt diet. Protein purification and LC-MS/MS analyses revealed this candidate protease as plasmin. Treatment of rat renal fibroblast cells with plasmin increased mRNA expression of profibrotic and inflammatory genes and they were significantly suppressed by CM. Furthermore, CM ameliorated the renal interstitial fibrosis in rats induced by aldosterone and a high-salt diet.Our current studies suggest that serine proteases have pivotal roles in the aldosterone-induced hypertension and renal fibrosis and that serine protease inhibitor could be a new strategy for the treatment of kidney diseases caused by aldosterone in humans. No COI.

Symposium 04
Role of STIM/Orai for physiological function

(March 16, 10:25–12:05, Room E)

1S4E-1

The roles of STIM/Orai and TRPC1/C6 channels in cell cycle progression of bone marrow stromal cells

Ichikawa, Jun; Inoue, Ryuji(*Department of Physiology, Fukuoka University School of Medicine*)

To question how the proliferative potential and cell cycle progression of adult stem cells are controlled is an attractive theme for regenerative medicine and bioengineering. However, the implications of non-voltage-gated Ca^{2+} entry channels therein are poorly elucidated. In this study, we investigated the roles of STIM/Orai and TRPC channels on the cell cycle progression of bone marrow stromal cells (BMSCs) which include mesenchymal stem cells. Cultured BMSCs were synchronized in the G_1, S, G_2 or M phases. In the S phase, the expression levels of STIM, Orai and TRPC1 were significantly enhanced. On the other hand, that of TRPC6 was greatly reduced in the S phase and enhanced in the G_1 phase. Store-operated Ca^{2+} entry (SOCE) was significantly enhanced in the S phase but suppressed in the G_1 phase. Flow cytometric analysis revealed that siRNA knockdown of STIM/Orai or TRPC1/C6 expression differentially affected the distribution of cell cycle stages. To seek a role of TRPC6 channels for cell cycle progression, we evaluated the resting membrane potential (RMP) of cells. siRNA knockdown of TRPC6 produced a significant negative shift of RMP. RMP of the S phase was deeper than of the other cell cycle stages. Chemical depolarization/hyperpolarization clamp experiments abrogated the differences in the magnitude of SOCE between cell cycle stages. From these observations, we suggest that TRPC6-mediated changes in RMP may alter driving force for Ca^{2+} influx at the G_1 and S phase, and modulate SOCE via STIM/Orai and TRPC1. No COI.

1S4E-2

Immune regulation by store-operated calcium entry

Oh-hora, Masatsugu[1]([1]*Division of Molecular Immunology, Medical Institute of Bioregulation, Kyushu University, Fukuoka, Japan,* [2]*Japan Science and Technology Agency (JST), Precursory Research for Embryonic Science and Technology, Fukuoka, Japan*)

T-cell receptor (TCR) signaling determines T cell fate in thymic development. Calcium signaling downstream of TCR plays a crucial role in the development of T cells. Store-operated calcium entry is the primary mechanism for calcium influx in T cells. Although it has been widely assumed that store-operated calcium entry is essential for T cell development in the thymus, there is no direct evidence for this assumption. To address this question, we have analyzed mice lacking STIM1 and STIM2, the two key mediators of store-operated calcium entry. We show here that unexpectedly, lack of store-operated calcium entry results in no effect on positive selection of conventional TCR$\alpha\beta^+$ T cell development and a mild impairment of negative selection. In contrast, store-operated calcium entry specifically controls the development of self-reactive agonist-selected T cells, such as regulatory T cells. Absence of STIM1 and STIM2 lead to a severe impairment of the post-selection proliferation and functional maturation of agonist-selected T cells. This defect was caused by inefficient expression of NFAT target genes including cytokine receptors. Consistently, double deletion of *Nfat1* and *Nfat2* in T cells with Lck-Cre transgenic mice led to a partial but specific reduction in the population of agonist-selected T cells. These findings indicate that STIM-mediated store-operated calcium entry, leading to efficient NFAT activation, is critical for the postselection maturation of agonist-selected T cells. No COI.

1S4E-3

Role of STIM1- and Orai1-mediated Ca^{2+} entry in epidermal keratinocyte physiology

Numaga-Tomita, Takuro[1]([1]*Inst Adv Studies, Kyushu Univ, Fukuoka, Japan,* [2]*NIEHS, NIH, RTP, NC, USA*)

The uppermost thin layer on the surface of the skin, called the epidermis, is responsible for the barrier function of the skin. The epidermis has a multilayered structure in which each layer consists of keratinocytes (KCs) of different differentiation status. Intracellular and extracellular Ca^{2+} is known to play important roles in KC differentiation. However, the molecular mechanisms underlying Ca^{2+} regulation of KC differentiation are still largely unknown. Store-operated Ca^{2+} entry (SOCE) is a major Ca^{2+} influx pathway in most non-excitable cells. In this study, we analyzed the contribution of SOCE to KC growth and differentiation in the human keratinocyte cell line, HaCaT. Knockdown of either STIM1 or Orai1, essential components of SOCE, strongly suppressed SOCE and Ca^{2+}-switch-induced Ca^{2+} responses, resulting in impaired expression of keratin1, an early KC differentiation marker. Furthermore, loss of either STIM1 or Orai1 suppressed normal growth of HaCaT cells in low Ca^{2+} and inhibited the growth arrest in response to a Ca^{2+} switch. These results demonstrate that SOCE plays multiple crucial roles in KC differentiation and function. We have investigated the physiological relevance of STIM1 in vivo with epidermis-specific STIM1 knockout mice (STIM1-epKO). The effect of STIM1 deficiency on skin wound healing was evaluated. Strikingly, STIM1-epKO mice showed faster wound healing compared to WT, which is concomitant with fewer inflammatory infiltrates, suggesting that STIM1 in epidermis is also important for the regulation of humoral factors production upon skin injury. No COI.

1S4E-4

Identification of *Stim1* as a candidate gene responsible for stress-induced hypertension in the stroke-prone spontaneously hypertensive rat

Ohara, Hiroki; Isomura, Minoru; Nabika, Toru(*Department of Functional Pathology, Shimane University School of Medicine, Izumo, Japan*)

The sympathetic nervous system (SNS) plays an important role in regulating the blood pressure (BP) in response to various environmental stimuli. Both in humans and in rodents, exaggerated SNS activity have been implied to be a putative cause of hypertension. Genes influencing sympathetic activity are, therefore, important when considering the genetic susceptibility to hypertension, especially in the context of gene-environmental interaction. The stroke-prone spontaneously hypertensive rat (SHRSP) is well known to have an exaggerated sympathetic response to stress, which may be causally related to its severe hypertension. Recently, based on the results of physiological analysis of congenic strains established between SHRSP and normotensive Wistar-kyoto (WKY) rat, we revealed that a gene (or genes) responsible for exaggerated sympathetic response to stress in SHRSP was located in a 1.2-Mbp fragment on chromosome (Chr) 1. Among the genes located in the Chr1 region, the whole-genome sequence analysis of SHRSP and WKY identified a nonsense mutation in the stromal interaction molecule 1 (*Stim1*) gene of SHRSP. The nonsense mutation found in SHRSP resulted in a truncation of 46 amino acids at the C-terminal end of the rat STIM1 that was indeed shown by western blot analysis. STIM1 plays a key role in store-operated calcium entry (SOCE) process. We hypothesize that abnormal SOCE activity induced by the mutated form of STIM1 results in exaggerated sympathetic response to stress in SHRSP. No COI.

Symposium 05
Challenge of synaptologists into understanding of the structure and function of the neural network

(March 16, 9:00–11:00, Room F)

1S5F-1
Retrograde signaling regulates synapse elimination in developing brain

Uesaka, Naofumi[1]; Uchigashima, Motokazu[3]; Mikuni, Takayasu[1]; Hirai, Hirokazu[2]; Watanabe, Masahiko[3]; Kano, Masanobu[1]([1]*Dept. of Neurophysiol., Grad. Sch. of Med., Univ. Tokyo, Tokyo, Japan,* [2]*Dept. of Neurophysiol., Grad. Sch. of Med., Gunma Univ., Maebashi, Japan,* [3]*Dept Anat., Grad Sch Med, Hokkaido Univ, Sapporo, Japan*)

Neurons form exuberant synapses with target cells early in development. Then, necessary synapses are selectively strengthened whereas unnercessary connections are weakened and eventually eliminated during the course of postnatal development. This process is known as synapse elimination, and is an important step to shape initial redundant neural circuits into functionally mature circuits. Moreover, the disruption is likely linked to mental disorder and brain dysfunction. Climbing fiber (CF) to Purkinje cell (PC) synapses in the cerebellum provide an excellent model to study the process of synapse elimination. Past studies identified several molecules required for the CF-PC synapses elimination. However, we can not still understand the molecular mechanisms of synapses elimination. To efficiently identify molecules required for synapse elimination, we planned the following approach. First, we developed an in vitro culture system which enable us to easily perform genetic manipulations. Second, using the culture system and a lentivirus-mediated knockdown technique, we screened molecules for the CF-PC synapse elimination. Third, we evaluated the function of the screened molecules in vivo. In this presentation, we are going to introduce screened molecules and their role in synapse elimination. No COI.

1S5F-2
Cerebellar ataxia and synaptic abnormality

Hosoi, Nobutake; Hirai, Hirokazu(*Department of Neurophysiology, Gunma University Graduate School of Medicine, Maebashi, Japan*)

Staggerer mutation causes a functional loss of a transcriptional factor, Retinoid-related Orphan Receptor α (RORα), which is abundantly expressed in cerebellar Purkinje cells. The homozygous *staggerer* mutant mice (*sg/sg*) show cerebellar hypoplasia and severe ataxia. The *sg/sg* mice serve as an important extreme mouse model for the herediatary spinocerebellar ataxia type 1 (SCA1), because RORα intereacts indirectly with a molecule called ataxin1, whose polyglutamine expansion leads to SCA1 pathogenesis, and RORα depletion is strongly correlated with severity of the disease. Morphological analyses of *sg/sg* cerebellum have been performed extensively, but functional synaptic abnormalities have not been examined in detail. In this presentation, we show that metabotropic glutamate receptor type 1 (mGluR1) signaling is completely disrupted at parallel fiber-Purkinje cell (PF-PC) synapses in SCA1-related *sg/sg* mice, although AMPA-receptor mediated fast synaptic transmission (fast EPSC) is relatively preserved. *Sg/sg* mice lacked mGluR1-mediated slow synaptic responses completely, and modulation of the fast EPSC caused by mGluR1-mediated endocannabinoid release from PCs was also abolished at *sg/sg* PF-PC synapses. In more realistic disease model mice, SCA1 transgenic mice with prolonged glutamine repeats of the ataxin1 gene, we preliminarily observed impairment of Ca^{2+} signaling mediated by mGluR in PCs. These data indicate that *sg/sg* and SCA1 mice have common deficiency in mGluR1 signaling and we hypothesize that disruption of mGluR1 signaling is a major shared mechanism of cerebellar ataxia between *sg/sg* and SCA1 mice. No COI.

1S5F-3
Regulation of the state of neuronal maturation by excitation/inhibition balance in adult hippocampus

Kobayashi, Katsunori[1,2](*Department of Pharmacology, Nippon Medical School, Tokyo, Japan,* [2]*JST,CREST, Kawaguchi, Japan*)

The aberrant or altered state of neuronal maturation has been implicated in the pathophysiology of psychiatric disorders and cellular mechanisms of antidepressant drugs. We have shown that the serotonergic antidepressant fluoxetine can reverse the state of maturation of the granule cells in the dentate gyrus of the adult mouse hippocampus. This dematuration is induced in a large population of the dentate granule cells and causes significant changes in physiological properties of the granule cells, including enhanced excitatory synaptic input, increased somatic excitability and reduced frequency facilitation at the output synapse. Therefore, the granule cell dematuration is supposed to have a substantial impact on the functioning of the neuronal circuit in the dentate gyrus. We recently found that electroconvulsive stimulation (ECS) can also induce the granule cell dematuration. ECS-induced dematuration can be partially reversed by enhancing synaptic inhibition, suggesting a potential importance of excitation/inhibition in the maintenance of dematured state. Our result suggests that the state of maturation of neurons in the adult brain is dynamically regulated by neuronal activity. The reciprocal relationship between the state of neuronal maturation and circuit functioning may play a critical role in sustained effects of antidepressant treatment on the brain. No COI.

1S5F-4

Distinct pH and buffering capacity between glutamatergic and GABAergic synaptic vesicles in hippocampal neurons

Egashira, Yoshihiro[1]; Takase, Miki[1]; Yanagawa, Yuchio[2]; Takamori, Shigeo[1]([1]*Laboratory of Neural Membrane Biology, Graduate School of Brain Science, Doshisha University, kyoto, Japan,* [2]*Department of Genetic and Behavioral Neuroscience, Gunma University Graduate School of Medicine, Gunma, Japan*)

Exocytotic release of neurotransmitters such as glutamate and GABA, first requires transport of neurotransmitter into synaptic vesicles (SVs), which critically depends on a proton electrochemical gradient generated by the vacuolar H^+-ATPase. Biochemical analyses using isolated SVs have demonstrated that transport of glutamate and GABA exhibits a differential dependence on the pH gradient across the SV membrane. However, whether gradient itself is different between the vesicle populations remains unknown. By using cultured hippocampal neurons derived from the VGAT-Venus transgenic mouse brain and lentivirus expression of synaptophysin-mOrange2 which is sensitive to the luminal pH of the SVs, we found that GABAergic SVs exhibit ~ 0.7 unit higher luminal pH and < 2-fold lower buffering capacity (BC) compared to glutamatergic SVs. These remarkable differences imply distinct susceptibilities of glutamatergic and GABAergic transmission to alterations in pH and BC under pathophysiological conditions. Moreover, since acidification of the synaptic cleft by proton release from SVs has been implicated in modulation of presynaptic calcium channels and ionotropic neurotransmitter receptors, our data demonstrate that glutamatergic SVs can release approximately 5 times more protons than GABAergic SVs, thereby implying more dynamic pH dependent synaptic modulation at glutamatergic synapses. No COI.

Symposium 06
Maintenance of Glucose Homeostasis through Tissue-tissue Interaction

(March 16, 8:40–10:20, Room G)

1S5F-5

Nociceptive Amygdala is Actively Involved in Fear Learning

Watabe, Ayako M; Sato, Masaru; Kato, Fusao(*Dept Neurosci, Jikei Univ Sch Med, Tokyo, Japan*)

Pavlovian fear conditioning is an associative form of learning depending on amygdala synaptic plasticity. The central amygdala (CeA) is known to play pivotal roles in this associative learning (Wilensky et al, 2007; Ciocchi et al, 2010; Li et al., 2013). The capsular part of the CeA, or CeC, receives aversive information from two distinct pathways: direct pathway arising from the dorsal horn via the external part of the lateral parabrachial nucleus (lPB) and indirect pathway from the basolateral nucleus of the amygdala (BLA) that carries highly processed polymodal signals. Such duplex organization would make the CeC strategically well positioned for convergence underlying this associative learning, a concept however remaining hypothetical. Here we provide two sets of evidence indicating that these pathways are integrated at a single neuron level, promoting synaptic plasticity and are involved in fear learning. First, we found that fear conditioning itself potentiates excitatory transmission at both lPB and BLA synapses. Interestingly, the synaptic weights of the two pathways were correlated in fear-conditioned mice, suggesting a heterosynaptic interaction. Second, we found that a transient pharmacological inactivation of the bilateral external lPB during conditioning significantly attenuated fear learning. On the basis of these lines of evidence, we propose that the CeC is actively involved in fear learning via serving as a convergence site for aversive signals of distinct origins and pathways. Synaptic plasticity of these pathways may regulate the sensitivity of emotional circuits to future aversive signals. No COI.

1S6G-1

Dynamic regulation of metabolite flow between adipose tissues and liver by insulin

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Insulin plays a key role in the regulation of carbohydrate and energy homeostasis and insulin resistance is the fundamental pathophysiology of diabetes mellitus. We found that a mouse harboring a loss-of-function mutation in insulin receptor (mIR mouse) develops overt hyperglycemia only under high fat diet (HFD). mIR mice under HFD (mIR/HFD) exhibited severely impaired glucose lowering effect of insulin as assessed by intraperitoneal insulin tolerance test, suggesting that gluconeogensis is increased in liver. As expected, we found that glucose-6-phosphatase (*G6pc*) expression in liver and gluconeogensis from glycerol *in vivo* was increased in mIR/HFD. Glycerol is generated through lipolysis of triacylglycerol in white adipose tissues (WAT) and insulin potently suppresses lipolysis by inactivating phosphorylation of hormone sensitive lipase (HSL). Notably, phospho-HSL levels were significantly increased in WAT of mIR/HFD. In addition, transplantation of wild-type WAT into mIR/HFD markedly ameliorated the hyperglycemia, suggesting that unsuppressed lipolysis in WAT and increased gluconeogensis from glycerol in liver triggers the development of diabetes mellitus. Since insulin is critical in suppressing both lipolysis in WAT and gluconeogensis in liver, defective insulin signaling in mIR mice and consequential increase in glycerol flow from WAT into liver contribute to a rise in blood glucose levels under HFD. Accordingly, maintenance of dynamic flow of metabolites between multiple organs is critical in the maintenance of glucose homeostasis. No COI.

1S6G-2

Role of adipose tissue inflammation in ectopic fat accumulation and insulin resistance

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Obesity may be viewed as a chronic low-grade inflammatory disease as well as a metabolic disease. Indeed, macrophages infiltrate into obese adipose tissue to induce inflammatory pathways, thereby inducing insulin resistance and ectopic fat accumulation. In response to nutritional conditions, lipid metabolism in adipose tissue is tightly regulated by insulin and the sympathetic nervous system. Adipocytes increase their size (hypertrophy) and number (hyperplasia) during the course of obesity to store triglyceride effectively in adipose tissue. When adipose tissue cannot meet the demand of storing excessive energy, triglyceride is accumulated in non-adipose tissues as ectopic fat, which may lead to insulin resistance in the liver and skeletal muscle and insufficient insulin secretion in the pancreas (lipotoxicity). Notably, chronic inflammation is capable of inducing insulin resistance, lipolysis, and interstitial fibrosis in adipose tissue, all of which may reduce the lipid-storing function. Recently, we found that Macrophage-inducible C-type lectin (Mincle), a pathogen sensor for pathogenic fungi and Mycobacterium tuberculosis, plays a critical role in adipose tissue fibrosis, thereby regulating ectopic fat accumulation in the liver. Understanding the molecular mechanism underlying adipose tissue fibrosis may offer a novel therapeutic strategy to prevent or treat obesity-induced metabolic derangement. No COI.

1S6G-3

Central insulin action induces IL-6-mediated suppression of hepatic glucose production

Inoue, Hiroshi; Kimura, Kumi(*Department of physiology and metabolism, Brain/Liver Interface Medicine Research Center, Kanazawa University, Japan*)

Central insulin action is not only involved in the regulation of energy metabolism via the regulation of food intake and heat production, but also engaged in glucose metabolism by regulating hepatic glucose production (HGP). Several papers have demonstrated that hyperpolarization of AgRP neurons induced by the activation of PI-3K signaling/KATP channels in the hypothalamus participates in the suppression of HGP mediated by central insulin action. The central insulin action-mediated suppression of HGP results from decreased gene expression of enzymes involved in hepatic gluconeogenesis, and both increased IL-6 expression in hepatic Kupffer cells induced by central insulin action and associated activation of hepatic STAT3 play an important role in the suppression of gene expression of hepatic gluconeogenesis-related enzymes.

Recently, we found that increased plasma histidine resulted in hepatic STAT3 activation. Intravenous and intracerebroventricular administration of histidine also activated hepatic STAT3 and augmented the suppression of HGP by insulin. The increment in HGP inhibition by histidine was blocked by inhibiting histamine H1 receptors in the central nervous system. Therefore, histidine activates hepatic STAT3 and suppresses hepatic glucose production via central histamine action. These results thus indicate that IL-6-STAT3 signaling in the liver contributes to central action of insulin and histamine, leading to the suppression of HGP. No COI.

1S6G-4

Role of the ventromedial hypothalamus in leptin-induced glucose metabolic regulation in skeletal muscle and the liver

Minokoshi, Yasuhiko; Toda, Chitoku(*Div Endocrinol Metab, Natl Inst Physiol Sci, Okazaki, Japan*)

Leptin is an adipocyte-derived hormone that plays an important role in glucose metabolism in mammals. Treatment with leptin ameliorates diabetes in lipodystrophic mice and humans as well as insulin deficient-diabetes in rodents. We previously showed that injection of leptin into the ventromedial hypothalamus (VMH) increases glucose uptake by skeletal muscle (mainly the red type), brown adipose tissue, and the heart, but not that by white adipose tissue, through activation of the melanocortin receptor (MCR) in the VMH. We now show that signaling by extracellular signal-regulated kinase (ERK) and its upstream kinase MEK in mediates the leptin-induced increase in glucose utilization as well as its insulin sensitivity in the whole body and in red-type skeletal muscle of mice through activation of the MCR in the VMH. In contrast, activation of signal transducer and activator of transcription 3 (STAT3), but not that of the MEK-ERK pathway, in the VMH by leptin enhances the insulin-induced suppression of endogenous glucose production in an MCR-independent manner, with this effect of leptin occurring only in the presence of an increased plasma concentration of insulin. Given that leptin, but not MCR agonist Melanotan-II, requires 6 h to increase muscle glucose uptake, the transient activation of MEK-ERK pathway in the VMH by leptin may play an role in the induction of synaptic plasticity in the VMH, resulting in the enhancement of MCR signaling in the nucleus, and leading to an increase in insulin sensitivity in red-type muscle. No COI.

Symposium 07
Advances in targeted genome editing and its application to physiology

(March 16, 10:25–12:05, Room G)

1S7G-1

Targeted genome editing using highly-active TALENs and CRISPR/Cas9

Yamamoto, Takashi(*Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Higashi-hiroshima, Japan*)

Genome editing with engineered nucleases is an emerging technology that enables manipulation of targeted genes in many organisms and cell lines. To date, two types of engineered nucleases have been developed. Zinc finger nucleases (ZFNs), which first emerged in 1996, have a long and successful history of genome editing. However, the construction of ZFNs is highly laborious and the success rate of active ZFN construction is very low. Transcription activator-like effector nucleases (TALENs), on the other hand, have been recently developed as a more user-friendly engineered nuclease, because TALENs are easy to construct in-house and the target site may be selected in any gene. Using these engineered nucleases, targeted gene disruptions have been achieved successfully in cultured cells and various organisms. Furthermore, gene correction or targeted gene addition have been reported mainly in cultured cells, and also in several organisms (Ochiai et al., 2012). Surprisingly, a third genome editing technology, known as CRISPR/Cas system, suddenly emerged last year and efficient genome editing using this system was reported in ES and iPS cells. In this way, genome editing technology is now becoming the most exciting field in life sciences.In this presentation, I introduce the recent research advances in genome editing using engineered nucleases and CRISPR/Cas9. I show the construction system for highly active TALENs that we named Platinum TALENs and results of genome editing in various organisms using Platinum TALENs. No COI.

1S7G-2

Rapid and highly efficient *in vivo* genome editing in mice

Aida, Tomomi[1]; Sakuma, Tetsushi[3]; Usami, Takako[2]; Ishikubo, Harumi[1]; Imahashi, Risa[1]; Yamamoto, Takashi[3]; Tanaka, Kohichi[1]([1]*Laboratory of Molecular Neuroscience, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan,* [2]*Laboratory of Recombinant Animals, Medical Research Institute, Tokyo Medical and Dental University, Tokyo, Japan,* [3]*Department of Mathematical and Life Sciences, Graduate School of Science, Hiroshima University, Hiroshima, Japan*)

Gene-targeted knockout or knockin mice have dramatically improved our understanding of the functions of genes *in vivo*. However, the generation of targeted mice relies on gene targeting in embryonic stem (ES) cells, which is a time-consuming, laborious, and expensive process. The recent groundbreaking development of genome editing technologies has enabled the targeted alteration of any sequence in any cell or organism. These technologies have now been directly applied to mouse zygotes (*in vivo* genome editing), thereby providing new avenues for simple, convenient, highly efficient and ultra-rapid production of targeted mice without ES cells or targeting vectors. We have generated several knockin mouse lines carrying precisely modified human single nucleotide variants as well as knockout mouse lines by *in vivo* genome editing with highly active Platinum TALENs (transcription activator-like effector nucleases) and oligo DNA donor. Because the efficiency of our method is high, several germline-competent knockout/knockin founders can be obtained within a month by single microinjection. Taken together, *in vivo* genome editing technology revolutionizes mouse gene targeting and provides exciting opportunities for the physiological research to freely generate gene-targeted mice. No COI.

1S7G-3

Optogenetics

Tanaka, Kenji F.(*Department of Neuropsychiatry, School of Medicine, Keio University, Tokyo, Japan*)

Optogenetics has proven to be a powerful tool capable of manipulating the activity of a specific population of cells in a complex multicellular organism. This approach is enthusiastically pursued in recent neuroscience field and the causal relationship between neural activity and behavior is finally starting to become unveiled. However, most studies utilize virus mediated gene transfer for the induction of light-sensitive proteins, such as channelrhodopsin-2 (ChR2), and such method inevitably introduces variability of expression between trials. Therefore, transgenic approach has long been sought, however, satisfying the demands of the specificity as well as the abundance of expression were difficult.Here, we established Knockin-mediated ENhanced Gene Expression by improved tetracycline-controlled gene induction system (KENGE-tet). We found that high levels of tTA-mediated transcription can be achieved by knocking in tetO-ChR2 cassette into a locus at a housekeeping gene, beta-actin. We crossed this tetO-ChR2 knockin mouse with 7 different tTA lines and in all cases the level of ChR2 expression was high enough to allow manipulation of cell activity.This technological improvement, KENGE-tet, enables us to expand the repertoire of optogenetically targeted cells and would provide resources beneficial to the neuroscience field; further generation of new lines of either tetO or tTA line would add even more repertoire for scientists to choose from. We believe that expanding repertoire will be of great interest to the large community of neuroscientists who are actively pursuing the causal relationship between cellular activity and behavior of complex organism. No COI.

1S7G-4

The problems and limitations associated with genetically engineered animals

Tanaka, Kohichi(*Lab of Mol Neurosc, Medical Research Insitute, Tokyo Medical & Dental University (TMDU), Tokyo, Japan*)

Gene modification technologies play a critical role in many fields of research, raging from physiology to neuroscience to genetics. Although genetically engineered mice have become invaluable tools for researchers trying to find out the in vivo functions of individual genes, genetically engineered mice have their limitations. The potential problems and limitations of gene targeting technology are as follows.

About 15% of conventional knockout mice are developmentally lethal.

Knockout mice may not show any observable change.

The genetic background of a knockout strain can have an influence on any observed phenotype.

Some genes, such as genes on Y chromosome, are difficult to knockout.

A complicated compensatory response to the gene's absence can mask the phenotypes of knockout mice.

Conditional gene targeting is an approach to overcoming these difficulties. However, there exist debate over the tissue specificity and the efficacy of conditional knockout mice. No COI.

Symposium 08
Progress of Retinal Research for Neural Circuits

(March 16, 8:40–10:20, Room H1)

1S8H1-1

Neuronal circuit for topographically focal facilitation of visual response in the avian retina

Uchiyama, Hiroyuki[1]; Matsutani, Shinji[2]; Ohno, Hiroshi[1]; Yamaoka, Seiya[1]; Mizokami, Takuya[1]([1]*Dept Informatics & Biomedical Engineering, Grad Sch, Kagoshima Univ,* [2]*Dept Functional Morphology, Sch Nursing, Kitasato Univ*)

Isthmo-optic (IO) neurons in the avian midbrain send their axons topographically to the contralateral retina. Because IO neurons vigorously fire prior to onset of goal-directed head movements in a particular direction, and lesion of those neurons impairs visual target selection for reaching with beak, IO neurons are thought to centrifugally send visual-selection-related signal in order to enhance retinal visual response focally and transiently. The IO neurons make synaptic contacts with a specific class of retinal intrinsic cells, IO target cells (IOTCs). IOTCs have an axon of various lengths that extends horizontally in the junction between the inner nuclear layer (INL) and the inner plexiform layer (IPL), and terminate at stratum 1 of the IPL. Because IOTCs do not contact directly with the retinal ganglion cells (RGCs), some retinal intrinsic cells (specifically amacrine or bipolar cells) must relay the centrifugal signals to RGCs in the IPL. Based on the immunohistochemical examination at the light microscopic level, we suppose that the bipolar cell that is immunoreactive for protein kinase C (PKC-BC) is a probable candidate of the target of IOTCs. Axon terminals of IOTCs appear to terminate mainly on a basal portion of the horizontal axonal branches of PKC-BCs at stratum 1 of the IPL. Thus it is possible that the centrifugal signal may transiently improve synaptic transmission efficacy between PKC-BCs and RGCs, perhaps through a signal transduction cascade within PKC-BCs. No COI.

1S8H1-2

Visualization of the glutamate release in the mouse retina

Ohkuma, Mahito[1]; Horio, Kayo[1]; Kaneda, Makoto[2]; Yosida, Sachiko[3]; Fukuda, Atsuo[4]; Miyachi, Ei-ichi[1]([1]*Dept. of Physiol., Sch. of Med., Fujita Health Univ., Aichi, Japan,* [2]*Dept. of Physiol., Nippon Medical School, Tokyo, Japan,* [3]*Dep. Env. & Life Sci., Toyohashi Univ. Tech., Aichi, Japan,* [4]*Dept. of Neurophysiol., Hamamatsu Univ. Sch. of Med., Shizuoka, Japan*)

Glutamate is a neurotransmitter of photoreceptors and bipolar cells in the retina. Because of the importance of glutamatergic circuits in the retina, many researchers challenged measurement of glutamate release from their axon terminals. Glutamate release from bipolar cells has been well examined by the bioassay system and that from photoreceptors has been detected by the fluometric method. However, detection of glutamate release in these studies has been limited at the single cell level. In the present experiment, we applied the enzyme-linked photo assay technique for slice preparation of the mouse retina to detect glutamate release at the circuit level. In this system, an application of glutamate induced an increase in fluorescent signals, while an application of acetylcholine, GABA or glycine did not. When high-K stimulation was used to evoke glutamate release, two types of increase in fluorescent signals were detected. An increase in fluorescence signal near the outer segment of photoreceptors was glutamate-independent. In the inner retina, an increase in fluorescent signal was glutamate-dependent. Application of 100 μM acetylcholine did not produce any increase in fluorescent signals. The enzyme-linked photo assay technique is useful to measure the glutamate release at the circuit level in the mouse retina. No COI.

1S8H1-3

Pathway-dependent modulation of cholinergic amacrine cells in the mouse retina.

Ishii, Toshiyuki; Kaneda, Makoto(*Dept. Physiol., Nippon Med. Sch., Tokyo, Japan*)

Cholinergic amacrine cells play a pivotal role for the formation of direction selectivity, which is a manifest feature of the dendritic computation in the retina. However, the input-output relationship of the cholinergic amacrine cells has not been elucidated. In this study, we examined how signals of purinergic, glycinergic and glutamatergic circuits are processed in cholinergic amacrine cells in the mouse retina. The purinergic inputs were dominant in the OFF-cholinergic amacrine cells, while the glycinergic inputs were dominant in the ON-cholinergic amacrine cells. Both ON- and OFF-cholinergic amacrine cells received glutamatergic inputs equally. Purinergic inputs were mediated by P2X2-purinoceptors and glycinergic inputs were mediated by, glycine receptors. Glutamatergic inputs were mediated by AMPA/KA and NMDA receptors. In the previous study, we have reported that the modulatory action of firing activity by P2-purinoceptor-mediated signals is distinct between ON- and OFF-ganglion cells. The retina is asked to adjust its visual activity to the wide range of light intensity, i.e. moon light level to shiny seaside level. To achieve this, retina is thought to shift the threshold of ON- or OFF-pathway independently. The presence of pathway-specific inputs to cholinergic amacrine cells might favor for the pathway-specific adjustment of dynamic range to achieve the best visual acuity in the mouse retina. No COI.

1S8H1-4

Coding of unstationary images by retinal ganglion cells

Matsumoto, Akihiro; Tachibana, Masao(*Department of Psychology, Graduate School of Humanities and Sociology, The University of Tokyo, Tokyo, Japan*)

Visual perception is stable. However, retinal images are not stabilized but change position by movements of eyes, head, and body. It is not evident how unstationary retinal images are coded by retinal ganglion cells. Applying the multi-electrode array to the isolated goldfish retina, we recorded spike discharges from multiple ganglion cells. Dynamic light stimuli presented on a CRT display were projected onto a wide area (visual angle: ~ 80 degree) of the retina through optics. A large square target was presented with or without a surrounding background. To mimic the movement of retinal images, the target and the background was jittered or rapidly moved together or independently. Based on the receptive field profile estimated by the spike-triggered average, we classified ganglion cells into six subtypes (Fast/Slow, transient/medium/sustained). We found in some subtypes that the latency of the first spike evoked by the rapidly moving target was shorter when the target moved together with the surrounding background than without background. Especially, the Fast-transient cells fired synchronously before the leading edge of the target reached their receptive fields. Cross-correlation analysis revealed that functional connectivity was newly established among the Fast-transient cells and other specific subtypes under this stimulus condition. These results suggest that eye movements may change the firing properties and functional connectivity of retinal ganglion cells, and that multiple subtypes of retinal ganglion cells may send visual information cooperatively to the brain. No COI.

Symposium 09
Retinal Signaling to the Brain: Modulation by Light, Correlation with Behavior, and Restoration of Vision

(March 16, 10:25–12:05, Room H1)

1S9H1-1

Retinal Ganglion Cell Spikes Are Not All-or-None Events

Ishida, Andrew; Ogata, Genki; Stradleigh, Tyler; Hayashida, Yuki; Oi, Hanako; Partida, Gloria(*NPB-OVS, University of California, Davis*)

Spike frequency and timing are commonly used to measure physiological responses. This treats spikes as digital signals and assumes that stimuli alter only the probability that spikes occur. We have explored other possibilities in the vertebrate retina, where some interneurons release modulatory neurotransmitters (e.g., biogenic amines) and the output neurons (retinal ganglion cells, RGCs) are equipped with a wider variety of ion channels than are needed to generate spikes. We have found that dopamine receptor agonists reduce the amplitude of voltage-gated sodium current in RGCs and that, consistent with this, dopamine alters the amplitude and duration of RGC spikes. We have also found that dopamine activates a mixture of classical and non-classical receptors in RGCs and, consistent with this, that dopamine receptor activation (by full-field illumination) elevates a cyclic nucleotide (cAMP) and activates a calcium/calmodulin-dependent protein kinase (CaMKII). These results are of interest because cAMP regulates some ion channels in RGCs; cAMP fuels a protein kinase (PKA) which modulates other ion channels in RGCs; the cAMP- and PKA-regulated channels gate at hyperpolarized and depolarized membrane potentials, respectively; and illumination induces the appearance of activated CaMKII in RGC nuclei. These results, along with results published by other laboratories, show that that RGC spikes are not all-or-none events and indicate that light may regulate spikes at different membrane potentials and speeds. Some, if not all, of these effects could alter the ability of RGCs to communicate with the brain. No COI.

1S9H1-2

Neural representation of visual information for escape behavior

Ishikane, Hiroshi[1,2](*Department of Psychology, Senshu University, [2]Center for Psychological Science, Institute for the Development of Social Intelligence, Senshu University*)

Recent studies have shown that retinal circuits are involved in more advanced information processing than that previously postulated by vision scientists. Various extracted stimulus features can be represented by the spike trains of retinal ganglion cells, although it is hard to demonstrate if the observed retinal codes are decoded and utilized in the next stage. In frog retina, it has been shown that synchronized oscillations among dimming detectors (class-4 neurons) encode visual information for escape behavior. Pharmacological manipulations of the strength of synchronized oscillations cause changes in the frog's escape behavior. However, it is still unclear what visual features are encoded by retinal oscillations. To elucidate this issue, the stimulus dependence of synchronized oscillations was investigated. The strength of synchronized oscillations depended both on the continuity and the size of visual stimuli. Furthermore, spike discharges were recorded from dimming detectors as well as the other three types of retinal ganglion cells in order to identify the retinal code that triggered the frog's escape behavior. The results of the behavioral and electrophysiological experiments suggested that the activities of class-1 and -2 neurons do not play a functional role in triggering escape behavior. Both synchronized oscillations among dimming detectors and the directional activities of class-3 neurons may encode visual information for escape behavior. No COI.

1S9H1-3

Microstimulation for Visual Prosthetic Interfaces

Hayashida, Yuki; Kameda, Seiji; Ishikawa, Naohiro;
Takeuchi, Kouzou; Tanaka, Hiroki; Okazaki, Yuka; Yagi, Tetsuya
(*Graduate school of Engineering, Osaka University*)

Visual prostheses utilize microstimulations to the visual pathway by which perceptible phosphenes can be induced in blind patients. Previous studies suggested that the number of stimulating electrodes as much as several hundred is required for providing a certain degree of useful visual sensation, although electrical current delivered from each electrode can spatially diffuse in the tissue, activating a mass of neurons at a time. Thus, the cortical-based prostheses can be advantageous because of the relatively large area available for implanting multiple electrodes with enough spacing. Besides the diffusive stimulus current, our experiments with a voltage-sensitive dye imaging in the mouse or rat visual cortex in vivo have shown that neural responses to even single-electrode stimulation significantly varied with the parameters such as pulse amplitude, inter-pulse interval, duty ratio of pulses in a train. Moreover, neural responses induced at spatially separated two stimulation sites can interact nonlinearly with each other. These implied the complex relationship between patterns of electrical stimulation and the resulting neural activities, which have remained largely unrevealed. In order to enable systematic analyses on such a relationship in preclinical animal experiments, we recently developed a CMOS-VLSI chip in which 64 channel current-pulse stimulators were implemented in an implantable size. The current pulses generated by the stimulator we fabricated were able to induce neural responses in the rodent visual cortical areas. Thus, our stimulator chip is expected to be useful for further animal studies, and in turn for optimizing the design of visual prosthetic interfaces. No COI.

Symposium 10
Renal tubular transport function in the 21st century: insights from transgenic and knockout animals

(March 16, 10:05–12:05, Room H2)

1S9H1-4

Application of optogenetic technologies to vision—Restoring vision for blind patients—

Tomina, Hiroshi; Sugano, Eriko; Murayama, Namie; Tabata, Kitako;
Takahashi, Maki; Saito, Takehiko; Nishiyama, Fumiaki;
Tamai, Makoto(*Dept. of Chemistry and Bioengineering, Iwate University*)

Channelrhodopsin 2 (ChR2) derived from the unicellular green algae Chlamydomonas reinhardtii has unique characteristics as a light-activated cation-selective channel. This specific feature allows us to generate photosensitive neurons by the transfer of a single gene, ChR2, to neuronal cells. The discovery of ChR2 and advances in techniques has introduced the field of optogenetics in neuroscience. A new strategy for restoring vision includes the transduction of the ChR2 gene into retinal ganglion cells (RGCs) or ON-bipolar cells in genetically blind mice and rats. These animals are models for retinitis pigmentosa (RP), which is a retinal degenerative disease associated with a loss of photoreceptors, arising from mutations in genes related to the phototransduction pathway in the retina. This progressive photoreceptor degeneration finally leads to blindness. Gene therapy using ChR2 is a potential method for restoring vision to RP patients. However, the visual function of ChR2 lacks sensitivity to wavelengths over 540 nm. VChR1 derived from Volvox cateri can detect longer wavelengths than those for ChR2 and is thus another candidate gene for restoring vision. We generated modified Volvox channelrhodopsin-1 (mVChR1), which is a chimera of Volvox channelrhodopsin-1 and Chlamydomonas channelrhodopsin-1 and demonstrated increased plasma membrane integration and dramatic improvement in its channel properties. We introduce the visual function of blind rats transduced newly developed mVChR1. No COI.

1S10H2-1

Regulation of water and electrolytes transport in kidney tubule cells: Vasopressin V1a receptor and calcium sensing receptor

Yasuoka, Yukiko; Kawahara, Katsumasa(*Dept of Physiology, Kitasato-univ. Sch, of Med, Sagamihara, Japan*)

AQP2 in the principal cells (PC) of kidney collecting ducts (CD) is stimulated by the vasopressin V2 receptor axis and is modified by the V1a receptor axis (Bankir. 2001). Moreover, luminal high [Ca^{2+}] causes downregulation of AQP2 expression in PC (Renkema et al. 2009) through activation of calcium sensing receptor signal pathway (CaSR-Ca^{2+}-PKC axis). Since Yasuoka et al. (2013) found that V1aR only localized in the intercalated cell type A (IC-A) along the CD, we have investigated the amount of AQP2 expression in WT and $V1aR^{-/-}$ mice. The luminal expression of AQP2 significantly increased both in WT and $V1aR^{-/-}$ during dehydration, although their levels of expression significantly decreased in $V1aR^{-/-}$ vs control. This suggests that V1aR in IC-A maintains and stimulates the expression of AQP2 through unknown cellular pathways. On the other hands, we found that CaSR, genetically same as CaSR in TAL, only localized in the basolateral membrane of intercalated cell type B (IC-B) through the CD (Yasuoka et al. 2011). We investigated the urine concentration and body pH metabolism in mice fed calcium and acid/base diets. The levels of the CaSR mRNA and protein expression in IC-B were increased and decreased, respectively, during alkali- and acid-loading. CaSR of IC-B may contribute to alkali secretion to prevent urolithiasis caused by hypercalciuria. V1aR and CaSR, respectively, may contribute to water and electrolytes transport by maintaining the basal expression of AQP2 and by stimulating alkali secretion in kidney collecting ducts. No COI.

1S10H2-2

Analysis of urate transporter gene-overexpressed mice for the study of renal tubular epithelial transport of urate

Anzai, Naohiko[1]; Jutabha, Promsuk[1]; Kimura, Toru[2]; Fukutomi, Toshiyuki[2]([1]*Department of Pharmacology and Toxicology, Dokkyo Medical University School of Medicine, Tochigi, Japan,* [2]*Department of Pharmacology, Kyorin University School of Medicine, Tokyo, Japan*)

Urate, the ionized form of uric acid, is a breakdown product of ingested and endogenously synthesized purine nucleotides in humans. Renal urate handling plays an important role in the determination of serum urate level. We have identified a urate-anion exchanger, URAT1, localized at the apical side and a voltage-driven urate efflux transporter, URATv1, expressed at the basolateral side of renal proximal tubules. Both URAT1 and URATv1 are fundamental to renal urate reabsorption because the loss-of-function mutations of these transporters cause hypouricemia. Since recent genome-wide association (GWA) studies have revealed close associations between serum urate levels and single nucleotide polymorphisms (SNPs) mainly in two transporter-related genes such as SLC2A9 (GLUT9/URATv1), and SLC22A12 (URAT1), we have constructed kidney-specific transgenic (Tg) mice for URAT1 or URATv1. Each transgene was under the control of the mouse Urat1 promoter so that transgene expression was directed to the kidney. Plasma urate concentrations in URAT1 and URATv1 Tg mice were not significantly different from that in wild-type (WT). Urate excretion in URAT1 Tg mice was similar to that in WT, while URATv1 Tg mice excreted more urate compared with WT. Our results suggest that increased URATv1 function in the kidneys can lead to increased urate reabsorption and may contribute to the development of hyperuricemia. No COI.

1S10H2-3

CARS microcopy and high-speed AFM: two emerging measurement techniques useful for epithelial transport physiology

Sohma, Yoshiro; Yamashita, Hayato; Yu, Ying-Chun; Yasui, Masato(*Department of Pharmacology, Keio University School of Medicine, Tokyo, Japan*)

Recent great advances in the nonlinear optical science and the nanotechnology give us novel kinds of information in the biomedical science field. In this symposium, we will introduce our challenges for the application of two emerging measurement techniques, Coherent Anti-stokes Raman Scattering (CARS) microcopy and High-Speed Atomic Force Microscopy (HS-AFM), to the epithelial transport physiology. The CARS laser-scanning microscopy gives us H_2O images in cells and tissues from the O-H stretch vibration-specific scattering. Applying this technique to 3D cysts formed by MDCK cells, we succeeded to make a direct observation of H_2O/D_2O exchange process across the MDCK epithelium and determined the diffusional water permeability of luminal and basolateral membranes by analyzing the time-lapse image data with a computer simulation model.

The HS-AFM has enabled, for the first time, direct visualization of dynamic structural changes and dynamic interactions occurring in individual biological molecules in a solution, which has been impossible with other techniques. The HS-AFM succeeded to image dynamic structural changes in membrane proteins including CFTR channels and also visualize the binding/unbinding process of autoantibodies to human aquaporins.

We expect that these two techniques will take a new turn in the epithelial transport physiology. No COI.

1S10H2-4

An attempt to create xenotransplantation-competent cloned piglets by the next-generation KO system CRISPR/Cas9

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The recent development of the CRISPR/Cas9 system has enabled genome editing of mammalian genomes; however, its applicability and efficiency in the pig have not been studied. Here, using this system, we aimed to destroy the function of the porcine α-1,3-galactosyltransferase (α-GalT) gene (*GGTA1*) whose product is responsible for the synthesis of the α-Gal epitope, a causative agent for hyperacute rejection upon pig-to-human xenotransplantation. Porcine embryonic fibroblasts were transfected with a Cas9 expression vector and guide RNA specifically designed to target *GGTA1*. After transfection, the cells were incubated with IB4 conjugated with saporin (IB4SAP), which eliminates α-Gal epitope-expressing cells. Almost all surviving colonies were negative for staining with IB4. Sequencing of the mutated portion of *GGTA1* revealed a frameshift of the α-GalT protein. Porcine blastocysts derived from the nuclear transfer of these α-Gal epitope-negative cells also lacked the α-Gal epitope on their surface. These results demonstrated that the CRISPR/Cas9 system can efficiently induce the biallelic conversion of *GGTA1* in the resulting somatic cells, and is thus a promising tool for the creation of KO cloned piglets. No COI.

Symposium 11
The time in physiology: Arrow of time and Biological rhythms

(March 16, 9:00–11:00, Room J)

1S11J-1

Misregulation of transcriptional program controlling the cellular differentiation disrupts the circadian clock development

Yagita, Kazuhiro(*Department of Physiology and Systems Bioscience, Kyoto Prefectural University of Medicine*)

In mammals, various physiological aspects are controlled by an internal time-keeping system called circadian clock. In vivo, the development of the circadian clock starts during embryonic stages. In vitro, the circadian clock formation occurs cell-autonomously in cultured mouse ES cells under differentiating conditions. Conversely, reprogramming clock oscillating somatic cells results in the disappearance of circadian molecular rhythms. These findings raise the hypothesis that the development of the cellular circadian clock closely correlates with cellular differentiation mechanisms. To address this issue, we here investigated the circadian clock in human Neuroblastoma cells, derived from an embryonic tumor with abnormal cellular differentiation, due to high-level expression of N-MYC. The circadian clock reporter monitoring assay revealed abolished circadian clock oscillations in individual neuroblastoma cells. Next, we established mouse ES cell lines exhibiting abnormal cellular differentiation because of altered transcriptional program through doxycyclin-inducible c-myc expression. Constitutive expression of c-myc resulted in the disruption of circadian clock development even after in vitro differentiation culture. Interestingly, all essential clock genes expressed in these cells without showing circadian cycle. Altogether, our data suggested that the misregulation of transcriptional program controlling cellular differentiation, rather than direct effect of MYC, might disrupt the cellular circadian clock development. No COI.

1S11J-2

Toward a comprehensive analysis of Ataxin-2 funtions for the understanding of a common mechanism underlying neurodegeneration

Kawahara, Yukio(*Laboratory of RNA Function, Graduate School of Medicine, Osaka University*)

Spinocerebellar ataxia type 2 is an autosomal dominant neurodegenerative disease. The disorder is caused by abnormal CAG repeat expansion in the coding region of the Ataxin-2 gene (ATXN2). Recently, moderate CAG repeat expansion in ATXN2 was identified as a risk factor for amyotrophic lateral sclerosis. This finding suggests a common pathogenic role of Ataxin-2 in these neurodegenerative diseases. Therefore, a comprehensive understanding of the physiological functions of Ataxin-2, especially related to disease pathogenesis, is necessary to elucidate the mechanism underlying Ataxin-2-mediated neurodegeneration. Ataxin-2 is known to directly bind to polyadenylate-binding protein 1 (PABPC1), which suggests that Ataxin-2 is likely involved in RNA metabolism. However, the functions of Ataxin-2 have not been characterized in detail. We have recently found that Ataxin-2 binds to some class of RNAs directly in a PABPC1-independent manner. Through deep sequencing analysis, we have elucidated that Ataxin-2 recognizes some distinct motifs situated in 3' untranslated region of mRNAs. In addition, we would like to show that Ataxin-2 promotes the translation of mRNA through the increase of RNA stability. Interaction with PABPC1 partially contributes to the promotion of translation; however, we identified a previously uncharacterized domain that is indispensable for this function. Furthermore, abnormal expansion of the polyglutamine stretch in Ataxin-2 significantly reduced the efficiency of translation, which may contribute to the pathogenesis of neurodegenerative diseases. No COI.

1S11J-3

Hypothalamic Sirt1 and energy balance regulation

Sasaki, Tsutomu; Kitamura, Tadahiro(*Laboratory of Metabolic Signals, Instituite for Molecular and Cellular Regulation, Gunma University, Maebashi, Japan*)

Obesity is associated with aging and increased caloric intake, while caloric restriction improves health and longevity in multiple organisms; the NAD^+-dependent deacetylase Sirt1 is implicated in this process. Proopiomelanocortin (POMC) and agouti-related peptide (AgRP) neurons in the arcuate nucleus of the hypothalamus (ARC) are critical for energy balance regulation, and Sirt1 protein level decreases with age in the ARC. Conditional Sirt1 overexpression in mouse POMC or AgRP neurons prevented age-associated weight gain; overexpression in POMC neurons stimulated energy expenditure via increased sympathetic activity in adipose tissue, whereas overexpression in AgRP neurons suppresses food intake. However, these phenotypes were absent in mice consuming a high-fat, high-sucrose diet due to decreases in ARC Sirt1 protein and hypothalamic NAD+ levels. Meanwhile, Sirt1 in the hypothalamus is regulated by nutrient/metabolic signals in a way different from the peripheral tissues. Hypothalamic Sirt1 protein level is decreased upon energy deficit and the ubiquitin/proteasome systems is involved in this process. The regulation of hypothalamic Sirt1 is disturbed by diet induced obesity. As an effort to elucidate the mechanisms for the dynamic regulation of energy homeostasis and how the disturbance of the system contributes to the pathophysiology of obesity, I will discuss how hypothalamic Sirt1 regulates energy balance through POMC and AgRP neurons, and how metabolic signals reflecting energy balance status of the body feed back to the hypothalamic Sirt1 and regulate it. No COI.

1S11J-4

Regulatory mechanism of wakefulness/non-REM sleep/REM sleep by the hypothalamic neurons

Yanamaka, Akihiro; Tsunematsu, Tomomi(*Department of Neuroscience II, Research Institute of Environmental Medicine, Nagoya University*)

Melanin-concentrating hormone (MCH) is a neuropeptide produced in neurons sparsely distributed in the lateral hypothalamic area. Recent studies have reported that MCH neurons are active during rapid eye movement (REM) sleep, but their physiological role in the regulation of sleep/wakefulness is not fully understood. To determine the physiological role of MCH neurons, newly developed transgenic mouse strains that enabled manipulation of the activity and fate of MCH neurons in vivo were generated using the recently developed KENGE-tet system. The activity of these cells was controlled by optogenetics by expressing channelrhodopsin2 (E123T/T159C) or Archaerhodopsin T in MCH neurons. Acute optogenetic activation of MCH neurons at 10Hz induced transitions from non-REM (NREM) to REM sleep and increased REM sleep time in conjunction with decreased NREM sleep. Activation of MCH neurons while mice were in NREM sleep induced REM sleep, but activation during wakefulness was ineffective. Acute optogenetic silencing of MCH neurons using ArchaerhodopsinT had no effect on any vigilance states. Temporally-controlled ablation of MCH neurons by cell-specific expression of diphtheria toxin A increased wakefulness and decreased NREM sleep duration without affecting REM sleep. Taken together, these results indicate that acute activation of MCH neurons is sufficient, but not necessary, to trigger the transition from NREM to REM sleep and that MCH neurons also play a role in the initiation and maintenance of NREM sleep. No COI.

1S11J-5

The circadian timing regulation of food intake

Nakamura, Wataru[1]([1]*Laboratory of Oral Chronobiology, Graduate School of Dentistry, Osaka University, Osaka, Japan,* [2]*JST, PRESTO, Saitama, Japan*)

An important aspect of daily homeostasis is the timing of daily rhythms in rest and activity, feeding behavior, energy utilization, and energy storage across the daily 24h cycles. The timing of food intake shows pronounced diurnal rhythm, most of which occur during the active phase of animals. The neural circadian clock located within the hypothalamic suprachiasmatic nucleus (SCN) orchestrates 24h cycles. Because restriction of food availability to a certain time of day elicits anticipatory behavior even after ablation of the SCN, such behavior has been assumed to be under the control of another circadian oscillator. In the present study, mice with SCN lesions or with mutant circadian periods were exposed to restricted feeding schedules at periods within and outside circadian range. Periodic feeding led to the entrainment of FAA rhythms only within a limited circadian range. *Cry1*$^{-/-}$ mice, which are known to be a short-period mutant, entrained to a shorter period of feeding cycles than did *Cry2*$^{-/-}$ mice. This result indicated that the intrinsic periods of FAA rhythms are also affected by *Cry* deficiency. *Bmal1*$^{-/-}$ mice, deficient in another essential element of the molecular clock machinery, exhibited a pre-feeding increase of activity far from circadian range, indicating a deficit in circadian oscillation. We propose that mice possess a food-entrainable pacemaker outside the SCN in which canonical clock genes such as *Cry1*, *Cry2* and *Bmal1* play essential roles in regulating FAA in a circadian oscillatory manner. No COI.

1S12K-1

Regulation of thick and thin filaments organization by smooth muscle myosin

Watanabe, Masaru[1]; Ishida, Yukisato[2]; Nakahara, Naoya[3]; Taguchi, Mika[3]; Kimura, Masako[3,4]; Takemori, Shigeru[3](*Graduate School of Human Health Sciences, Tokyo Metropolitan University, Tokyo, Japan,* [2]*Bunkyo Gakuin University,* [3]*The Jikei University School of Medicine,* [4]*Kagawa Education Institute of Nutrition*)

Thick (myosin) filaments are known to exist in the living smooth muscle even when regulatory light chain of myosin (MLC_{20}) is dephosphorylated. However, several groups including us have presented that organization of thick and thin filaments in the vertebrate smooth muscle was more labile than that in the skeletal muscle. We found that blebbistatin, a potent inhibitor of myosin II, disrupted organization of the thick filaments in the skinned (cell membrane permeabilized) smooth muscle from guinea pig taenia cecum (Watanabe et al., 2010). X-ray diffraction studies on the skinned taenia cecum in the resting state, showed that blebbistatin induced decrease in the intensity of both meridional reflection 14.4 nm arisen from the arrangement of myosin molecules in the thick filaments and diffuse equatorial peak 1/11.4 nm^{-1} originated from lattice-like arrangement of the thin filaments. These results indicate that functional changes in smooth muscle myosin molecules regulate the organization of thick-filaments, resulting in the alteration of thin-filaments structure and/or organization even in the absence of cross-bridge formation. No COI.

Symposium 12
Smooth muscle myosin; novel regulatory mechanisms in smooth muscle contraction

(March 16, 8:40–10:20, Room K)

1S12K-2

Smooth muscle myosin: Novel roles in smooth muscle contraction

SATO, OSAMU; Ikebe, Mitsuo(*Department of Cellular and Molecular Biology, University of Texas Health Science Center, Tyler, Texas, USA*)

Myosins are known as molecular motors that convert the energy of ATP hydrolysis to the mechanical force to move along actin filaments and diversified in more than 20 different classes, thus constituting a superfamily. It is known that smooth muscle myosin is classified into type 2 myosin in its superfamily. While myosin II is called "conventional myosin", the properties of myosin II are quite unique to produce force. Myosin II is a low duty ratio motor, which is critical for myosin II to function as a force producing motor protein. Another critical issue of myosin II is the large thick filament formation, which enables myosin II to reside in actin filaments during the power stroke cycle. Thus, myosin II plays roles in muscle contraction, cell motility, cell formation, maintenance of cell structure, and so on. It has been widely accepted that smooth muscle myosin is regulated by phosphorylation of its regulatory light chain (RLC). However, it is still controversial how smooth muscle myosin molecule is regulated by phosphorylation. While single-headed smooth muscle myosin has been considered to be fully active regardless of RLC phosphorylation, current study proposed that the single-headed myosin is neither fully active nor fully inhibited. Recent studies have also shown how RLC kinase causes phosphorylation of RLC, and myosin phosphatase reverses the phosphorylation level. It also remains to be established whether the formation of smooth muscle thick filaments is regulated in situ. I will address those issues from biochemical aspects in this symposium. No COI.

1S12K-3

Myosin phosphorylation dependent and independent smooth muscle contraction

Takeya, Kosuke; Takai, Akira(*Dept. of Physiology, Asahikawa Med. Univ., Asahikawa, Japan*)

Signal transduction in the regulation of smooth muscle contraction has been studied by using physiological, pharmacological and biochemical techniques. In pharmacological study, specific and selective drugs are not always available, and complex results lead to controversial interpretation. Biochemical analysis can present direct evidence at molecular level (e.g. phosphorylation) to support the pharmacological interpretation. However, due to the lack of sensitivity, the application of biochemical techniques has been limited to relatively large smooth muscle tissues. We have overcome this limitation by introducing phostag methods, and measured myosin phosphorylation in small smooth muscles. We have found, in some tissues, signal pathways proposed by pharmacological studies were not consistent with the biochemical changes. For example, pharmacological study showed PKC inhibition in rat cerebral artery (RCA) resulted in attenuation of myogenic response, suggesting the involvement of CPI-17 phosphorylation in the myogenic response. However, we could not find any change in CPI-17 phosphorylation during myogenic response. Moreover, myosin phosphorylation did not decrease in the RCA dilated by PKC inhibitor. These results suggest the existence of unknown phosphorylation-independent pathways in myogenic response. Another example of phosphorylation independence is ciliary muscle. Ciliary muscle contraction requires increase in intracellular Ca^{2+}. However, we could not find significant change in myosin phosphorylation during contraction, suggesting the involvement of phosphorylation-independent mechanisms. No COI.

Symposium 13
New aspects of the hierarchical study on function and morphology of epithelial membrane
[Collaboration Symposium with The Membrane Society of Japan]

(March 16, 10:25–12:05, Room K)

1S12K-4

The role of direct phosphorylation of myosin light chain by Rho-kinase in Ca^{2+}-sensitization of smooth muscle contraction

Kobayashi, Sei(*Department of Molecular Physiology and Medical Bioregulation, Yamaguchi University Graduate School of Medicine, Ube, Japan*)

Rho-kinase (ROK) plays a central role in Ca^{2+}-sensitization of vascular smooth muscle (VSM) contraction leading to abnormal VSM contraction such as vasospasm. The ROK-mediated elevation of myosin light chain (MLC) phosphorylation has been well documented, and in vitro studies showed that 1) ROK indirectly increases MLC phosphorylation through the inhibition of MLC phosphatase, and 2) ROK directly phosphorylates MLC. However, the latter possibility has been excluded without any strong evidence and the former mechanism is regarded as sole pathway for Ca^{2+}-sensitization. In this study we tested the latter possibility, using in vitro motility assay with highly purified contractile proteins of smooth muscle, but in the complete absence of the MLC kinase (MLCK) and phosphatase, both of which are essential for the former mechanism. Smooth muscle myosins were purified as recombinant proteins in a baculovirus expression system and confirmed by MALDI-TOF MS. The myosin MLC was phosphorylated by either ROK or MLCK. The in vitro motility assay revealed that average velocity of actin sliding on ROK-phosphorylated myosin was 30 times faster than that on unphosphorylated MLC and was comparable to the maximum value obtained with MLCK-phosphorylated myosin. Finally, in the permeabilized VSM constitutively active ROK induced large Ca^{2+}-sensitization and MLC phosphorylation in the absence of cytosolic Ca^{2+} and MLCK activity. These results suggest that direct phosphorylation of MLC by ROK is alone sufficient for the Ca^{2+}-sensitization. No COI.

1S13K-1

Pathophysiological roles of an actin-binding protein, ezrin in epithelial tissues.

Asano, Shinji; Hatano, Ryo; Matsumoto, Yosuke; Yoshida, Saori (*College of Pharmaceutical Sciences, Ritsumeikan University, Kusatsu, Japan*)

Ezrin is an actin-binding protein, which cross-links membrane proteins and F-actin directly or indirectly through scaffold proteins. It is concentrated on apical surface of many epithelial cells especially in small intestines, stomachs, and kidneys. Tamura *et al.* prepared ezrin knockdown (*Vil2*$^{kd/kd}$) mice in which expression level of ezrin was decreased to less than 10% compared with wild-type. Here, we introduce the phenotypes of these mice in epithelial tissues. In stomachs, ezrin is expressed on apical canalicular membrane of parietal cells. The *Vil2*$^{kd/kd}$ mice showed achlorhydria due to impairment of membrane fusion of tubulovesicles with apical membrane. They showed foveolar hyperplasia and atrophy in the fundic glands with the number of parietal and chief cells being decreased, and the number of neck cells being increased. The neck cells expressed spasmolytic polypeptide (SP), representing SP-expressing metaplasia, SPEM. Therefore, ezrin is involved not only in gastric acid secretion but also in architecture and development of gastric glandular epithelia. In the kidney, ezrin is located at the brush border membrane of proximal tubules where it interacts with a Na^+/phosphate cotransporter, Npt2a, through a scaffolding protein, NHERF1. The *Vil2*$^{kd/kd}$ mice exhibited hypophosphatemia, osteomalacia, and urinary loss of phosphate. The Npt2a and NHERF1 expressions at brush border membrane were reduced in the *Vil2*$^{kd/kd}$ mice. These results suggest that ezrin is involved in cell surface expression of Npt2a, and required for the regulation of systemic P_i homeostasis. No COI.

1S13K-2

Mechanisms for ABCB4- and PEMT-mediated resistance of hepatocytes against bile salts

Morita, Shin-ya(*Department of Pharmacy, Shiga University of Medical Science Hospital, Otsu City, Shiga, Japan*)

Bile salts have potent detergent properties and damage hepatocytes by affecting the integrity of cellular membranes, which are responsible for cholestasis and hepatocellular necrosis. However, it is not fully understood how the canalicular membrane of hepatocytes acquire the resistance against bile salts.

ABCB4, a member of the ABC transporter family, is present in the canalicular membranes of hepatocytes. Lipid rafts are plasma membrane microdomains containing high levels of sphingomyelin (SM) and cholesterol. We showed that ABCB4 was localized mainly to nonraft membranes, and that taurocholate significantly stimulated the ABCB4-mediated efflux of phosphatidylcholine (PC), phosphatidylethanolamine (PE) and SM, while the efflux of PE and SM was much less than that of PC. The biliary phospholipid secretion protects the canalicular membranes of hepatocytes against bile salts.

PE N-methyltransferase (PEMT) converts PE to PC in the liver. We demonstrated that PEMT expression elevated the levels of PC species containing longer polyunsaturated acyl chains, increased the microvillus diameter, and resulted in increased resistance to conjugated bile salts.

These findings suggest that ABCB4 and PEMT play a crucial role in the resistance of hepatocytes against bile salt-induced cytotoxicity and in cholestasis and hepatocellular injury. No COI.

Ref. [1] Morita et al. (2007) Hepatology, 46, 188–199. [2] Morita et al. (2010) Biochem. J., 432, 387–398. [3] Morita et al. (2011) FEBS J., 278, 4768–4781. [4] Morita et al. (2013) J. Lipid Res., 54, 1221–1230.

1S13K-3

Regulatory mechanism of Na,K-ATPase function in plasma membrane microdomains

Sakai, Hideki; Fujii, Takuto; Shimizu, Takahiro(*Department of Pharmaceutical Physiology, University of Toyama, Toyama, Japan*)

Lipid rafts and caveolae are glycosphingolipid- and cholesterol-enriched microdomains and are thought to be the functional domains involved in membrane trafficking and signal transduction. Here, we will talk about our recent findings about novel functions of Na,K-ATPase in the plasma membrane microdomains. In gastric parietal cells, we found that Na,K-ATPase and K,Cl-cotransporter 3a (KCC3a) were coimmunoprecipitated, and that both of them were highly localized in a lipid raft fraction. The Na,K-ATPase activity was significantly inhibited by a KCC inhibitor. The stable exogenous expression of KCC3a in LLC-PK1 cells resulted in association of KCC3a with Na,K-ATPase and it recruited Na,K-ATPase in lipid rafts, accompanying an increase in Na,K-ATPase activity. We suggest that KCC3a forms a functional complex with Na,K-ATPase in lipid rafts of the cells. In caveolae of LLC-PK1 cells, it has been reported that non-pumping Na,K-ATPase but not pumping ATPase is predominantly localized. In human cancer cells, we found that disruption of caveolae by methylβ-cyclodextrin (MβCD) significantly inhibited the activation of volume-sensitive outwardly rectitfying anion channels (VSOR) induced by low concentration of ouabain (100 nM). MβCD also inhibited the ouabain-induced mitochondrial dysfunction and decrease in cell proliferation. Our results suggest that binding of ouabain to non-pumping Na,K-ATPase in caveolae upregulates the VSOR activity and inhibits the cancer cell proliferation. No COI.

1S13K-4

Molecular mechanism of regulation of epithelial Na+ channel (ENaC) activity at the plasma membrane - Roles of raft domain

Yokoyama, Noriko[1]; Marunaka, Yoshinori[1,2,3](*[1]Department of Molecular Cell Physiology, Kyoto Prefectural University of Medicine Graduate School of Medical Science, [2]Department of Bio-Ionomics, Kyoto Prefectural University of Medicine Graduate School of Medical Science, [3]Japan Inst for Food Education & Health, St. Agnes Univ.*)

ENaC expressed in the apical membrane of sodium-transporting epithelial cells plays a critical role in homeostasis of fluid content and blood pressure. The amount of ENaC-mediated Na^+ transport depends on the open probability of the channel and the number of channel expressed on the apical membrane. ENaC is cleaved by aldosterone-induced proteases. A releasing inhibitory tract from the extracellular loop of ENaC resulted in an increase in open probability. The number of ENaC located at the apical membrane is regulated by ubiquitin ligase (Nedd4-2). In this study we quantified the abundance and localization of ENaC at the plasma membrane with/without aldosterone stimulation in renal epithelial cells. Aldosterone stimulated expression of both full-length and cleaved ENaCs; i.e., the full-length ENaC was expressed in both cytoplasm and plasma membrane (raft domain), whereas a large part of the cleaved ENaC was observed at the non-raft plasma membrane. Depletion of cholesterol from the raft domain diminished the movement of ENaC by aldosterone stimulation. Aldosterone also increased expression of Nedd4-2 and SGK1 in raft domain, suggesting that the ubiquitination-mediated regulation of ENaC lifetime would occur in raft domain. Based on our findings, we propose novel roles of the lipid raft domain on the regulation of ENaC activity/trafficking. No COI.

Symposium 14
Neurobiology of glutamate synaps

(March 16, 15:05–17:05, Room D1)

1S14D1-1

Physiological importance of the rate of synaptic vesicle filling for maintaining fast synaptic transmission in the mammalian CNS

Hori, Tetsuya[1,2]; Takahashi, Tomoyuki[1,2]([1]*Cellular and Molecular Synaptic Function Unit, Okinawa Institute of Science and Technology Graduate University, Okinawa, Japan,* [2]*Laboratory of Molecular Synaptic Function, Graduate School of Brain Sciences, Doshisha University*)

At the presynaptic terminal, after exocytosis of synaptic vesicles, fused vesicle membranes are re-internalized by endocytosis, and synaptic vesicles are refilled with neurotransmitter and recycled for reuse. At glutamatergic presynaptic terminals, vesicles are refilled with glutamate by vesicular glutamate transporters by using a H^+ electrochemical gradient produced by the H^+-ATPase. As we have previously reported, vesicle refilling with glutamate has a mean time constant of 15 s, and this time constant varies depending upon temperature, developmental stage of animals, and presynaptic Cl^- concentrations ($[Cl^-]$). In isolated vesicles, magnitudes of glutamate uptake depend on extra-vesicular $[Cl^-]$ concentrations, with less uptake both at low and high Cl^- concentrations (Naito & Ueda, 1985). Likewise, at the calyx of Held glutamatergic presynaptic terminals, vesicles are optimally refilled at 30mM $[Cl^-]$, but refilling is less efficient at lower or higher $[Cl^-]$ (Hori & Takahashi, 2012). Here, at the calyx of Held-MNTB synapse, we asked whether the slowing of the glutamate refilling rate by Cl^- would affect fast synaptic transmission during high frequency firing. My presentation highlights the importance of vesicle filling rate for maintaining the fidelity of postsynaptic firing in the mammalian CNS. No COI.

1S14D1-2

Imaging of glutamate release at synapses

Hirose, Kenzo(*Department of Neurobiology, Graduate School of Medicine, the University of Tokyo, Tokyo, Japan*)

Glutamatergic synapses play essential roles in brain functions. To study properties of glutamatergic synapses, we have developed fluorescent glutamate probes named EOS probes. EOS probe is a hybrid type probe which consists of a synthetic fluorescent dye and a glutamate binding protein derived from AMPA receptor subunit, GluA2. EOS when applied in cultured hippocampal neurons visualized glutamate release from glutamatergic synapses at a single synapse resolution. From quantal analysis for the glutamate release data, we found that each synapse has multiple release sites. The number of release sites (N) was found to be heterogeneous among synapses. We propose that the heterogeneity in N provides unique weights in synaptic computation. No COI.

1S14D1-3

Roles of postsynaptic density in spatiotemporal control of glutamate receptors

Suzuki, Etsuko[1,2]; Kamiya, Haruyuki[1]([1]*Department of Neurobiology, Graduate School of Medicine, Hokkaido University,* [2]*JSPS*)

Spatial and temporal distribution of synaptic glutamate receptors are controlled by multiplex molecular interactions between glutamate receptor subunits and postsynaptic scaffolds. There is accumulating evidence showing that PSD95, one of the major proteins in a macromolecular complex of the postsynaptic density, play a key role in controlling subcellular localization of synaptic glutamate receptors. In this symposium, we will introduce our recent attempts to identifying the roles of PSD95 in synaptic AMPA receptor dynamics in the hippocampal CA1 synapses, as well as in specific localization of synaptic kainate receptors at the mossy fiber-CA3 synapses. Recently we developed a photochemical inactivation technique, using a photoreactive AMPA receptor blocker ANQX, to monitor temporal dynamics of synaptic AMPA receptors. We applied this method to hippocampal slices obtained from knockout mice of PSD95. Our result supports the notion that PSD95 limit mobility and trafficking of AMPA receptors. Since PSD95 also have shown to bind directly to kainate receptor subunits, we also examined changes in kainate receptor-mediated EPSCs in PSD95 knockout mice. Stimulation of hippocampal mossy fibers elicited mixed EPSCs composed of both AMPA receptor-mediated and much slower kainate receptor-mediated components in CA3 neurons. Preliminary results have suggested that the relative contribution of the two components was affected in PSD95 knock out. We will discuss about our model of the roles of PSD95 in the spatially restricted distribution of kainate receptors on the mossy fiber-CA3 synapses. No COI.

1S14D1-4

The C1q complement family complements glutamatergic synapses on cerebellar Purkinje cells.

Kakegawa, Wataru[1,2]; Yuzaki, Michisuke[1,2](*Department of Physiology, Keio University Schook of Medicine, Tokyo, Japan,* [2]*JST-CREST, Saitama, Japan*)

Cerebellar Purkinje cells receive two major glutamatergic inputs, parallel fibers from granule cells (PF synapses) and climbing fibers from inferior olive (CF synapses). Cbln1, a member of C1q complement family characterized by a conserved global C1q domain, is highly expressed in granule cells and released from PF terminals to regulate PF synapse formation and long-term depression (LTD), a synaptic plasticity underlying cerebellar motor learning (Hirai et al., *Nat Neurosci*, '05; Matsuda et al., *Science*, '10). Recently, we identified a novel C1q-family protein, C1q-like protein 1 (C1qL1), which is selectively expressed in inferior olive (Iijima et al., *Eur J Neurosci*, '10). In this study, to examine whether C1qL1 controls CF synapse formation and functions, we analyzed a mutant mouse lacking C1qL1 gene (C1qL1 KO mouse). Immunohistochemistry using anti-C1qL1 antibodies revealed that C1qL1 proteins were restricted on CF synapses in wild-type (WT) mice. Interestingly, CFs of C1qL1 KO mice were severely retracted compared to WT and had a decreased number of vesicular glutamate transporter II, a presynaptic marker of CF synapses. C1qL1 KO mice also showed significantly small excitatory postsynaptic currents at CF synapses with several steps of amplitudes, reflecting a multiple CF innervation, a characteristic of immature types of CFs in WT. Furthermore, these KO mice impaired LTD at PF synapses and cerebellar motor learning. These results indicate that, like Cbln1 at PF synapses, C1qL1 complements CF synapse integrity and cerebellar motor learning. No COI.

Symposium 15
New Aspects of Zinc Ion Biology in Homeostasis, Diseases and Signal transduction

(March16, 15:05–17:05, Room D2)

1S15D2-1
Zinc signaling: New regulatory system in physiology and diseases

Fukada, Toshiyuki(*RIKEN, IMS, Homeostatic networks*)

Zinc is an essential trace element that is required for numbers of cellular functions. Impairment of its homeostasis exerts various problems including growth retardation, abnormal bone formation, fragile skin, and immunodeficiency, etc. Zinc homeostasis is regulated by zinc transporters and channels, and recent genetic and molecular approaches on these molecules revealed the important roles of zinc ion as a signaling mediator. The emerging attentions have been drawn to actions of intracellular and extracellular zinc ions, which has become a focus of its potential to identify novel targets for therapeutic intervention in human disease. I Firstly will introduce the significant contribution of zinc transporter-mediated zinc signaling in our health, and discuss updated information by highlighting zinc signal that selectively controls molecules and the following cellular events, called **Zn signal axis** which maintain the physiological phenomena. No COI.

Reference:
Fukada et al, ***J. Bone Miner. Meta.*** 31: 129–135, 2013 Review.

1S15D2-2
Intracellular Zn2+ signal in the dentate gyrus is required for object recognition memory

Takeda, Atsushi(*Department of Bioorganic Chemistry, School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, Japan*)

Brain zinc homeostasis is strictly controlled under healthy condition, indicating the importance of zinc homeostasis in physiological function in the brain. Zinc is relatively concentrated in the hippocampus. The hippocampus is required for memory retention for a limited period of time after learning. Hippocampal three synapses, i.e., perforant pathway-dentate granule cell, mossy fiber-CA3 pyramidal cell and Schaffer collateral-CA1 pyramidal cell, are glutamatergic (zincergic). The three synapses contain zinc in the presynaptic vesicles, which serves as a Zn^{2+} signal. Zn^{2+} is co-released with glutamate and modulates glutamate signaling at zincergic synapses. However, the role of synaptic Zn^{2+} signal in learning and memory is poorly understood. Synaptic plasticity such as long-term potentiation (LTP) is believed to be a cellular model for memory. On the basis of the evidence that Zn^{2+} signal multi-functionally modulates LTP induction in hippocampal slices, in vivo real-time correlation between LTP induction and memory was examined using young rats. The role of perforant pathway-dentate granule cell synapses in cognitive behavior was examined focusing on the selective loss of synaptic Zn^{2+} signal in the dentate gyrus, which was induced by injection of zinc chelators into the dentate gyrus. The present study indicated that intracellular Zn^{2+} signal in the dentate gyrus is required for object recognition memory, probably via dentate gyrus LTP expression. It is likely that dentate gyrus LTP is real-timely linked to object recognition memory. No COI.

1S15D2-3
The diabetes susceptible gene SLC30A8/ZnT8 regulates hepatic insulin clearance

Fujitani, Yoshio(*Department of Metabolism & Endocrinology, Juntendo University, Tokyo, Japan*)

Recent genome-wide association studies revealed that common variants of SLC30A8 increase susceptibility to type 2 diabetes. SLC30A8 encodes zinc transporter-8 (ZnT8), which transports zinc ion from the cytoplasm into insulin granules in pancreatic beta cells. While it is well known that insulin granule contains high amount of zinc, its physiological meaning has not been fully elucidated. Here we show that zinc co-secreted with insulin regulates hepatic insulin clearance. To determine the role of ZnT8 in glucose metabolism, we characterized beta-cell-specific Slc30a8-deficient mice. Low peripheral blood insulin levels were observed in mutant mice after glucose challenge, although insulin secretion from pancreatic beta cells was enhanced. A series of experiments assessing in vivo zinc flux revealed that loss of zinc secretion from pancreas failed to suppress hepatic insulin clearance in postprandial state. Consistent with these findings, human individuals with rs13266634, a major risk allele of SLC30A8, exhibited increased insulin clearance assessed by c-peptide/insulin ratio. The results indicate that zinc-mediated pancreas-to-liver communication regulates hepatic insulin clearance, and that dysregulation of this system could play a role in the pathogenesis of type 2 diabetes. No COI.

1S15D2-4

Zinc transporter ZnT2/Slc30a2 is involved in skin-wound healing

Nishida, Keigo[1,2](*Lab. for Homeostatic Network., Center for IMS., (IMS-RCAI) RIKEN, [2]Immune system., Grad. Sch. Frontier Biosci., Osaka Univ.*)

Zinc (Zn) is an essential nutrient, and its deficiency causes immunodeficiency and skin disorders. Many metalloenzymes and transcription factors require Zn to exert their functions. Zn also acts as an intracellular signaling molecule. A change in Zn's intracellular status in response to extracellular stimuli affects a variety of signaling pathways; thus, Zn is critically involved in various physiological functions, including immune responses and wound healing. In addition to these intracellular roles, Zn appears to act as a neurotransmitter. Mast cell granules are rich in Zn, which is released when the cell is stimulated, as do neurons at synapses. However, the roles of the Zn released from mast cells are unknown. Here we report that the Zn transporter Slc30a2/ZnT2 is localized to the membrane of the Zn-containing granules in mast cells. *ZnT2*-KO mice do not contain Zn in their mast-cell granules and show defective Zn release upon stimulation. Importantly, by using these mice, we found that Zn released by mast cells plays a major role in skin wound repair, functioning as a first messenger. We showed that Zn promotes the production of inflammatory cytokines such as IL-6 in macrophages and fibroblasts via the Zn receptor G-protein-coupled receptor 39 (GPR39). We obtained genetic evidence that not only IL-6 but also GPR39 are required for skin-wound healing. Collectively, our findings indicate that Zn released by mast cells is an inflammatory "first messenger" and that the Zn/GPR39/IL-6 axis plays a major role in wound healing, in which inflammation is a key process. No COI.

Symposium 16
Late-breaking topics in the ion transport system
[Collaboration Symposium with The Japanese Pharmacological Society]

(March 16, 15:05–17:05, Room E)

1S15D2-5

Zinc deficiency and dermatitis: From the standpoint of zinc transporter functions

Kambe, Taiho(*Graduate School of Biostudies, Kyoto University, Kyoto, Japan*)

Severe zinc deficiency causes a broad range of defects including immune system dysfunction, taste dysfunction, and skin lesions of dermatitis as particular symptoms. In the last four decades, inherited zinc deficiency disorder has been investigated, and two zinc transporter genes have been identified, mutations of which cause severe dermatitis. One is the rare autosomal recessive genetic disorder acrodermatitis enteropathica, which is caused by mutations of zinc transporter ZIP4/SLC39A4. ZIP4 functions as an essential component for zinc absorption in the small intestine. A number of studies have revealed that expression of ZIP4 is dynamically regulated by multiple post-transcriptional mechanisms, and we found ZIP4 protein undergoes processing by removal of the long extracellular amino-terminal half via proteolytic cleavage during prolonged zinc deficiency. The similar processing has been detected in other ZIP transporter proteins, which suggests its importance to regulate activity of ZIP transporters. The other is the transient neonatal zinc deficiency with severe dermatitis. This type of zinc deficiency is caused by low zinc breast milk only during breast-feeding and does not reoccur after weaning. Recently, we found that novel compound heterozygous mutations in ZnT2/SLC30A2 cause the low zinc levels in the breast milk of the Japanese mother, which caused severe zinc deficiency in her infant.As pointed out above, the study of zinc transporters is now of great clinical interest. Here, the inherited zinc deficiency and zinc transporters functions are reviewed from the molecular standpoint. No COI.

1S16E-1

Pathophysiological roles of HCN channel family in the heart

Takano, Makoto[1]; Ohshita, Kensuke[1]; Ito, Masayuki[1]; Ishihara, Keiko[1]; Kuwahara, Koichiro[2]([1]*Dept.Physiol., Sch. Med., Kurume University, Kurume, Japan,* [2]*Dept.Cardiovasc.Med., Grad.Sch.Med., Kyoto Univ., Kyoto, Japan*)

Among hyperpolarization-activated cyclic nucleotide-modulated (HCN1-4) channel family, HCN4 is highly expressed in the pacemaker cells of sino-atrial node. In the ventricular myocytes, HCN2 and HCN4 are expressed in the fetal heart, but down-regulated during development. They are re-expressed in the hypertrophied heart, and thought to increase the susceptibility to arrhythmia. Recent studies using transgenic mice have revealed unexpected roles of HCN channels, both in physiological- and pathophysiological condition. In the pacemaker cells, functional consequence of HCN4 knockout appeared controversial; Baruscotti et al. reported HCN4 knockout caused deep bradycardia and heart block, whereas Hermann et al. suggested that HCN4 only provided depolarization reserve and was not required for heart rate acceleration. In ventricular myocytes, overexpression of HCN2 channel reduced repolarization reserve and prolonged the action potential duration, but this was not sufficient to induce lethal arrhythmia in physiological condition. In the transgenic mice overexpressing dominant negative mutant of neuron-restricted silencing factor (dnNRSF), multiple fetal type cardiac channels including HCN2 were up-regulated, and died suddenly from tachyarrhythmia. Most notably, ivabradine, a blocker of HCN channels were reported to improve the survival of dnNRSF mice. These results suggested that HCN channels may be a therapeutic target of ventricular arrhythmia in cardiac hypertrophy. COI properly declared.

1S16E-2

Selective modulation of TRPA1 channels by O_2 and trans-nitrosylation

Kozai, Daisuke[1]; Kiyonaka, Shigeki[1,2]; Numata, Tomohiro[1,2]; Takahashi, Nobuaki[1]; Ohwada, Tomohiko[3]; Mori, Yasuo[1,2]([1]*Dept. Synth. Chem. and Biol. Chem., Grad. Sch. Engineer., Kyoto Univ.,* [2]*Hall of Global Environmental Studies, Kyoto Univ.*)

Certain members of transient receptor potential (TRP) cation channels act as cell sensors for changes in redox status. Our systematic evaluation of TRP channels using reactive disulfides with different redox potentials revealed the capability of TRPA1 to sense O_2. We previously demonstrated that sensitivity to activation by synthetic NO-releasing agents via *S*-nitrosylation is a common feature of members of TRP channels. However, strategies to confer subtype selectivity to nitrosylating agents targeted to TRP channels are yet to be developed. Here, we show selective activation of TRPA1 channels by novel NO donors derived from the 7-azabenzobicyclo[2.2.1]heptane (ABBH) *N*-nitrosamines, which exhibit trans-nitrosylation reactivity to thiols without releasing NO. The *N*-nitrosamine elicits *S*-nitrosylation of TRPA1 proteins, and dose-dependently induces robust Ca^{2+} influx via recombinant TRPA1 channels, but not via other NO-activated TRP channels. TRPA1 activation by the *N*-nitrosamine is suppressed by specific cysteine mutations but not by NO scavenging. Interestingly, non-electrophilic derivatives of ABBH also activate TRPA1 selectively but less potently compared to the *N*-nitrosamine. Thus, ABBH *N*-nitrosamines confer subtype selectivity on *S*-nitrosylation in TRP channels through synergetic effects of two chemical processes: cysteine trans-nitrosylation and molecular recognition of the non-electrophilic moiety. No COI.

1S16E-3

Involvement of Na^+/Ca^{2+} exchanger in Ca^{2+} regulation of pancreatic β cells

Kita, Satomi; Iwamoto, Takahiro(*Department of Pharmacology, Faculty of Medicine, Fukuoka University*)

Na^+/Ca^{2+} exchanger type1 (NCX1) is a plasma membrane transporter involved in intracellular Ca^{2+} regulation. NCX1 exchanges Na^+ and Ca^{2+} in either Ca^{2+} efflux or Ca^{2+} influx mode, depending on membrane potential and transmembrane ion gradients. Pancreatic islet transplantation is an attractive therapy for the treatment of insulin-dependent diabetes mellitus, but the low efficiency of this procedure mainly due to the early loss of transplanted islets is a major obstacle. Previous study showed that a large amount of high-mobility group box 1 protein (HMGB1) released from islets soon after their transplantation in mice triggers innate immune rejection with activation of DC, NKT cells and neutrophils to produce IFN-γ, ultimately leading to the early loss of transplanted islets. Thus, HMGB1 release plays an initial pivotal role in this process, however, its mechanism remains unclear. Here we demonstrate that release of HMGB1 from transplanted islets is due to hypoxic damage resulting from Ca^{2+} influx into β cells through NCX1 which isoform mainly expressed in β cells. Moreover, the hypoxia-induced β cell damage was prevented by pretreatment with a specific NCX1 inhibitor prior to transplantation, resulting in protection and long-term survival of transplanted mouse and human islets when grafted into mice. These findings suggest that NCX1 inhibitor could be applied to human donor islets to improve efficiency of islet transplantation. No COI.

1S16E-4

Inhibition of metastatic cell growth by MagEx

Funato, Yosuke; Yamazaki, Daisuke; Miki, Hiroaki(*Department of Cellular Regulation, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan*)

PRL overexpression is associated with cancer progression, but the molecular mechanism of inducing metastatic cell growth by PRL has remained unknown. We here perform a comprehensive search for in vivo binding proteins for PRL, and identify an uncharacterized membrane protein Magnesium-Exporting protein (MagEx). Live imaging analyses reveal that MagEx stimulates Mg^{2+}-efflux by Na^+/Mg^{2+} exchange and decreases intracellular Mg^{2+} levels. PRL directly binds to MagEx and inhibits its Mg^{2+}-efflux function. Overexpression and knockdown analyses of PRL and/or MagEx reveal that the Mg^{2+} level is correlated to that of ATP, and augmented Mg^{2+} and ATP levels by PRL overexpression confers cell resistance to energy stress under glucose starvation. Also, the importance of MagEx in metastatic growth is corroborated by experimental metastasis analyses in mice, and downregulation of MagEx expression is correlated with cancer malignancy in human colon cancers. These results present a novel role of Mg^{2+} in controlling the energy status and growth of cancer cells. No COI.

Symposium 17
Tissue homeostasis of the myocardium and its disruption

(March 16, 15:05–17:05, Room F)

1S17F-1

Inhibition of hypoxia-induced apoptosis of cardiomyocytes by synthetic peptide from ischemia-inducible activator of G-protein signaling 8 (AGS8)

Sato, Motohiko; Suzuki, Hiroko; Sakima, Miho; Mamun, Abdullah Al; Hayashi, Hisaki; Iwase, Satoshi; Inukai, Yoko; Nishimura, Naoki; Sato, Maki; Shimizu, Yuuki(*Department of Physiology, Aichi Medical University, Nagakute, Aichi, Japan*)

Ischemic injury of the heart is associated with activation of multiple signaling pathways including those mediated by heterotrimeric G-protein. Previously, we identified an ischemia-inducible G-protein activator, activator of G-protein signaling 8 (AGS8), which interacted with G$\beta\gamma$ and triggered the apoptotic cascades of cardiomyocytes under hypoxia. Here, we report the protection of cardiomyocytes from hypoxia-induced apoptosis by a synthetic peptide (AGS8-peptide), based on amino acids of the G$\beta\gamma$ interacting domain of AGS8. AGS8-peptide successfully blocked the interaction of G$\beta\gamma$ with AGS8. FITC conjugated AGS8-peptide was successfully delivered into cardiomyocytes by chemical reagent. AGS8-peptide effectively blocked apoptosis in cardiomyocytes, determined by DNA end-labeling (57.7±5.1% of apoptotic group, $p<0.05$, mean±SEM) and by cleaved caspase-3 (57.9±3.9% of apoptotic group, $p<0.05$, mean±SEM) following exposure of cultured cardiomyocytes to hypoxia (6 h)/reoxygenation (18 h). In contrast with general inhibition of G$\beta\gamma$-signaling by small compound, AGS8-pepetide did not show cytotoxicity in MTT assay (62.5±2.3% of control, $p<0.05$, mean±SEM), suggesting an advantage of AGS8-peptide for the protection of cardiomyocytes. These data indicated an importance of accessory proteins for heterotrimeric G-protein in patho-physiological challenge and their potential as novel therapeutic target. No COI.

1S17F-2

The role of cardiac fibroblasts in inflammation after myocardial infarction

Kuorse, Hitoshi; Nakaya, Michio(*Department of Pharmacology and Toxicology, Graduate School of Pharmaceutical Sciences, Kyushu University*)

At myocardial infarction, many cells are dead by limitation of oxygen supple from the coronary artery. These dead cells should be removed properly. Otherwise, cellular components released from the dead cells cause inflammation. It has been recognized that excess inflammation leads to detrimental effects on the heart. However, inflammation should be stopped by a mechanism, which is not well established. Although inflammatory cells such as macrophages and neutrophils are believed to be responsible for removal of dead cells, it is unknown whether these inflammatory cells are actually involved in removal of dead cells. After removal of dead cells, space that was dropped out at infarction area should be filled up with collagen. Accumulation of collagen is called as fibrosis. Collagen is produced by myofibroblasts that are differentiated from fibroblasts. Therefore, differentiation from fibroblasts to myofibroblasts should be tightly regulated to avoid excess fibrosis. However, the trigger of differentiation of fibroblasts into myofibroblasts is not well understood, and the relationship between differentiation of fibroblasts and inflammation at infarct area is not well defined. In my presentation, I will show the important role of fibroblasts in inflammation at myocardial infarction. No COI.

1S17F-3

Another pathophysiological aspect of stress-related cardiomyopathy

Kakinuma, Yoshihiko[1]; Tsuda, Masayuki[2]; Akiyama, Tsuyoshi[3]; Okazaki, Kayo[4]; Iketani, Mitsue[1]; Oikawa, Shino[1]; Sato, Takayuki[4] ([1]*Department of Physiology, Nippon Medical School Graduate School of Medicine, Tokyo, Japan,* [2]*Institute for Laboratory Animal Research, Kochi Medical School, Nankoku, Japan,* [3]*Department of Cardiac Physiology, National Cardiovascular Center Research Institute, Osaka, Japan ,* [4]*Department of Cardiovascular Control, Kochi Medical School, Nankoku, Japan,* [5]*Department of Physiology, Nippon Medical School Graduate School of Medicine, Tokyo, Japan*)

The pathophysiology of stress-related cardiomyopathy, initially reported in Japan as Tako-tsubo cardiomyopathy, remains to be elucidated. Despite the catecholamine surge as one of triggers, it remains to be investigated how catecholamine could disturb cardiac function. To disclose the pathophysiology, a FDG-PET study in humans was performed to reveal that the prevalence of healthy human females with maximal FDG uptake was more than that of males specifically in postmenopausal ages. Estrogen replacement in patients with oophorectomy also enhanced cardiac FDG uptake, suggesting that the female heart more depends on glucose. The transgenic mice with the heart-specific overexpression of a non-neuronal cholinergic system was developed, whose heart showed a glucose preference with upregulated transporters. Moreover, estrogen itself activated the non-neuronal cholinergic system. In a rat model of stress-related cardiomyopathy, glucose administration blunted catecholamine surge and attenuated impairment of cardiac performance. These results suggest that female sexuality, the cardiac cholinergic system, which regulates glucose utilization, is involved in the cardiopmyopathy. No COI.

1S17F-4

The role of TCTP, a novel endogenous inhibitor of p53, in the development of doxorubicin-induced heart failure

Fujita, Takayuki; Cai, Wenqian; Hidaka, yuko; Jin, Hui-Lin; Suita, Kenji; Ishikawa, Yoshihiro(*Yokohama City University Graduate School of Medicine, Cardiovascular Research Institute*)

Anthracycline antibiotics, including doxorubicin (DOX), are widely used chemotherapeutic agents for cancer therapy. However, their clinical usage has been limited by their serious cardiotoxicity. p53-induced apoptosis and production of reactive oxygen species (ROS) have been recognized as mechanisms of the cardiotoxicity. TCTP (translationally controlled tumor protein) is an anti-apoptotic protein. Recently, we demonstrated that TCTP is a p53 inhibitor. TCTP binds directly to the DNA binding domain of p53, thereby interfering the interaction between DNA and p53, a transcription factor. In addition, it is reported that p53 and TCTP downregulate each other's expression. Moreover, TCTP is reported to protect cells from oxidative stress induced cell death. Based on these findings, we hypothesize that TCTP plays important role in the development of DOX-induced heart failure. We found that DOX treatment downregulates cardiac TCTP expression in vitro and in vivo. Downregulation of TCTP by SiRNA results in increased susceptibility to apoptosis in cardiomyocytes. In addition, TCTP downregulation enhanced DOX induced production of ROS, at least in part, through p53 dependent mechanism. Moreover, overexpression of TCTP attenuated DOX-induced apoptosis of cardiomyocytes. The transgenic mice with cardiomyocyte-specific overexpression of TCTP are resistant to the development of DOX-induced heart failure.

These findings indicate that TCTP may be a potent therapeutic target for DOX-induced heart failure. No COI.

1S17F-5

Fusion of prion protein (PrP)-expressing cardiac progenitor cells forms atypically-shaped cardiomyocytes (ACMs), a new subpopulation of self-beating heart cells in mouse

Omatsu-Kanbe, Mariko; Matsuura, Hiroshi(*Department of Physiology, Shiga University of Medical Science, Otsu, Japan*)

We have recently identified atypically-shaped cardiomyocytes (ACMs), a new subpopulation of spontaneously beating heart cells observed within a culture of cardiac myocyte-depleted fraction cells from adult mouse heart. ACMs are mostly multinucleated and respond β-adrenergic stimulation on the spontaneous Ca^{2+} transients. However, many of the characteristics are still unclear. It has been recently reported that prion protein (PrP) serves as a surface marker for isolating cardiomyogenic progenitors from murine embryonic stem cells. The present study examined the origins and the development of multinuclear beating ACMs. ACMs were found to express PrP mostly in the plasma membrane even in the very early stage of culture. We observed that the small cells expressing PrP migrated towards the beating ACMs and fused with them to become larger cells. Furthermore, some of the ACMs connected at the edges of the plasma membrane often migrated towards the different directions until they were torn in the individually beating cells. Immunohistochemical analyses revealed that the cells co-expressing PrP and cardiac troponin T (cTnT) were resident in the interstitial small space among ventricular myocytes. The results suggest that the PrP(+)/cTnT(+) cells identified in the mouse heart are cardiac progenitor cells and their fusion results in the development of multinuclear beating ACMs, while there are some possibilities that these cells play a harmful role, such as a cause of arrhythmia, in injured heart. No COI.

Symposium 18
Central circuitries for psychologically induced physiological behaviors and responses

[Collaboration Symposium with The Japan Neuroscience Society]

(March 16, 15:05–17:05, Room G)

1S18G-1

A direct hypothalamo-medullary pathway for sociopsychological stress-induced brown fat thermogenesis and hyperthermia

Kataoka, Naoya[1]; Nakamura, Kazuhiro[1,2]([1]*Career-Path Prom. Uni. for Young Life Sci., Kyoto Univ., Kyoto, Japan,* [2]*PRESTO, JST, Japan*)

Psychological stress-induced hyperthermia is a fundamental autonomic response in mammals. We have previously shown that stress induces heat production in brown adipose tissue (BAT) by activating sympathetic premotor neurons in the rostral medullary raphe (rMR) (Lkhagvasuren *et al*., 2011). In this study, we further investigated the central circuit mechanism that drives stress-induced BAT thermogenesis and hyperthermia. Exposure of rats to social defeat stress, a sociopsychological stress model, exhibited an immediate increase in BAT temperature and a delayed elevation of abdominal temperature, as detected with a telemetry system. This response was eliminated by a nanoinjection into the rMR with a mixture of AP5 and CNQX, glutamate receptor antagonists. The stress-driven glutamatergic input to the rMR might be provided from the dorsomedial hypothalamus (DMH), since rMR-projecting neurons in the DMH were activated in response to the stress. Consistent with this hypothesis, inhibition of neurons in the DMH with muscimol injections eliminated the stress-induced BAT thermogenesis and hyperthermia. Supporting the functional contribution of the DMH-rMR monosynaptic pathway in the stress-driven BAT thermogenesis, *in vivo* optogenetic stimulation of DMH-derived nerve endings in the rMR elicited BAT thermogenesis, which was mediated by glutamate receptors in the rMR. These results indicate that a DMH-rMR glutamatergic monosynaptic pathway likely mediates stress signaling to drive BAT thermogenesis, contributing to stress-induced hyperthermia. No COI.

1S18G-2

Neuronal basis of pain-induced aversion

Minami, Masabumi(*Dept of Pharmacol, Grad Sch of Pharm Sci, Hokkaido Univ, Sapporo, Japan*)

The neural basis underlying the sensory component of pain have been studied extensively, but we are only beginning to understand that underlying its emotional component. The bed nucleus of the stria terminalis (BNST) has been implicated in negative affective states, such as anxiety, fear, and aversion. In the present study, we first examined the role of the CRFergic transmission within the dorsolateral part of the BNST (dlBNST) in pain-induced aversion. In vivo microdialysis demonstrated the increased release of CRF within the dlBNST by noxious stimulation. Intra-dlBNST injection of CRF antagonists attenuated the pain-induced conditioned place aversion (CPA). Intra-dlBNST injection of CRF produced CPA even in the absence of noxious stimulation. Taken together, these results reveal that enhanced CRFergic transmissions within the dlBNST are important for pain-induced aversion. Next, in order to address cellular mechanisms for this effect of CRF, we examined the effect of CRF on neuronal activity in dlBNST neurons using a whole-cell patch-clamp recording. We found that CRF modulated the resting membrane potential in a particular type of neurons, type II neurons, in the dlBNST. To clarify the neuronal circuit(s) involved in pain-induced aversion, we characterized VTA-projecting BNST neurons using combined neurotracing and histochemical techniques. The major BNST-VTA projection originates from GAD67-expressing GABAergic neurons in the BNST, and preferentially targets GABAergic interneurons in the VTA, suggesting the disinhibitory control of VTA dopaminergic neurons by BNST-VTA projection. I will discuss the neuronal circuits for pain-induced aversion. No COI.

1S18G-3

The septo-habenular pathway in anxiety- and fear-related behaviors

Yamaguchi, Takashi[1]; Danjo, teruko[1]; Ira, Pastan[2]; Hikida, Takatoshi[1]; Nakanishi, Shigetada[1]([1]*Department of Systems Biology, Osaka Bioscience Institute, Suita, Japan,* [2]*Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, USA*)

The posterior septum consisting of the triangular septum (TS) and the bed nucleus of the anterior commissure (BAC) is predominantly linked with the medial habenula (MHb) and has been implicated in the control of anxiety and fear responses. However, its anatomical and functional linkage has largely remained elusive. We established a transgenic model mouse in which the TS and BAC projection neurons were visualized by GFP fluorescence and selectively eliminated by immunotoxin-mediated cell targeting. The linkage between the TS/BAC and the MHb constitutes 2 parallel pathways composed of the TS-ventral MHb-the core part of the interpeduncular nucleus (IPN), and the BAC-dorsal MHb-the peripheral part of the IPN. Ablation of the TS and BAC projection neurons selectively impaired anxiety and enhanced fear responses and learning, respectively. Inputs from the TS and BAC to the MHb are thus segregated by 2 parallel pathways and play specialized roles in controlling emotional behaviors. No COI.

Symposium 19
Regulatory logic of stress response in blood vessels

(March 16, 15:05–17:05, Room H1)

1S18G-4

Regulatory mechanism of basal ganglia circuit in reward and aversive behavior

Hikida, Takatoshi(*Medical Innovation Center, Kyoto University Graduate School of Medicine, Kyoto, Japan*)

The basal ganglia-thalamocortical circuitry plays a critical role in reward and aversive learning and decision-making. The inputs of the nucleus accumbens in this circuitry are transmitted through two parallel direct and indirect pathways and controlled by dopamine transmitter. To explore how the associative learning behavior is controlled in the basal ganglia circuit, we developed a reversible neurotransmission blocking (RNB) technique, in which transmission-blocking tetanus toxin was specifically expressed in the direct striatonigral or the indirect striatopallidal pathway and, in turn, blocked each pathway in a doxycycline-dependent manner. We have revealed the distinct role of the two striatal pathways: the direct pathway critical for reward-based learning and the indirect pathway for aversive behavior and flexibility of learning. We also addressed the regulatory mechanisms of the basal ganglia circuit, suggesting the dynamic shift of pathway-specific neural plasticity via selective transmitter receptors is essential for reward and aversive behavior. No COI.

1S19H1-1

Stretch-induced responses of Human Umbilical Vein Endothelial Cells

Naruse, Keiji(*Cardiovascular Physiology, Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, Okayama University*)

Human umbilical vein endothelial cells (HUVEC) are located at the inner surface of vessel wall and are continuously subjected to mechanical stimulations in vivo, such as shear stress, hydrostatic pressure and stretch, and it is widely recognized that they are playing an important physiological role in cardiovascular system. In response to stretch, we reported that intracellular Ca^{2+} increased transiently through the activation of mechanosensitive (MS) cation channel in HUVEC. However, the molecular entities that form the MS cation channel in the cell remain unknown. Recent papers report some members of the transient receptor potential (TRP) channels have been suggested as one of components of MS channels.Since HUVEC expresses several types of mechanosensitive TRP channels including TRPV2, TRPC1, and TRPM7, we suppressed the expression of these channels in HUVEC. In the TRPV2-knocked down cells, the stretch induced Ca^{2+} increase measured by fluorescence imaging using fura2 was completely abolished. After the cells were subjected to 20% uniaxial cyclic stretch at 1Hz for 1h, neither a stretch-enhanced stress fiber formation nor a change in the cell orientation perpendicular to the strain direction could not be observed. Finally, TRPV2-knocked down HUVEC did not show stretch-induced nitric oxide (NO) production. These observations strongly suggest that TRPV2 is a responsible ion channel for stretch-induced Ca2+ increase, which leads the cytoskeletal reorganization and NO production in HUVEC. Thus, TRPV2 would be a key component of MS channel complex in HUVEC. COI properly declared.

1S19H1-2

Molecular mechanism of elastic laminae development

Nakamura, Tomoyuki(*Department of Pharmacology, Kansai Medical University*)

Elastic laminae comprise as much as 50 % of the dry weight in large arteries, and are necessary for elastic recoil of stretched arterial wall. Aging-related deterioration of elastic laminae causes increase in pulse pressure. Because degradation of elastic laminae is a pathological feature of aneurysms, it has been of interest whether elastic laminae degradation is a cause of aneurysmal enlargement of arterial wall. We and others found that three secreted proteins, fibulin-4, 5, and LTBP-4 are required for elastic fiber development. Genetically engineered mice deficient in any of these proteins showed severely disorganized elastic laminae in aortae. However, only fibulin-4 mutant mice, but not fibulin-5 or LTBP-4 deficient mice showed aortic aneurysms. My talk will focus on the differences of functions of these elastogenic proteins. The possibility of elastic fiber regeneration will also be discussed. No COI.

1S19H1-3

Significant roles of matricellular protein in vascular development and disease.

Imanaka-Yoshida, Kyoko[1,2](*The Department of Pathology and Matrix Biology, Mie University Graduate School of Medicine, Tsu, Japan, [2]Mie University Research Center for Matrix Biology*)

The extracellular matrix (ECM) is well recognized to provide not only structural support but also important biological signaling which influences various cellular function in embryonic development and physiological / pathological response to extrinsic stimuli. Among matrix molecules, increased attention has been focused on matricellular proteins. Matricellular proteins are a group of non-structural ECM proteins highly up-regulated at active tissue of remodeling, serving as biological mediators by interacting directly with cells or regulating the activities of growth factors, cytokines, proteases, and other ECM molecules. Tenascin-C(TN-C) is a typical matricellular protein, strongly expressed during embryonic development, wound healing, inflammation and cancer invasion. In vascular system, strong expression is detected in medial smooth muscle cells during maturation of vascular wall, and also inked with several pathological condition such as cerebral vasospasm, intimal hyperplasia, pulmonary artery hypertension, and aortic aneurysm/ dissection. Our recent results suggest that TN-C synthesized in response to developmental and environmental cue including growth factors and mechanostress maybe a determinant for mechanical property of tissue and modulate inflammation, synthesis of other matrix molecules. The discussion will be focused on several roles and their regulatory pathway of TN-C in adaptation during normal vascular development as well as tissue remodeling in pathological condition. No COI.

1S19H1-4

Hemodynamic stress on aortic walls: adaptation and maladaptation

Aoki, Hiroki(*Cardiovascular Research Institute, Kurume University, Kurume, Japan*)

Aorta needs to cope with the hemodynamic stress through the remodeling of its wall. This is accomplished by the dynamic interaction between the resident smooth muscle cells and the inflammatory cells that infiltrate into the aortic wall under the abnormal stress. To better understand the adaptive and maladaptive responses of the aortic wall, we created a mouse model of aortic wall stiffening and augmented hemodynamic stress by periaortic CaCl2 application and angiotensin II infusion. The aortic stress caused a minor injury with macrophage infiltration in the aortic walls, which healed in 6 weeks. When JAK/STAT signaling was augmented specifically in macrophages by deleting Socs3, an endogenous inhibitor of JAK/STAT, the minor aortic injury progressed to aortic dissection in 6 weeks. The aortic dissection was associated with the decrease in extracellular structural proteins and cell adhesion molecules, suggesting the dysregulation of the tissue integrity. Thus, adequate regulation of the proinflammatory response is essential for the protection of aortic walls under the stress. No COI.

1S19H1-5

Extracellular matrix remodeling is critical for closure of the ductus arterioles

Minamisawa, Susumu[1]; Yokoyama, Utako[2]([1]*Department of Cell Physiology, The Jikei University School of Medicine, Tokyo, Japan, [2]Cardiovascular Research Institute, Yokohama City Univ. Yokohama, Japan*)

Vascular cells are defined by the ways in which they regulate their extracellular matrix (ECM), and the ECM, in turn, changes vascular cell phenotype, i.e. the ability to differentiate, proliferate, and migrate. Elastin, collagens, proteoglycans and structural glycoproteins are synthesized during fetal development and their three dimensional organization is optimal for vascular functions. Thus, the ECM is a key component in the development and remodeling of specific vascular structure. Here we demonstrate how cell signaling pathways such as prostaglandin E (PGE) regulate the ECM in the ductus arteriosus, an essential bypass artery between the aortic arch and the pulmonary artery during fetal development. The proper organization of the ECM is crucial for closure of the ductus arteriosus after birth. We also would like to discuss about a potential therapeutic strategy to regulate the ECM for vascular diseases such as the persistent patent ductus arteriosus. No COI.

Symposium 20
Metabolic regulation of renal physiology and pathophysiology
[FAOPS Joint Symposium- Japan/China]

(March 16, 15:05–17:05, Room H2)

1S20H2-1
Farnesoid X receptor (FXR) gene deficiency impairs urine concentration in mice.

Zhang Xiaoyan[1,2]; Huang, Shizheng[1]; Guan, Youfei[1,2]([1]*Peking University Health Science Center,* [2]*Shenzhen University Health Science Center, China*)

The Farnesoid X receptor (FXR) is a ligand-activated transcription factor belonging to the nuclear receptor superfamily. FXR is mainly expressed in liver and small intestine, where it plays an important role in bile acid, lipid and glucose metabolism. The kidney also has a high FXR expression level with its physiological function unknown. Here we demonstrate that FXR is ubiquitously distributed in renal tubules. FXR agonist treatment significantly lowered urine volume and increased urine osmolality, while FXR knockout mice exhibited an impaired urine concentrating ability which led to a polyuria phenotype. We further found that treatment of C57Bl/6 mice with chenodeoxycholic acid (CDCA), an FXR endogenous ligand, significantly upregulated renal aquaporin 2 (AQP2) expression,while FXR gene deficiency markedly reduced AQP2 expression levels in the kidney. In vitro studies showed that the AQP2 gene promoter contained a putative FXRE site, which can be bound and activated by FXR resulting in a significant increase of AQP2 transcription in cultured primary inner medullary collecting duct cells. In conclusion, the present study demonstrates that FXR plays a critical role in the regulation of urine volume and its activation increases urinary concentrating capacity mainly via upregulating its target gene AQP2 expression in the collecting ducts. No COI.

1S20H2-2
Interaction between renal medullary (pro)renin receptor and PGE2 in AngII-induced hypertension

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(Pro)renin receptor (PRR), a newly discovered component of the renin-angiotensin system (RAS), is viewed as a potential regulator of tissues RAS in light of its capability of binding renin and prorenin to increase their catalytic activity. Within the kidney, PRR expression is predominantly expressed in the intercalated cells of the CD where its expression is induced by AngII. The goal of our study was to investigate the mechanism of how AngII activated PRR in the CD cells and further explore the functional implication of this phenomenon. Our focus was placed on defining the COX-2/PGE2/EP4 pathway in regulation of PRR in the CD cells by AngII. In vitro studies demonstrated that AngII stimulated PRR protein expression in primary rat IMCD cells, which was completely abolished by COX-2 inhibitor or EP4 antagonism and to less extent by EP1 but not EP3 antagonism. The medium renin activity varied in parallel with PRR expression level. In vivo studies showed that chronic AngII infusion elevated rat renal medullary PRR expression and the active and total renin levels, all of which were abolished by COX-2 inhibition or EP4 antagonism, accompanied by a significant attention of hypertension development. Overall, these results have established a crucial role of COX-2/PGE2/EP4 pathway in mediating the upregulation of renal medullary PRR expression and renin activity during AngII hypertension. No COI.

1S20H2-3
Crosstalk between cholesterol homeostasis and TLR4/6 pathway ikidney

Xiong Zhong Ruan[1]; Li, Andrew[2]([1]*Centre for Lipid Research, Chongqing Medical University, China,* [2]*John Moorhead Renal Research Laboratory, Centre for Nephrology, University College London (UCL) Medical School, Royal Free Campus, London, UK*)

Both macrophages and renal proximal tubular cells play important role in the progression of chronic kidney disease (CKD). Sterol regulatory element binding protein (SREBP) cleavage-activating protein (SCAP) is a cholesterol sensor that regulates LDL receptor (LDLr) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCoAR) transcription and maintains the intracellular cholesterol homeostasis. The aim of this study is to investigate if SCAP regulates inflammatory response in PMA activated THP-1 and HK2 cells. Over-expression of SCAP increased, while knockdown of SCAP decreased LDLr and HMGCoAR expression in both cells. Intracellular cholesterol content was significantly increased after over-expression of SCAP and remarkably reduced after knocking down SCAP. Interestingly, over-expression of SCAP also increased the pro-inflammatory cytokines IL-6 and TNF-α. LPS increased IL-6 and TNF-α expression as expected. However, knocking-down SCAP abolished the up-regulatory effects by LPS on IL-6 and TNF-α, accompanying with reduction of phosphorylation of IκB (rather than JNK phosphorylation) and nuclear p65 levels, indicating that SCAP mediates inflammatory response in THP-1 and HK2 cells via IκB phosphorylation. This suggests that SCAP is not only a cholesterol sensor but also a key regulator for inflammatory response in THP-1 and HK2 cells. SCAP may serve as a novel target for both lipid-lowering and anti-inflammatory therapies in renal diseases. No COI.

1S20H2-4

Molecular Mechanisms of Regulation of ENaC Expression and Intracellular Trafficking in Renal Epithelium

Marunaka, Yoshinori; Niisato, Naomi; Yokoyama, Noriko; Sasamoto, Kouhei(*Kyoto Prefectural University of Medicine*)

Epithelial Na+ channel (ENaC)-mediated Na+ reabsorption in the cortical collecting duct plays an important role in regulation of extracelullar fluid volume and blood pressure. The Na+ entry step via ENaC located at the apical membrane is the rate-limiting step for transepithelial Na+ reabsorption. The amount of Na+ entry via ENaC depends on: 1) the activity of individual ENaC in the apical membrane, and 2) the amount of ENaC surface expression in the apical membrane. To investigate regulation of ENaC activity and trafficking, we established a mathematical model of ENaC trafficking including 4 steps involved in ENaC translocation: i.e. 1) insertion, 2) endocytosis, 3) recycling, and 4) degradation. Combing this mathematical model with electrophysiological and biochemical observations, we obtained the amount of recycled ENaCs depending on quality control of ENaC in the intracellular store site. In our talk, we provide information on: 1) regulation of ENaC activity by phosphorylation; 2) regulation of ENaC surface expression in the apical membrane and roles of lipid rafts, and 3) the established mathematical model of ENaC trafficking and regulation of ENaC recycling. Supported by JSPS (24590283, 25670111) and Salt Science Research Foundation (1235). No COI

Symposium 21
Circadian clock signaling for adaptation to environments

(March 16, 15:05–17:05, Room J)

1S21J-1

Role of HIF1α in the Mammalian Circadian System

Ikeda, Masaaki[1,3]; Kumagai, Megumi[1,3]; Ueno, Munehisa[2]; Okabe, Takashi[2,3]([1]*Department of Physiology, Saitama Medical University, Moroyama, Saitama, Japan,* [2]*Department of Uro-oncology, International Hospital, Saitama Medical University,* [3]*Molecular Clock Project, Research Center for Genomic Medicine, Saitama Medical University, Hidaka, Saitama, Japan*)

PAS factors are so named after the discovery of a domain common to Per, Arnt, and Sim proteins. These factors sense and bind small molecules, such as oxygen, dioxins, and cellular metabolites. They are involved in the adaptation to external and internal changes in the environment, such as circadian, hypoxic, and toxic state changes in mammals. In the circadian system, the core feedback loop that accounts for the generation of an approximately 24-h rhythm involves up to four PAS factors (BMAL1, CLOCK, NPAS2, and BMAL2) and three PER proteins. Cross-talk between the PAS factors involved in hypoxic responses and circadian systems has been reported. While studying the cross-talk among the adaptation systems that make use of PAS factors, we discovered the transcriptional regulation of *Per2* genes by HIF1*α*. We will discuss the mechanism of the transcriptional regulation of the *Per2* gene by HIF1*α* and the role of HIF1*α* in the *Per2* regulation of tumor cells. No COI.

1S21J-2

ROS stress resets circadian clocks to coordinate pro-survival signals

Tamaru, Teruya(*Dept. of Physiol., Toho Univ. Sch. of Med.*)

As an adaptive response to daily environmental changes, the biological clock (circadian system) confers temporal order as a multi-cellular/tissue synchronization state evoked by resetting cues and daily anticipatory rhythmic physiological processes driven by circadian system. Dysfunction of circadian clocks exacerbates various diseases, in part likely due to impaired stress resistance. It is unclear how mammalian circadian system responds to reactive oxygen species (ROS) -induced stress. To address this question, we sought to identify a novel clock-related adaptive signaling system evoked at the life-death boundary to mediate protection from the stress. We identified a ROS -responsive circadian pathway in mammals. Near-lethal doses of ROS-induced critical oxidative stress (cOS) at the branch point of life and death resets circadian clocks, synergistically evoking protective responses for cell survival. The cOS-triggered clock resetting and pro-survival responses are mediated by transcription factor, central clock-regulatory BMAL1 and heat shock stress-responsive (HSR) HSF1. Casein kinase II (CK2) -mediated phosphorylation regulates dimerization and function of BMAL1 and HSF1 to control the cOS-evoked responses. The core cOS-responsive transcriptome includes CK2-regulated cross-talk between the circadian, HSR, NFκB-mediated anti-apoptotic, and Nrf2-mediated anti-oxidant pathways. This novel circadian-adaptive signaling system likely plays fundamental protective roles in various ROS-inducible disorders, diseases such as cancer and neurodegenerative diseases, and death. No COI.

1S21J-3

Phase shift of cancer clock by hypoxia

Masubuchi, Satoru[1]; Yagita, Kazuhiro[2]; Nakamura, Wataru[3]; Honma, Sato[4]; Honma, Ken-ichi[4](*Department of Physiology, Aichi Medical University, Nagakute, Aichi, Japan, [2]Department of Physiology and Systems Bioscience, Kyoto Prefectural University of Medicine, Kyoto, Japan, [3]Laboratory of Oral Chronobiology, Graduate School of Dentistry, Osaka University, Suita, Osaka, Japan, [4]Department of Chronomedicine, Hokkaido University Graduate School of Medicine, Sapporo, Japan*)

Mammalian circadian physiological and behavioral functions are driven by intracellular transcriptional negative-positive feedback loops involving several clock genes. Clock genes are expressed in a circadian fashion not only in the master clock, the hypothalamic suprachiasmatic nucleus (SCN) , and peripheral clocks in extra-SCN organs but also in cancer cells. Partial pressure of oxygen (pO2) in the solid tumor declines depending on the distance from blood vessels. Since hypoxic cancer cells are resistant to therapies, information about circadian rhythm of these hypoxic cancer cells is critical for designing chronotherapy. Previous study on drosophilla eclosion rhythm suggests that low pO2 affects the circadian rhythms of cancer cells . In the present study, synchronized colon cancer cell line (HCT116: DBP-luc) was exposed to 6h hypoxia of different pO2. Hypoxia phase shifted cancer clock depending on exposure timing and oxygen concentration. In contrast, the same treatment had little effect on fibroblast clock. Buffering of hypoxia-induced pH decrease attenuated phase shifts of cancer clock. These results suggest that cancer specific metabolic response to hypoxia modifies clock function in solid tumor. No COI.

1S21J-4

Dissection of circadian clock system in humans

Hida, Akiko(*Department of Psychophysiology, National Institute of Mental Health, National Center of Neurology and Psychiatry, Tokyo, Japan*)

Circadian rhythms of behavior and physiology are driven by a system of self-sustained clocks and are entrained by external cues. The mammalian central oscillator, SCN incorporates environmental information and orchestrates slave oscillators in peripheral cells. The circadian clock system is composed of a hierarchy of oscillators that involve transcription and translation feedback loops of multiple clock genes. Disorganization of the circadian system is known to be related to many diseases including sleep, mood and metabolic disorders. Evaluation of circadian phenotypes is crucial for understanding the pathophysiology of diseases associated with disturbed biological rhythms. We measured clock gene expression in fibroblasts derived from individual subjects and observed circadian rhythms in the cells (*in vitro* rhythms). Period length of the *in vitro* rhythm (*in vitro* period) was compared with the intrinsic circadian period, τ, measured under a forced desynchrony protocol (*in vivo* period) and circadian/sleep parameters. Although no significant correlation was observed between the *in vitro* and *in vivo* periods, the *in vitro* period was correlated with chronotype. Evaluating rhythmic expression of clock genes in isolated fibroblast cells might therefore be an appropriate method to assess an individual's circadian clock phenotype. Here, we have examined *in vitro* rhythms in patients with disturbed sleep-wake rhythms and will discuss circadian phenotypes of the patients. No COI.

1S21J-5

Stress and circadian clock

Takumi, Toru(*RIKEN Brain Science Institute, Wako, Japan*)

Several lines of indirect evidence suggest that mood disorders and dysfunctions in circadian timing are linked. Learned helplessness (LH) rats, a model of depression, exhibit shorter periods of circadian rhythms and these shortened periods are lengthened by lithium, a mood stabilizer and inhibitor of glycogen synthesis kinase 3β (GSK3β). Circadian expression of phosphorylated GSK3β (pGSK3β) disappears in the suprachiasmatic nucleus and prefrontal cortex of LH rats and in fibroblasts. The altered pGSK3β level is also observed in different depression models. We identify a phosphorylation site on PERIOD2 (PER2) by GSK3β and present evidence that phosphorylation on PER2 modulates both circadian and depression-related phenotypes. The altered pGSK3β and a new signaling pathway specified in this study open the door to develop new diagnostic and therapeutic tools for mood disorders. No COI.

Symposium 22
Presentation skills for researchers
[Japan Young Physiologist Association Symposium]

(March 16, 15:05–17:05, Room K)

1S22K-1

Presentation skills for scientists, in a view of present situation and tasks

Kamikubo, Yuji(*Department of Pharmacology, Faculty of Medicine, Juntendo University, Tokyo, Japan*)

The presentation of scientific knowledge and research is an important part of a researcher's career, yet scientists are rarely offered professional instruction on how to present their achievement. In this presentation, I talk about presentation instructions for young scientists.

1S22K-2

Presentation skills for researchers

Hayashi, Hidetoshi(*Tokyo JTECT Bldg.,[11-15], Ginza[7], Chuo-ku Tokyo Japan*)

Hidetoshi HayashiRICOH HUMAN CREATES CO., LTDPresentation skills for researchersThe following required since an effective presentation is put on explains three points. 1. Persuasion by logos "logic" language, scenario, and reason. 2. Ethos "reliance" speaker's character and personal character, sense of ethics, credibility. 3. Feeling of pathos "passion" opinion, earnestness, thought.

1S22K-3

Easy to Understand and Beautiful Visual Design for Research Presentations

Tanaka, Sayoko(*University of Tsukuba, Tsukuba, Japan*)

Visual expressions have power of transmitting contents easily even though difficult by language. Visual expressions for research presentations that help understandings are occupying positions more and more important for researchers. Based on this situation, I talk visual design methods for research presentations that are easy to understand and beautiful. The main contents are as follows.1. Drawing: How to create complicated figures easily by PowerPoint or Keynote. 2. Color: Simply and contrasty color scheme, color universal design for transmitting to more people.3. Typography: Recommendation and non-recommendation font styles of Japanese and English, suitable font size, suitable space between lines, and suitable length of lines.4. Layout: Natural eyes flow, alignment, closing and separating, white space, hierarchy of information.

Symposium 23
Regulation of brain functions by meals and systemic metabolism

(March17, 9:00–11:00, Room B)

2S23B-1

Facilitation of spatial memory and hippocampal plasticity during food intake through satiety signals

Oomura, Yutaka[1]; Katafuchi, Toshihiko[1]; Aou, Shuji[2]; Moriguchi, Shigeki[3]; Fukunaga, Koji[3]([1]*Dept Integr Physiol, Sch Med, Kyushu Univ, Fukuoka,* [2]*Dept Brain Sci Engineer, Kyushu Inst Technol, Kitakyushu,* [3]*Dept Pharmacol, Sch Pharmacy, Tohoku Univ, Sendai*)

During food intake 3 mM glucose concentration in CSF becomes twice. Intrahippocampal injection of 6 mM glucose facilitats spatial learning and memory. In vitro CA1 slice preparation with 3 mM glucose in Ringer solution, EPSPs amplitudes generated by the Schaffer collateral/commissural stimulation markedly increased in 6 mM glucose. NMDA and AMPA evoked currents were significantly augmented by 6 mM glucose. The paired-pulse facilitation experiments indicated augmentation of presynaptic transmitter release by 6 mM glucose. These augmentations were associated with enhanced phosphorylations of CAMKII, PKC and presynaptic synapsin. High frequency stimulation of the Schaffer pathway failed to induce LTP in the CA1 region in 3 mM glucose but facilitated by 6 mM glucose. The LTP induction in the 6 mM glucose was associated with further increase in CAMKII and PKC autophosphorylations. On the above mentioned glucose effects there is one question whether these effects are direct glucose or glucose converted ATP effects. Then we are now experimenting using pinacidil and dehydorepiandrosterone on the glucose effects, since these are making opening the ATP sensitive K channels. If the glucose is still effective on these conditions, we can sagely mention the direct glucose effects. Taken together food intake is valuable for the brain plasticity enhancing spatial memory of food rich places. No COI.

2S23B-2

Pancreatic hormones affect the brain and regulate food intake via vagal afferent neural pathway

Iwasaki, Yusaku; Yada, Toshihiko(*Department of Physiology, Jichi Medical University School of Medicine, Tochigi, Japan*)

Peripheral hormones are thought to signal to the brain via penetrating through the blood-brain barrier and/or acting on the vagal afferents. Pancreatic hormones insulin and pancreatic polypeptide (PP) are released in response to meal intake and contribute to formation of satiety. This study aimed to determine whether insulin and PP directly act on the vagal afferents. We monitored the activities of isolated vagal afferent nodose ganglion neurons (NGNs) by measuring cytosolic Ca^{2+} ($[Ca^{2+}]_i$) and membrane potential. PP increased $[Ca^{2+}]_i$ in NGNs. The anorexigenic effect of PP is reportedly abolished by vagotomy. Hence, the activation of vagal afferents by PP is linked to inhibition of feeding. Insulin depolarized and increased $[Ca^{2+}]_i$ in 8% of NGNs. We examined whether the vagal afferents innervating the pancreas sense insulin. The neural fibers innervating pancreatic islets expressed the insulin receptor. The NGNs innervating the pancreas responded to insulin at 10^{-7} M, the estimated insulin concentration in the pancreas, with high response incidence (20%). Intraperitoneal injection of glibenclamide rapidly induced insulin secretion and phosphorylated AKT in NGNs. Moreover, the insulin action was markedly attenuated in NGNs isolated from IRS-2 KO mice that exhibit hyperphagia and obesity. These results suggest that vagal afferent neurons innervating the pancreas sense the released insulin within the pancreas to rapidly inform the brain of metabolic changes, thereby controlling appetite, and possibly energy and glucose metabolism. No COI.

2S23B-3

Regulation of longevity and cognitive function through neural insulin-like signaling

Taguchi, Akiko[1,2]; Makinodan, Manabu[3]; Fukuokaya, Wataru[1]; Kurata, Eiko[1]; Nakazato, Masamitsu[1]; Corfas, Gabriel[3]; White, Morris[2]([1]*Div. of Neurology, Endocrinology, and Metabolism, Faculty of Medicine, Univ. of Miyazaki, Japan,* [2]*Div. of Endocrinology, Childrens Hosp Boston, Harvard Med Sch, USA,* [3]*Div. of Neurology, Childrens Hosp Boston, Harvard Med Sch, USA*)

Diabetes and obesity may contribute to cognitive impairment and reduced adult hippocampal neurogenesis that is associated with memory function, suggesting that metabolic disturbances promote aging-like effects. Previous studies show that less neural IRS2 (Insulin Receptor Substrates2) increases lifespan and reverses premature mortality with reducing aggregated Aβ in Alzheimer disease model mice, while increasing blood insulin level. However, it remains unknown how neural IRS2 deficiency influences cognitive decline and adult hippocampal neurogenesis. Here we show that IRS2 is expressed in both mature neurons and neural stem/progenitor cells in the hippocampal dentate gyrus (DG). Proliferation and neuronal differentiation remarkably augmented in aged mice lacking neural IRS2 (BIRS2ko). In the process of neuronal differentiation, neural IRS2 depletion increased dendritic complexity and decreased microglial activation in the DG of aged mice. Moreover, the Water T-maze test demonstrated that the test accuracy ratios in aged BIrs2ko mice were significantly greater than those in age-matched controls. These findings suggest that less neural IRS2 promotes adult neurogenesis and retains cognitive functions in aged mice without age-associated reduction in dendritic complexity and increased neuroinflammation, regardless of hyperinsulinemia. No COI.

2S23B-4

The role of hypothalamic brain-derived neurotrophic factor (BDNF) in energy metabolism: therapeutic potential for visceral obesity

Maekawa, Fumihiko[1,2]; Fujiwara, Ken[2,3]; Toriya, Masako[2]; Nohara, Keiko[1]; Yada, Toshihiko[2](*Center for Environmental Health Sciences, National Institute for Environmental Studies, Tsukuba, Japan,* [2]*Department of Physiology, Jichi Medical University, Shimotsuke, Japan,* [3]*Department of Anatomy, Jichi Medical University, Shimotsuke, Japan*)

BDNF is implicated in feeding behavior and energy balance. In this study, we focused on examining 1) how BDNF regulates energy metabolism and 2) whether impaired expression of BDNF plays a role in the disorder of metabolism. Firstly, we found that BDNF regulated the neurons expressing corticotrophin-releasing hormone (CRH) in the paraventricular nucleus of hypothalamus. The CRH neurons expressed TrkB, a receptor for BDNF, and intracerebroventricular (icv) injection of BDNF increased CRH mRNA. In addition, the increased energy expenditure by BDNF treatment was antagonized by simultaneous treatment with a CRH receptor antagonist. These results demonstrated the possibility that the effect of BDNF is mediated via the CRH neural pathway. Secondly, we investigated BDNF expression in type 2 diabetic GK rats exhibiting visceral obesity. In middle-aged GK rats, BDNF and glucose transporter-2 expressions were significantly decreased in the ventromedial hypothalamus (VMH), suggesting that impaired glucose metabolism caused BDNF reduction. Since BDNF treatment by icv injection ameliorated visceral fat accumulation and hyperleptinemia, the visceral obesity is at least in part due to the reduction of BDNF in VMH. The BDNF supplementation could provide an effective treatment of visceral obesity in type 2 diabetes. No COI.

Symposium 24
Frontline of molecular physiology of hair cells in the inner ear

(March 17, 10:05–12:05, Room C)

2S24C-1
Introduction of hair cell function

Hibino, Hiroshi(*Department of Molecular Physiology, Niigata University School of Medicine, Niigata, Japan*)

Hair cells in the cochlea of the mammalian inner ear are sensory epithelia that lie above the basilar membrane and play central roles in hearing system. Sound-induced vibration of the basilar membrane deflects the stereocilia of the hair cells and opens the mechnoelectrical transduction channels at their top. Substantially cation in the extracellular solution enters into the hair cell, which depolarizes them. This process transforms the mechanical energy of sounds into electrical signals. Excitation of hair cells secretes the neurotransmitter glutamate by unique molecular architecture in their presynaptic region. Furthermore, hair cells amplify faint sound energy by dynamic physiological phenomena that emerge in the stereocilia and in the cell bodies. In this talk, I will explain how hair cells work in the cochlea as the introduction of this symposium. No COI.

2S24C-2
Mechanoelectrical transduction channels in the inner ear: TMC1 and TMC2

Kawashima, Yoshiyuki(*Department of Otolaryngology, Tokyo Medical and Dental University, Tokyo, Japan*)

Transmembrane channel-like 1 (*TMC1*) gene was identified through positional cloning of the gene underlying dominant and recessive nonsyndromic hearing loss at the DFNA36 and DFNB7/B11 loci, respectively. Mouse *Tmc1* mRNA is expressed in inner ear hair cells and p.M412K point mutation in TMC1 cause hearing loss in Beethoven ($Tmc1^{Bth}$) mice. The closely related *Tmc2* gene is also expressed in hair cells. *Tmc1* and *Tmc2* encode six-pass integral membrane proteins with sequence and topology similar to each other. A recent report suggests that *C. elegance tmc-1* forms non-selective cation channels when expressed in heterologous cells. The onset of *Tmc2* expression in the inner ear coincides with development of hair cell transduction. Mice with a target deletion of *Tmc1* are deaf, while those with a deletion of *Tmc2* are phenotypically normal. Mice that are double homozygotes for targeted deletions of *Tmc1* and *Tmc2* exhibit vestibular dysfunction as well as profound deafness. Double homozygous mutant hair cells show intact stereocilia yet have no transduction. Exogenously expressed TMC proteins can be localized near tips of stereocilia and either TMC1 or TMC2 can rescue transduction in the double homozygous mutant hair cells. Hair cells that expressed *Tmc2* alone has large single-channel currents relative to those recorded from hair cells that expressed *Tmc1*. Importantly, hair cells that expressed $Tmc1^{Bth}$ has significantly small single-channel currents relative to those recorded from hair cells that expressed wild-type *Tmc1*. The data indicate that TMC1 and TMC2 are components of mechanoelectrical transduction channels. No COI.

2S24C-3
Tip link is an asymmetric filament formed by heterophilic interaction of cadherin 23 and protocadherin 15

Sakaguchi, Hirofumi(*Department of Otolaryngology-Head and Neck Surgery, Kyoto Prefectural University of Medicine, Japan*)

Hair cells are specialized mechanosensors that conducts mechanoelectrical transduction (MET). MET in hair cells occurs in hair bundles, comprised of dozens of stereocilia. MET channels are localized close to tip links, the extracellular filaments connecting the tip of each stereocilium to its neighbor. Hair bundle deflection generates tension in tip links, which rapidly increase in the open probability of MET channels. Two members of the cadherin family, cadherin 23 (CDH23) and protocadherin 15 (PCDH15), had been identified as integral components of tip links, but its true entity was still unknown. We first demonstrated that heterophilic interaction of CDH23 homodimers and PCDH15 homodimers forms a tip link filament using structural and biochemical assays. Immuno-TEM study showed that both CDH23 and PCDH15 extracellular cadherin domains (ECDs) localize at the upper and lower part of a tip link, respectively. Labeling of N-termini of CDH23 and PCDH15 colocalized in the tip link, suggesting that the two cadherins interact via their N-termini. Negative-staining TEM showed that recombinant ECD of CDH23 and PCDH15 form coiled homodimers that interact at their N-termini, resembling tip link structure. Interaction of the CDH23 and PCDH15 ECDs was also confirmed by biochemical assay. Our data identified the constituents of the tip link and revealed its asymmetric molecular property, which shed new light on the molecular basis of MET and the mechanisms causing hereditary deafness, noise-induced hearing loss and presbycusis. No COI.

2S24C-4

Dissection of synaptic function of otoferlin at the mouse inner hair cell afferent synapse

Takago, Hideki[1,2,3]; Moser, Tobias[2,3]([1]*Perceptual Funtions Section, Department of Rehabilitation for Sensory Functions, Research Institute, National Rehabilitation Center for Persons with Disabilites, Tokorozawa, Japan,* [2]*InnerEarLab, Department of Otolaryngology, Univerisity Medical Center Göttingen, Göttingen, Germany,* [3]*Bernstein Centor for Computational Neuroscience, Univeristy of Göttingen, Göttingen, Germany*)

Ca^{2+} signals and subsequent exocytosis at the inner hair cell (IHC) afferent synapse underlie the encoding of sound. The multi-C_2 domain protein otoferlin, whose mutations cause the autosomal recessive deafness DFNB9, has been believed to control Ca^{2+}-triggered exocytosis at this synapse by means of neuronal SNARE proteins. However, a recent study has argued that the hair cell afferent synapse apparently operates without neuronal SNARE proteins, requesting a detailed evaluation of the synaptic function of otoferlin at a single synapse level. Therefore, we performed patch-clamp recordings from postsynaptic boutons of spiral ganglion neurons in wild-type and otoferlin mutant mice, each of which contacts a single IHC through a single ribbon-type synapse. We found that deletion of otoferlin decreased frequency of both spontaneous and high potassium-evoked release, confirming the role of otoferlin for priming and/or fusion of synaptic vesicles. Furthermore, we found that deletion of otoferlin decreased EPSC amplitude, but not abolished large and varied amplitude of EPSCs, supporting the hypothesis that uniquantal release of synaptic vesicles is a fundamental mechanism at the IHC afferent synapse. No COI.

Symposium 25
Pathophysiological roles of TRPM subfamily in stressed heart

(March 17, 10:05–12:05, Room D1)

2S24C-5

Contributions of two force-generating mechanisms of hair cell to nonlinear amplification in the mammalian cochlea

Nin, Fumiaki[1]; Reichenbach, Tobias[2]; Fisher, Jonathan A.N.[2]; Hudspeth, J. A.[2]([1]*Department of Molecular Physiology, Niigata University School of Medicine, Niigata, Japan,* [2]*Rockefeller University, New York, US*)

The cochlear traveling waves' high sensitivity stems from the active process of outer hair cells. The active process possesses two force-generating mechanisms: active hair-bundle motility elicited by Ca^{2+} influx and somatic motility mediated by the voltage-sensitive protein prestin. Although interference with prestin has demonstrated a role for somatic motility in the active process, it remains unclear whether hair-bundle motility contributes in vivo. We selectively perturbed the two mechanisms by infusing substances into the endolymph or perilymph of the chinchilla's cochlea and then used scanning laser interferometry to measure vibrations of the basilar membrane. Blocking somatic motility, damaging the tip links of hair bundles, or depolarizing hair cells eliminated amplification. While reducing amplification to a lesser degree, pharmacological perturbation of active hair-bundle motility diminished or eliminated the nonlinear compression underlying the broad dynamic range associated with normal hearing. The results suggest that not only somatic motility but also active hair-bundle motility plays a significant role in the amplification, and active hair-bundle motility contributes compressive nonlinearity of the cochlear traveling wave. No COI.

2S25D1-1

9-Phenanthrol, a TRPM4 Inhibitor, protects rat hearts from ischemia-reperfusion injury

Takahashi, Ken[1]; Piao, Hulin[1,2]; Wang, Jing[1,3]; Naruse, Keiji[1]
([1]*Okayama University Graduate School of Medicine, Dentistry and Pharmacological Sciences,* [2]*Department of Cardiovascular Surgery, The Second Affiliated Hospital of Jilin University, Changchun, China,* [3]*Qingdao Municipal Hospital, Qingdao, China*)

Ischemic heart disease not only remains the leading cause of mortality, but also leaves complications such as angina pectoris or arrhythmias. TRPM4 channels are involved in the pathogenesis of various cardiovascular diseases. Here we explored the possible involvement of TRPM4 channels in the development of ischemia-reperfusion (I/R) injury. Pretreatment with 9-phenanthrol (9-Phe), a specific inhibitor of TRPM4, improved the survival rate of rats underwent left anterior descending coronary artery occlusion followed by reperfusion *in vivo*. In isolated rat hearts, 9-Phe pretreatment recovered cardiac contractility dramatically, with reduced tissue damage and arrhythmic events. Surprisingly, simultaneous application of 5-HD, a blocker of the mitochondrial K_{ATP} channel, did not abolish the cardioprotective effect produced by 9-Phe, suggesting that the cardioprotection by 9-Phe is not derived from K_{ATP} channel necessary for classic cardioprotection pathway. Furthermore, a rat cardiomyocyte cell line H9c2 was used to test whether cardiomyocytes have protective function against I/R injury by themselves. 9-Phe pretreatment kept viability of H9c2 cells underwent oxidative stress by H_2O_2 (mimicking I/R damage) and anoxia. TRPM4 channels may serve as an effective pharmacological target for cardioprotective treatment strategies against I/R injury. No COI.

2S25D1-2

Enhanced PIP_2 sensitivity of human TRPM4 channel with an arrhythmic mutation

Hu, Yaopeng[1]; Kurahara, Rin[1]; Okamura, Yasushi[2]; Mori, Masayuki[3]; Inoue, Ryuji[1]([1]*Dept. Physi., Grad.Sch. Med. Sci., Fukuoka Univ.,* [2]*Lab. Integ. Physiol., Dept. Physiol., Grad. Sch. Med., Osaka Univ.,* [3]*Dept. Synth. Biol.Chem., Grad. Sch. Engineer., Kyoto Univ.*)

TRPM4 is a Ca^{2+}-activated monovalent cation-selective channel and known to be involved in both congenital and acquired forms of cardiac arrhythmias. In this study, we explored how this channel is regulated by endogenous PIP_2 level which would fluctuate under neurohormonal stresses, and also the functional impact of an arrhythmic mutation E7K thereon, by using the Danio rerio voltage-sensing phosphatase (VSP). VSP was coexpressed in HEK293 cells with human TRPM4 [$EKE^{5\text{-}7}$ (wild-type), $EKK^{5\text{-}7}$ (E7K mutant), or $ENE^{5\text{-}7}$ mutant] and currents induced by intracellular perfusion of 1μM Ca^{2+} were recorded by the patch clamp technique. When the Ca^{2+}-activated currents were stabilized, a set of depolarizing pulses (from -60 to +120mV; 300–2000ms in duration) were repetitively applied to reduce endogenous PIP_2 content via VSP activation. Prolonging the duration of the pulses progressively inhibited the Ca^{2+}-activated currents, and recovery from it required several seconds. The apparent sensitivity to PIP_2 reduction caused by VSP activation was stronger in the rank order of $ENE^{5\text{-}7}$>wild-type>E7K, whereas the recovery from the inhibition was more rapid; E7K>wild-type> $ENE^{5\text{-}7}$. These results suggest that endogenous PIP_2 is essential to maintain TRPM4 channel activity presumably through electrostatic interactions dependent on the charged status of the 5th to 7th N-terminal amino acid residues, which might be pathologically enhanced in the E7K mutation. No COI.

2S25D1-3

Cardiac TRPM2 aggravate ischemia reperfusion injury

Numata, Tomohiro[1]; Shimizu, Shunichi[2]; Mori, Yasuo[1]([1]*Department of Technology and Ecology, Hall of Global Environmental Research, Kyoto University, Kyoto, Japan,* [2]*Department of Pathophysiology, Showa University School of Pharmacy, Hatanodai, Shinagawa-ku, Tokyo, Japan*)

Transient receptor potential melastatin 2 (TRPM2) is a Ca^{2+}-permeable nonselective cation channel activated by oxidatative stress. However, it remains unknown whether TRPM2 contributes to heart function during ischemia-reperfusion (I/R). Here we show that the TRPM2 aggravate I/R injury in cardiac myocyte. In isolated cardiac cells from adult mouse hearts, whole cell recordings revealed that the ADP ribose-induced macroscopic cationic currents exhibit TRPM2-like properties such as linear rectification and sensitivity to econazol. The whole cell cation current was also activated by H_2O_2 and oxygen glucose deprivation (OGD)/reperfusion. RT-PCR and real time PCR demonstrated molecular expression of TRPM2 in isolated cardiac cells from adult mouse hearts. In isolated cardiac cells from TRPM2-deficient mice, H_2O_2- or OGD-induced whole-cell currents and cell death were suppressed. In the Langendorff perfusion model, we found that left ventricular pressure, heart rate, rate pressure were recovered earlier after reperfusion in TRPM2-deficient mice than in wild type mice. It is concluded that activation of endogenous myocardial TRPM2 is involved in myocardial injury induced by I/R *ex vivo* and *in vitro*. Cardiac TRPM2 may serve as a target accessible even after ischemic attack for pharmacotherapeutic intervention in I/R-induced myocardial infarction.
No COI.

2S25D1-4

Activation of TRPM2 channels in neutrophils enhances myocardial ischemia/reperfusion injury

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Transient receptor potential melastatin 2 (TRPM2) is a Ca^{2+}-permeable nonselective cation channel activated by oxidative stress, and is expressed in neutrophils and cardiomyocytes. We examined whether TRPM2 contributes to myocardial ischemia-reperfusion (I/R) injury. Wild-type (WT) and Trpm2 knockout (KO) mice were exposed to I/R by ligation of the left coronary artery. Myocardial infarction following I/R but not ischemia alone was reduced in Trpm2 KO mice, and cardiac contractile function was also improved in Trpm2 KO mice. Moreover, neutrophil accumulation in the reperfused area was lowered in Trpm2 KO mice. When WT or Trpm2 KO polymorphonuclear leukocytes (PMNs) were administered to the Trpm2 KO heart ex vivo through perfusate or in vivo by intravenous injection, WT PMNs induced more severe cardiac injury following I/R compared with Trpm2 KO PMNs. In WT but not in Trpm2 KO PMNs, the combination of H_2O_2 and leukotriene B4 (LTB4) resulted in enhancement of the increase in intracellular Ca^{2+} and their adhesion to endothelial cells. These findings indicate that TRPM2 is implicated in the development of myocardial reperfusion injury. Accumulation of neutrophils in the heart triggered by activation of neutrophil TRPM2 by H_2O_2 and LTB4 is likely to have a crucial role in myocardial I/R injury. No COI.

Symposium 26
Modulation of cellular functions by heat

[Collaboration Symposium with The Biophysical Society of Japan]

(March 17, 9:00–11:00, Room E)

2S26E-1

Detection of temperature change in norepinephrine-stimulated brown adipocytes with a bimaterial microcantilever

Sato, Masaaki; Ishijima, Akihiko(*Institute of Multidisciplinary Research for Advanced Materials, Tohoku University, Sendai, Japan*)

Temperature globally affects biological activities and chemical reactions in living things. In mammals, brown adipocytes generate heat to maintain body temperature. Although the mechanism of heat generation has been studied in cell suspensions containing 10^5 or more adipocytes, heat generation and temperature elevation in individual cells has not been measured. In addition, measuring the rate of oxygen consumption in a closed vessel would disrupt the cell's natural condition, by the depletion of substances required for generating heat, such as oxygen dissolved in the solution.
In view of the need for a method to investigate thermogenic capacity non-invasively at the cellular level with keeping cell's natural condition, we fabricated a bimaterial microcantilever that can detect millikelvin-order temperature changes in solution. We observed bending in the microcantilever in response to a temperature rise of about 0.1 K per cell, when it was placed with 4–7 norepinephrine-stimulated brown adipocytes. Our experimental system revealed that when adequate dissolved oxygen was available, the amount of heat generated by the brown adipocytes gradually increased over a period of several hours. Our bimaterial microcantilever can be used to observe changes in cellular temperature in a wide range of cell types, including virus-infected and cancer cells, and our study of brown adipocytes at the single-cell level may contribute to medical approaches to treating metabolic syndromes and lifestyle-related diseases. No COI.

2S26E-2

Microscopic heat pulses reveal novel temperature-sensitivities of living cells

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The chemical reactions of living cells cause local and temporary temperature changes, which have the potential to modulate the activity of both the cells themselves and also the surrounding cells. The viewpoint of "Thermo-Chemical Signaling" should be essential for understanding how the living cells efficiently use the surrounding local temperature changes and those within the cell itself. We have revealed novel temperature-sensitivities of living cells using the microheating systems and local temperature measurements. In this presentation, we would like to introduce the following 3 topics.
(1) A microscopic heat pulse induces Ca^{2+} burst in human cancer cells.
(2) Ca^{2+}-independent contractions of cardiomyocytes induced by microscopic heat pulses.
(3) "Walking nanothermometers" reveal temperature-sensitivity of transported acidic organelle in living cells.
No COI.

2S26E-3

Thermometry in aqueous solution at single cell-scale using fluorescent nanothermometers

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Carrier materials used in drug delivery systems (DDS) should target the certain regions in a body while avoiding undesirable entrapments by cells and organs. In addition to the targeting ability, the controlled release of drugs in the right place at the right time supports the system's efficiency and versatility. Current stimuli-responsive DDS in clinical trials rely on the externally applied temperature change. Yet the details of heating such as the temperature distribution and its time course are not fully clarified, especially at the scale of single cell. I will present our newly developed methods for the temperature measurement in aqueous condition under the fluorescence microscope. I will show that our fluorescent thermometers are compatible with living cells, and discuss our new findings in HeLa cells obtained by combining our thermometry with [Ca2+] imaging. We envisage these methods improving our understanding in local temperature dynamics from sub-cellular scale to the spheroid cultures, possibly towards live animals in near future. No COI.

2S26E-4

In vivo gene manipulation in the targeted single cells using an infrared laser

Kimura, Eiji[1]; Kamei, Yasuhiro[2](*[1]Dept. of Anat., Iwate Med. Univ., Iwate, Japan, [2]Spectrography and Bioimaging Facility, NIBB, Aichi, Japan*)

In vivo spatiotemporal gene regulation in a single cell is obligatory to analyze specific gene functions associated with morphogenesis in developing embryos. The infrared laser-evoked gene operator (IR-LEGO) system is a new microscope optimized to heat cells using an infrared (IR) laser, which has a superior ability to heat water. A combination of the heat shock protein (hsp) promoter and IR laser irradiation enables the spatiotemporal gene induction in targeted cells. We reported this system was efficient in regulating spatiotemporal gene expression, and IR laser-mediated gene induction in single targeted cells in nematodes (*Caenorhabditis elegans*), and the local gene expression in various tissues, such as the muscle, notochord, and retina in some living vertebrates, for example, zebrafish (*Danio rerio*) and medaka (*Oryzias latipes*). More recently, we applied this system to the vascular biology in zebrafish and established an excellent method to induce laser-mediated gene expression in single targeted endothelial cells of vertebrate organism *in vivo*. We optimized the irradiation conditions, which resulted in raising the efficiency of laser-mediated gene induction up to 60%. Furthermore, we applied this method to the endothelial cells of the first intersegmental arteries (SeAs) to evaluate the system, and revealed their contribution in connecting the vascular systems of the brain and spinal cord. Our achievement in this study will lead us to the prospect of uncovering the mechanisms underlying morphogenesis of vertebrate model organisms. No COI.

2S26E-5

The physiological roles of thermosensitive channel TRPM2

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The gene encoding the capsaicin receptor as a noxious heat sensor, which is now called TRPV1, was isolated from a rodent sensory neuron cDNA library in 1997 and was considered to be a breakthrough for the research concerning temperature sensing. Since then, several TRP channels having thermosensitive ability have been identified in mammals, with ten thermosensitive TRP channels reported in mammals to date. While searching for a novel thermosensitive TRP channel, we found that TRPM2 has thermosensitivity, and its temperature-dependent activation is drastically enhanced by co-application of ligands such as cyclic adenosine 5-diphosphoribose (cADPR). Interestingly, TRPM2 is mainly expressed in the tissues not exposed to the drastic temperature changes. TRPM2 was originally cloned as the target of ADPR that causes an intracellular Ca^{2+} increase, and has been reported to be activated by nicotinamide adenine dinucleotide, hydrogen peroxide and intracellular Ca^{2+}. In addition, TRPM2 has high Ca^{2+}-permeability, indicating that its physiological roles might depend on intracellular Ca^{2+} increases. In this symposium, I would like to talk about the physiological role of TRPM2 in pancreas and immunocytes. Especially, I focus on the involvement of TRPM2 in the development of diabetes and degranulation from mast cells. No COI.

Symposium 27
New developments of mathematical physiology

(March 17, 9:00–11:00, Room F)

2S27F-1

Modeling analysis of inositol 1,4,5-trisphosphate receptors (IP3R)-mediated calcium mobilization from intracellular stores in pancreatic beta cells.

Takeda, Yukari; Takeda, Yukari; Noma, Akinori(*Ritsumeikan University, Faculty of Bioinformatics, Kusatsu, Japan*)

Upon elevation of plasma glucose concentration, pancreatic β-cells generate bursts of action potentials and induce cyclic changes in $[Ca^{2+}]_i$ regulating pulsatile insulin release. GLP-1, an incretin hormone, synergistically enhances glucose-dependent insulin secretion by modulating multiple ion channels and exocytotic machinery proteins through PKA- and EPAC-dependent mechanisms. PKA-mediated intracellular Ca^{2+} mobilization via IP3Rs was suggested to be part of the mechanism by which cAMP amplifies insulin release. Increase in $[Ca^{2+}]_i$ would modulate activities of multiple Ca^{2+}-sensitive ion channels and transporters as well as mitochondrial and membrane enzymes which regulate $[ATP]_i$ and $[cAMP]_i$, thus intricately affecting cellular responsiveness. Intracellular Ca^{2+} relese channels in conventional β-cell models, however, have been described as a simple leak channel which would not be able to reproduce dynamic ca release from stores. Here we developed a mathematical model of IP3R and reconstructed Ca^{2+} transients and oscillations that are generated during GLP-1 stimulation in pancreatic β-cells. Simulation studies indicated that these events of Ca mobilization were generated by positive feedback on the Ca^{2+}-dependent activation of the channel. Slow rate of Ca^{2+}-dependent inactivation was also suggested to determine the time course of decay of Ca^{2+} transients. Interestingly, mathematical analysis revealed that the slow rate of Ca^{2+}-dependent inactivation of the channel is the key to generating Ca^{2+} oscillations. No COI.

2S27F-2

An updated model of interstitial cells of Cajal reproducing intestinal pacemaker activity

Youm, Jae Boum[1]; Kim, Hyoung Kyu[1]; Heo, Hye-Jin[1]; Kim, Nari[1]; Leem, Chae Hun[2]; Han, Jin[1]([1]*Department of Physiology, College of Medicine, Inje University,* [2]*Department of Physiology, University of Ulsan College of Medicine, Seoul, Korea*)

Interstitial cells of Cajal play as a pacemaker in gastrointestinal system. Previously, we constructed a biophysically based model of interstitial cells of Cajal in mouse small intestine. Since ion channels contributing to the pacemaker activity have been substantially updated, we tried to improve our mathematical model. We incorporated 5 more ion channels into our previous model. They are voltage-gated Na^+ channel (Nav 1.5), Ca^{2+}-activated Cl^- channel, ERG K^+ channel, Ca^{2+}-activated K^+ (BK) channels, and Na^+-leak channel (NALCN). The IP_3-mediated Ca^{2+} release is a key event to drive regenerating pacemaker potentials and was updated to reproduce its stochastic behavior. The stochastic currents were reproduced by simulating the random openings and closing of individual ion channel. The updated model was able to reproduce stochastic features of pacemaker potentials. They were not uniform in size, duration, and frequency, which correspond to those of experimental recordings. The model suggests that the Na^+-leak channel contributes to depolarization about 10 mV in resting membrane potential. The model also suggests that Ca^{2+}-activated Cl^- channel is more likely to stabilize membrane potential rather than to excite under the physiological condition. We conclude that this improved mathematical model could give an insight how ion channels and stochastic IP_3-mediated Ca^{2+} release drive pacemaker activity in gastrointestinal system. No COI.

2S27F-3

Simulation study of Ca2+ response in lymphocytes

Matsuoka, Satoshi[1]; Bongju, Kim[2]; Ayako, Takeuchi[1]; Orie, Koga[3]; Masaki, Hikida[3](*[1]Integr. Physiol. Fac. Med. Sci. Univ. Fukui, [2]Inst. Genome Res., Univ. Tokushima, [3]Cent. Innov. Immunoreg. Tech. and Therap. Kyoto Univ.*)

To clarify the molecular mechanisms underlying antigen-receptor induced Ca^{2+} response of lymphocytes, we created a mathematical model of the lymphocyte Ca^{2+} dynamics. Following processes are modeled. 1) Antigen-receptor mediated production of inositol 1,4,5-trisphosphate (IP3) by phospholipase C and the degradation by inositol phosphate 5-phosphatases and IP3 3-kinase. 2) Endoplasmic reticulum (ER) Ca^{2+} uptake by Ca^{2+} pump, Ca^{2+} release by IP3 receptor. 3) Mitochondrial Ca^{2+} influx by Ca^{2+} uniporter and Ca^{2+} efflux by Na-Ca exchange (NCXm). 4) ER Ca^{2+} dependent transition of stromal interaction molecule 1 and its activation of CRAC channel. 5) Plasma membrane Ca^{2+} extrusion by Ca^{2+} pump and Na-Ca exchange, and cytoplasmic Ca^{2+} ($Ca^{2+}{}_i$) buffer by calmodulin. Ordinary differential equations were integrated by Runge-Kutta methods. The model well reproduced antigen-receptor mediated $^{2+}{}_i$ rise of B lymphocytes. Systematic analysis of the model predicted that NCXm activity affects ER Ca^{2+} content and antigen-receptor mediated $Ca^{2+}{}_i$ rise. The model prediction was validated in DT 40 and A20 B lymphocytes. Pharmacological inhibition of NCXm by CGP-37157 and a targeted knockout or knockdown of NCXm gene (NCLX) markedly decreased ER Ca^{2+} content, suppressing the antigen-receptor induced $Ca^{2+}{}_i$ rise. We concluded that NCXm functions as a Ca^{2+} provider to ER and is important in antigen-receptor induced Ca^{2+} response of lymphocytes. No COI.

2S27F-4

Model prediction and experimental validation of mechanisms regulating synaptic plasticity in the cerebellum

Kawaguchi, Shin-ya(*Graduate School of Brain Science, Doshisha University, Kyoto, Japan*)

Synaptic plasticity, neuronal activity-dependent sustained alteration of the efficacy of synaptic transmission, is a cellular basis for learning and memory. Many forms of synaptic plasticity are induced by an increase in intracellular Ca2+ concentration ([Ca2+]i) through activation of complicated intracellular signaling network including several feedback loops. To intuitively understand the dynamic behavior of synaptic plasticity induction, quantitative simulation of the molecular network is useful. We have constructed a kinetic simulation model of inhibitory synaptic plasticity in the cerebellum, and systematically analyzed the behavior of intricate molecular networks composed of protein kinases, phosphatases, etc. Simulation analyses have provided several predictions, such as the mechanisms regulating Ca2+ threshold for the plasticity induction, the dynamic plasticity regulation by the temporal context of [Ca2+]i increase, and the cooperatively regulation of different forms of plasticity at excitatory and inhibitory synapses. In this symposium, I will show dynamic regulation mechanisms of synaptic plasticity revealed by combined application of modeling and experimental validation. No COI.

2S27F-5

EAD and DAD mechanisms analyzed by developing a new ventricular cell model; quantitative analysis by applying mathematical analyses

Asakura, Keiichi[1,2]; Asakura, Keiichi[1,2]; Cha, ChaeYoung[3]; Horikawa, Yuusuke[2]; Memida, Hiraku[2]; Yamaoka, Hiroyo[2]; Trevor, Powell[4]; Noma, Akinori[2](*[1]Pharmacokinetics and Safety Assessment Dept., Nippon Shinyaku Co., Ltd., Kyoto, Japan, [2]Department of Bioinformatics, Ritsumeikan University, Japan, [3]Oxford Centre for Diabetes, Oxford University, UK, [4]Laboratory of Physiology, Oxford University,UK*)

Drugs that prolong the QT interval present a major safety concern for pharmaceutical companies. The QT prolongation has been linked with the potentially lethal ventricular arrhythmia Torsades de Pointes (TdP). However, hERG-channel block and QT prolongation are neither necessary nor sufficient conditions for a torsadogenic risk, despite a strong association. Because a precise mechanisms that lead to initiation of TdP remain undetermined. Proarrhythmic vulnerability linked to impairment of repolarization that supports early- (EAD) or delayed-afterdepolarizations (DAD). Here, we focused on the membrane- and Ca^{2+}-mechanisms underlying both EAD and DAD in a quantitative manner by constructing a new ventricular cell model. The ion channel models were mostly based on electrophysiological data from human myocytes and the LCC-RyR model based on local control theory was adopted. EADs and DADs were evoked by applying several condition. Contributions of ion channels and transporters were quantified with the lead potential (VL) analysis, which revealed that I_{K1} and I_{Kr} played the primary role of EAD initiation. I_{CaL} and I_{NCX} amplified EAD. We believe that such application of mathematical analyses to a realistic cell model is a powerful tool to evaluate and predict proarrhythmic potential. No COI.

Symposium 28
Leading edge of research on neuronal circuitry underlying cognitive functions

(March 17, 9:00–11:00, Room G)

2S28G-1

Computational principles of microcircuit operations for representation and memory retrieval of objects in macaque temporal cortex

Hirabayashi, Toshiyuki(*Dept. of Physiol., The Univ. of Tokyo Sch. of Med., Tokyo, Japan*)

Inferior temporal (IT) cortex in monkeys locates at the final stage of visual object processing. Although neuronal responses in this cortical region have been characterized with various task paradigms, it remains largely unknown how individual neurons interact to form functional circuits for implementing cognitive demands. Here, I will discuss microcircuit operations in macaque IT cortex in an object-association memory task, focusing on two different topics. The first topic is the circuit operation for object memory retrieval. We found the directed signal flow between functionally different classes of memory neurons that generates memory retrieval signal of visual objects. The second topic is hierarchical object processing across cortical areas. The representation of a given feature of visual objects has been believed to be constructed in the cortical area where that representation is prevalent. However, another possible scheme is that preparatory codes of a given feature are created in a lower-order area before their increase to become predominant in a higher-order area. In the object association task paradigm, we found the microcircuits that generate and increase the representations of object associations in successive lower- and higher-order areas in the IT cortex, respectively, consistent with the latter hypothesis. Together, these results demonstrate that examinations of microcircuit operations in monkeys performing an object association memory task provided the principles of cortical computations for representation and memory retrieval of visual objects. No COI.

2S28G-2

Cognitive signals in midbrain dopamine neurons

Matsumoto, Masayuki(*Laboratory of Cognitive and Behavioral Neuroscience, Division of Biomedical Science, Faculty of Medicine, University of Tsukuba*)

Dopamine neurons in the substantia nigra pars compacta (SNc) and the ventral tegmental area (VTA) are well known for their crucial roles in reward processing. A separate line of research, on the other hand, has established that neurotransmitter dopamine is essential to cognitive functions such as working memory and attention. However, despite abundant studies demonstrating dopamine neuron activity related to reinforcement and motivation, little is known about what signals dopamine neurons convey to promote cognitive processing. In order to identify cognitive signals carried by dopamine neurons, we recently conducted single-unit recording from dopamine neurons in the SNc and VTA while monkeys were engaged in a cognitive task that required working memory and visual search. We found that the activity of dopamine neurons at different locations reflected signals suitable for distinct roles in cognitive processing. A subset of dopamine neurons were activated by visual stimuli if the monkey had to store the stimuli in working memory. These neurons were located dorsolaterally in the SNc, whereas ventromedial dopamine neurons, some in the VTA, represented conventional reward prediction signals. Furthermore, dopamine neurons monitored visual search performance, becoming active when the monkey made an internal judgment that the search was successfully completed. Our findings suggest an anatomical gradient of dopamine signals along the dorsolateral-ventromedial axis of the ventral midbrain. No COI.

2S28G-3

Clarifying the neural mechanisms of social action monitoring using macaques

Isoda, Masaki(*Department of Physiology, Kansai Medical University School of Medicine, Osaka, Japan*)

The last decade has seen a surge of interest in the study of social brain functions. Research in this field, called social neuroscience, has been carried out mostly using a neuroimaging technique for human subjects. However, given the limited spatiotemporal resolution inherent in the methodology, a systems neuroscience approach using macaque monkeys may provide a useful platform for understanding social brain functions at the cellular level, thereby complementing neuroimaging techniques. We recently developed an experimental paradigm in which two monkeys actively monitored each other's actions for their own action selection. Using this model, we recorded single-unit activity in the medial frontal cortex (MFC), a critical node in the social brain networks. Results revealed that the MFC encompassed a selective neural code for the action of the partner, in addition to a selective neural code for one's own actions and a shared neural code for the self and partner's actions. Further, a sizable number of MFC neurons selectively responded to partner's errors. These partner-error neurons were distributed in two segregated populations: the dorsomedial convexity, where other's errors were detected, and cingulate sulcus patches, where one's correct behavior following other's errors was encoded. These findings suggest that the MFC plays a role in self-other differentiation and monitoring others' behavior for adaptive social decision making. The continuing effort in this research direction will uncover the neural basis whereby primates have become such a successful social being in the animal kingdom. No COI.

2S28G-4

What's a function of the pulvinar as a subcotical hub for the visual system?

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The pulvinar, a visual thalamic nucleus, does not exist in the rodent brain and shows a marked evolutionary expansion in the primate brain. It interconnects with multiple visual cortices. Despite of its central position in visual system, the functional role of the pulvinar in vision remains elusive. Using a combination of psychophysics, neural recordings, pharmacological inactivation and computational modeling, we have found the neural correlates and causes of perceptual confidence in the pulvinar. We trained the monkeys to perform a categorization task for the visual stimuli with color-motion pairing. In a categorization task, the pulvinar responses correlated with stimulus ambiguity. In an opt-out task, the pulvinar responses to the same physical stimulus decreased when the monkeys chose escape options, indicating less confidence in visual categorization. Functional silencing of the unilateral pulvinar caused to increase frequencies of the opt-out behaviors, only when the visual target appeared in the contralateral visual field to the inactivation site of the pulvinar. These results indicate that the pulvinar plays a crucial role in a subject's certainty of the perceived world, thereby potentially underlying visual consciousness. No COI.

2S28G-5

Neural basis of temporal processing: a role of the cerebellum

Tanaka, Masaki(*Department of Physiology, Hokkaido University School of Medicine*)

Temporal processing over a short period of time is important for both motor control and perception. For example, we are able to detect slight changes in musical rhythm, which requires temporal prediction of regular beat. Previous studies suggest that the cerebellum may play a role in such processes. However, the underlying neuronal mechanism remains largely unknown.
To address this, we performed experiments in monkeys. When the animals were required to detect a sudden omission of isochronous repetitive stimuli (missing oddball paradigm), neurons in the deep cerebellar nuclei exhibited firing modulation that gradually increased as the repetition progressed. The magnitude of firing modulation for each stimulus was positively correlated with the length of the inter-stimulus interval in a given trial, indicating that the sensory gain depended on the time elapsed from the preceding stimulus. Because inactivation of recording sites delayed the detection of stimulus omission, the temporally specific sensory signals in the cerebellum might be converted into the prediction error signals for stimulus omission in the downstream thalamocortical networks. Taken together with the data obtained from the ventrolateral thalamus, I will discuss the possible information processing through the cerebello-thalamocortical pathways for temporal prediction. No COI.

Symposium 29
Relationship between cardiovascular physiology and physical therapy
[Collaboration Symposium with Japanese Physical Therapy Association]

(March 17, 8:40–10:20, Room H1)

2S29H1-1

Central regulation of skeletal muscle blood flow during exercise

Ishii, Kei; Matsukawa, Kanji(*Department of Integrative Physiology, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan*)

Whether a centrally-induced neural mechanism plays a role in exercise hyperemia in skeletal muscle is controversial. Some animal studies have reported centrally-induced sympathetic cholinergic vasodilatation in skeletal muscle at start of exercise, which may serve as a feedforward regulatory signal for increasing muscle blood flow. However, such neural mechanism has been denied in humans, because cervical sympathectomy and muscarinic blockade had no impact on exercise hyperemia. On the other hand, the sympathetic nervous system predominantly regulates blood flow to non-contracting muscle. Recently we have demonstrated that blood flow to non-contracting muscle increased at start of voluntary one-legged cycling and during imagery of the exercise, suggesting that central command contributes to the muscle hyperemia. Since imagery of the exercise increased blood flow in bilateral muscles to the same extent, the centrally-induced vasodilator signal appears to be transmitted equally to the bilateral muscles. In this context, we found that blood flow in contracting muscle as well as non-contracting muscle increased at start of one-legged cycling and that the hyperemic responses in both muscles were blunted by atropine. Thus it is concluded that central command causes cholinergic vasodilatation equally in bilateral muscles, which contributes to muscle hyperemia at start of voluntary exercise. Such central vasodilator mechanism may be applied to a new rehabilitation approach to improve muscle circulation, disorder of which may result in exercise intolerance. No COI.

2S29H1-2

Effect of exercise intensities on endothelial function in chronic and acute phases

Goto, Chikara(*Department of Rehabilitation, Hiroshima International University, Hiroshima, Japan*)

Background: It is well known that endothelial dysfunction is an initial step of atherosclerosis process, leading to the onset of cardiovascular event. Also, physical training and exercise have been established to improve endothelial function not only in patients with hypertension, coronary artery disease, and chronic heart failure, but also in healthy individuals. However, there is no information of the relationship between exercise intensities and endothelial function so far. Method and result: To determine the effect of different exercise intensities on endothelial function, we evaluated the endothelial function by measuring forearm blood flow (FBF) response before and after different intensities for 12 weeks (mild, 25% V02max; moderate, 50% V02max; and high, 75% V02max; bicycle ergometers) in healthy young men. FBF response was significantly increased after moderate-intensity exercise for 12 weeks, but not after mild- and high-intensity. Interestingly, moderate-intensity exercise tended to decrease both indices of oxidative stress, whereas high-intensity exercise increased. Next, we sought to determine the acute effect of different exercise intensities on endothelial function. Then, similarly, we also found that the exercise of moderate-intensity, but not of mild- and high-intensities, could substantially improve FBF even in an acute phase. Conclusions: These findings suggest that moderate-intensity exercise could improve endothelial function even in healthy humans through a decrease of oxidative stress and subsequent increase of NO production not only in chronic, but also acute phases. No COI.

2S29H1-3

Effect of exercise for Left Ventricular Assist Device patients

Yanase, Masanobu[1]; Nakatani, Takeshi[1]; Kumasaka, Reon[2]; Arakawa, Tetuso[2]; Nakanishi, Michio[2]; Noguchi, Teruo[2]; Oohara, Takahiro[2]; Fukui, Shigefumi[2]; Goto, Yoichi[2]([1]*Department of Transplantation, National Cerebral and Cardiovascular Center,* [2]*Department of Cardiovascular Medicine, National Cerebral and Cardiovascular Center*)

The Left ventricular assist device (LVAD) is an alternative therapy for the patient with advanced heart failure who does not respond to conventional medical treatments. We have previously reported that in addition to improve exercise tolerance, exercise therapy for the patient with paracorporeal LVAD (p-LVAD) could potentially improve survival on LVAD. From April 2011, implantable LVAD (i-LVAD) has been widely used as bridge to transplant in Japan. However, period of LVAD support in our country is longer than that of Europe and United States. Therefore, exercise training is important for LVAD patients to improve their physical activity and quality of life of their LVAD support period. In the patient with i-LVAD, pulse pressure often increase after exercise therapy. This phenomenon is partially accounted for the physiological feather of continuous-flow pump that pump flow is changed by beat to beat along with native cardiac cycle to make pulse pressure and exercise contributes to increase in left ventricular contraction through activation of sympathetic activity and secretion of catecholamine. Further, exercise also contributes to enhance pulse pressure through increasing cardiac preload by driving muscle pump. There remains much to be elucidated about the physiological role of exercise therapy in i-LVAD patients. But, we consider that exercises training may be useful to improve outcome with i-LVAD patients. No COI.

2S29H1-4

Quadriceps isometric strength as a predictor of exercise capacity and mortality in patients with coronary artery disease

Kamiya, Kentaro(*Department of Rehabilitation, Kitasato University Hospital, Sagamihara, Japan*)

Background: Improvement in exercise capacity is one of the main goals of cardiovascular physiotherapy, resulting in both a reduction of mortality risk and an increased level of everyday habitual activities. Quadriceps strength is related to exercise capacity in normal subjects and different patient populations, but the relationship between maximal quadriceps isometric strength (QIS) and different exercise capacity levels in coronary artery disease (CAD) patients has not been systematically evaluated yet. **Method:** We studied 621 patients (60.6 ± 9.9 years) who were admitted to the hospital because of CAD. The QIS was expressed as percentage of body weight (%BW). Multivariate logistic regression analysis determined a predictor of exercise capacity. The cut-off values of QIS for the attainment of exercises of 5, 7 and 10 METs were analyzed using receiver-operating characteristics (ROC) curves. **Results:** ROC curves revealed that the QIS was 46%BW, 51%BW and 59%BW as predictive cut-off values for the attainment of exercises of 5, 7 and 10 METs, respectively. Moreover, we followed this cohort for totally 2,027 person-years, and the patients who had lower QIS had significantly lower survival. **Conclusion:** The predictive cut-off values of quadriceps strength were 30 %BW for prognosis, 46%, 51% and 59%BW in 5, 7 and 10 METs exercise capacities, respectively. These findings can be used in cardiovascular physiotherapy for the definition of training goals according to patients' needs about their physical activity levels. No COI.

Symposium 30
Cardiovascular diseases and autonomic nerve intervention: Go back from clinical to basic science

(March 17, 10:25–12:05, Room H1)

2S30H1-1

Open-loop analysis of the arterial baroreflex in heart failure and hypertension

Kawada, Toru; Shimizu, Shuji; Turner, James Michael; Sugimachi, Masaru(*Department of Cardiovascular Dynamics, National Cerebral and Cardiovascular Center*)

The arterial baroreflex system can be divided into the neural arc subsystem from pressure input to efferent sympathetic nerve activity (SNA) and the peripheral arc subsystem from SNA to arterial pressure (AP). The arterial baroreflex function is reported to be altered in diseased states such as heart failure and hypertension. However, because any changes in mean AP associated with the disease can reflexly alter the level of SNA even if the baroreflex function is unaltered, it is hard to determine whether the baroreflex function is truly altered in diseased states. To resolve this question, we have employed an open-loop systems analysis where the arterial baroreflex function is compared between normal and diseased states by exactly controlling the baroreceptor input pressure to predefined levels. The total-loop baroreflex function approximates an inverse sigmoidal curve. In rats with chronic heart failure, the response range of AP is significantly narrowed and the baroreflex maximum gain is decreased compared with normal rats, indicating the loss of AP buffering function around the operating point. In spontaneously hypertensive rats, the sigmoid curve is shifted toward higher input and output pressures with the baroreflex maximum gain unchanged, indicating the shift of the operating point toward higher pressure (resetting) without the loss of AP buffering function around the operating point. To gain insights into the development of disease, the neural and peripheral arc characteristics are to be compared between normal and diseased states. No COI.

2S30H1-2

Effects of dihydropyridine calcium channel blockers on baroreflex sympathetic regulation

Yamamoto, Hiromi(*Division of Cardiology, Depertment of Medicine, Faculty of Medicine, Kinki University, Osaka, Japan*)

Calcium channel blockers are widely used for the treatment of hypertension. First generation dihydropyridine calcium blockers (DHPs) exert a strong and rapid atrial pressure (AP)-lowering effect via relaxation of vascular smooth muscles. The hypotensive state may evoke baroreflex-mediated sympathetic excitation and vagal withdrawal, leading to increased heart rate (HR) known as "reflex tachycardia". Second generation DHPs have slow-onset and reduce AP while either maintaining or actually decreasing HR. Third generation DHPs have long-acting property in addition to the slow-onset property. Although lack of tachycardia in response to the 2nd and 3rd generation DHPs may indicate possible sympathoinhibitory effects, it remains unknown whether sympathetic outflow from the central nervous system is suppressed by DHPs. We examined the acute effect of intravenous nifedipine (1st generation), cilnidipine (2nd generation) and azelnidipine (3rd generation) on postganglionic splanchnic sympathetic nerve activity (SNA) using a method of baroreflex open-loop analysis in anesthetized Wistar Kyoto rats with vagotomy. When the baroreceptor input pressure was exactly controlled at predefined pressure levels, nifedipine, cilnidipine and azelnidipine did not significantly affect the SNA response over the entire operating range of the carotid sinus baroreflex. These results suggest that the acute hypotensive effects of intravenous DHPs may be chiefly attributable to the peripheral vasodilator action but not to the direct inhibition of the sympathetic outflow from the central nervous system. No COI.

2S30H1-3

Renal Denervation Alters Neural Arc in Spontaneously Hypertensive Rats

Sata, Yusuke[1]; Kawada, Toru[2]; Shimizu, Shuji[2]; Schlaich, Markus[1]; Esler, Murray[1]; Sugimachi, Masaru[2](*[1]Baker IDI Heart and Diabetes Institute, Melbourne, Australia, [2]National Cerebral and Cardiovascular Center, Department of Cardiovascular Dynamics*)

Catheter-based renal sympathetic nerve ablation has demonstrated a significant blood pressure drop in patients with resistant hypertension. Through the modulation of renal afferent and efferent sympathetic nerve activities, renal denervation may provide centrally-mediated anti-hypertensive effects. To elucidate how neural and peripheral characteristics are altered by renal sympathetic denervation (RDN), we examined the open-loop static characteristics of the carotid sinus baroreflex in renal-denervated spontaneously hypertensive rats (RD-SHR) and compared with normotensive Wistar Kyoto rats (WKY) and non-denervated SHR. The operating-point arterial pressure (AP) was determined from the intersection between the baroreflex neural and peripheral arc in a baroreflex equilibrium diagram. RD-SHR showed the operating-point AP of 129.8 ± 6.9 mmHg, which was between that of WKY (111.3 ± 4.4 mmHg) and that of SHR (145.1 ± 5.7 mmHg). Renal denervation modulates the baroreflex control of sympathetic nerve activity, and decreased the operating-point AP in RD-SHR compared to SHR. However, RDN did not completely normalize SNA or AP regulation compared to WKY. No COI.

2S30H1-4

Water drinking-related muscle contraction induces the pressor response via mechanoreceptors in conscious rats.

Abe, Chikara; Morita, Hironobu(*Department of Physiology, Gifu University Graduate School of Medicine, Gifu, Japan*)

Water drinking is known to induce the pressor response. The efferent pathway in this response involves sympathoexcitation, because the pressor response was completely abolished by ganglionic blockade or an α(1)-adrenergic antagonist. However, the afferent pathway in this response has not been identified. In the present study, we hypothesized that water itself stimulates the upper digestive tract to induce the pressor response, and/or drinking-related muscle contraction induces the pressor response via mechanoreceptors. To examine this, we evaluated the pressor response induced by spontaneous or passive water drinking in conscious rats. Since the baroreflex modulates and obscures the pressor response, the experiments were conducted using rats with sinoaortic denervation. The pressor response was not suppressed by 1) transient oral surface anesthesia using lidocaine, 2) bilateral denervation of the glossopharyngeal nerve and sensory branch of the superior laryngeal nerve, or 3) denervation of the tunica adventitia in the esophagus. However, the pressor response was significantly suppressed (by -52%) by intravenous gadolinium chloride administration. Electrical stimulation of the hypoglossal nerve induced the pressor response, which was significantly suppressed (by -57%) by intravenous gadolinium chloride administration and completely abolished by severing the distal end of this nerve. These results indicate that afferent signals from mechanoreceptors in drinking-related muscles are involved in the water drinking-induced pressor response. No COI.

Symposium 31
Pathophysiology for immunity-related gastrointestinal dysfunctions

(March 17, 8:40–10:20, Room J)

2S31J-1

Significant contribution of TRPC6-mediated Ca2+ influx to the pathogenesis of Crohn's disease fibrotic stenosis

Kurahara Hai, Lin[1]; Sumiyoshi, Miho[1]; Hung, Hsichin[1]; Aoyagi, Kunihiko[2]; Inoue, Ryuji[1] (*[1]Department of Physiology, Fukuoka University, Fukuoka, Japan , [2]Department of Gastroenterology, Fukuoka University, Fukuoka, Japan*)

Crohn's disease is characterized by repeated cycles of inflammation and healing of the gut which ultimately progress into intestinal fibrosis. We examined the mRNA expression of biopsy samples from non-stenotic or stenotic intestinal areas of Crohn's disease's patients. The mRNA levels of canonical transient receptor potential protein 6 (TRPC6), N-cadherin, collagen types 1A1 and 3A1, and interleukins (IL-1β, IL-6, IL-8, IL-10, IL-11 and IL-17β) were significantly increased in the fibrotic stenotic area. Amongst them, the mRNA level of IL-11, an important anti-fibrosis, anti-inflammation cytokine, was most prominently increased (218 fold). We next explored a potential role of TRPC6 channels for intestinal inflammation by using a human colonic myofibroblast cell line InMyoFib. TGF-β1, the major stimulator of intestinal fibrosis, activated TRPC6-mediated Ca^{2+} influx, which was requisite for the differentiation and interleukin synthesis of the myofibroblast. In TGF-β1 treated InMyoFibs, TRPC6 strongly interacted with α-SMA and N-cadherin and promoted mechanically-induced Ca^{2+} entry. Furthermore, TRPC6-mediated Ca^{2+} entry significantly suppressed IL-11 expression by down-regulating ERK and p38-MAPK phosphorylation. These results suggest that TRPC6-mediated myofibroblastic differentiation could be an important process promoting intestinal fibrogenesis, and thus a promising target for future anti-fibrotic and anti-inflammatory therapies in the gut. No COI.

2S31J-2

Up-regulation of Tenascin-C in subepithelial myofibroblasts is critical for intestinal mucosal protection in mice

Islam, Shafiqul Md[1]; Kusakabe, Moriaki[2]; Horiguchi, Kazuhide[3]; Iino, Satoshi[3]; Nakamura, Tatsuro[1]; Iwanaga, Koichi[1]; Hashimoto, Hisashi[1,4]; Matsumoto, Hisashi[5]; Murata, Takahisa[1]; Hori, Masatoshi[1]; Ozaki, Hiroshi[1] (*[1]Dept. of Vet. Pharmacol., Grad. Sch. of Agri. and Life Sci., The Univ. of Tokyo, Tokyo, Japan, [2]Develop. of Adv. Tech. Lab, Res. Center for Food Safety, The Univ. of Tokyo, Tokyo, Japan, [3]Dept. of Anatomy, Fac. of Med. Sci., Univ. of Fukui, Fukui, Japan, [4]Dept. of Anatomy, Jikei Univ. Sch. of Med., Tokyo, Japan, [5]Yakult Central Institute*)

Tenascin-C (TnC) is a multi-domain extracellular matrix glycoprotein. DSS-induced colitis mice greatly expressed TnC in the damaged mucosal areas and significantly up-regulated mRNA of TnC, pro-inflammatory cytokines and growth factors in colitis tissues. In addition, TNBS-induced colitis and SAMP1/Yit spontaneous Crohn's disease model mice also exhibited an up-regulation of mucosal TnC in colon and ilea, respectively. PDGFRα positive ISEMF were found to be the primary TnC-producing cells in the colon tissues. Accordingly, ISEMF collected from the rat colon constitutively expressed both TnC and PDGFRα. PDGF-BB and TGF-β1 up-regulated both TnC mRNA and protein levels in ISEMF. Knockdown of TnC gene made mice more susceptible to DSS-induced colitis and remarkably showed abrasion of intestinal mucosal barrier. Moreover, TnC accelerated both trans-well migration and wound healing in epithelial cells. Conclusions: The pharmacological profiles of PDGF-BB and TGF-β in colitis tissues and ISEMF suggest that increased TnC production during inflammation contributes epithelial cell migration, remodeling and protection of intestinal barrier. No COI.

2S31J-3

Leptin receptor signaling pathways is required for high-fat diet-induced atrophic gastritis in mice.

Inagaki-Ohara, Kyoko[1,2]; Okamoto, Shiki[2]; Minokoshi, Yasuhiko[2] (*[1]Department of Gastroenterology, Research Center for Hepatitis and Immunology, Research Institute, National Center for Global Health and Medicine (NCGM), Japan, [2]Division of Endocrinology and Metabolism, Department of Developmental Physiology, National Institute for Physiological Sciences (NIPS)*)

Obesity is associated with gastrointestinal diseases in addition to cardiovascular disease, diabetes, and dyslipidemia. Atrophic gastritis is one of obesity-related diseases, however, the mechanisms of its onset have remained uncertain. We now show that mice fed a high-fat diet (HFD) developed atrophic gastritis with enhancing expression of gastric leptin and its receptor signaling pathway. HFD induced a gastric hyperplasia with increased leptin expression at 1wk-feeding and the progression of mucosal hyperplasia was developed with a higher Ki67-positive proliferating cell frequency and led to atrophy in the presence of inflammation in an age-dependent manner. Activation of ObR, STAT3, Akt and ERK, which are molecules in leptin receptor signaling was detected in the gastric mucosa in HFD fed mice. Atrophy in gastric mucosa displayed pathology and molecular alterations, such as cdx2 and muc2, resembling human intestinal metaplasia, which is considered to be most progressive feature of atrophic gastritis. Unlike WT mice, leptin-deficient ob/ob mice and leptin receptor mutated db/db mice did not show significant increase in cdx2 expression due to HFD-feeding. These results suggested that enhancement of leptin signaling in the stomach is required to development of obesity-associated atrophic gastritis. No COI.

2S31J-4

Gastrointestinal immune activation and esophageal motility disorders

Ihara, Eikichi; Tanaka, Yoshimasa; Muta, Kazumasa; Bai, Xiaopeng; Akiho, Hirotada; Nakamura, Kazuhiko; Takayanagi, Ryoichi (*Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University*)

Coordinated regulation of gastrointestinal (GI) motility is indispensable for maintaining general health and wellness. GI inflammation and immune activation are accompanied by alteration of GI motility with impaired function of GI smooth muscle. The delicate balance between microbes and host defensive responses at the mucosal barrier has a pivotal role in the pathogenesis of GI inflammation. Adaptive immune response seems to play an important role in GI smooth muscle function where Th1 and Th2 immune response is associated with hypocontractility and hypercontractility of inflamed GI smooth muscle, respectively. Based on basic research, we will review a current understanding of relationships between immune activation and GI smooth muscle function, focusing on cytokine-induced alteration of GI smooth muscle contractility. From clinical viewpoint, some motility disorders are associated with the immune activation, such as inflammatory bowel disease, esophageal motility disorders and functional gastrointestinal disorders. It is very important to testify whether the evidence led by basic research can be also applied to clinical conditions. In this regard we will present here some clinical data from patients with esophageal motility disorders including achalasia, esophagogastric junction outflow obstruction (EGOO) and eosinophilic esophagitis, and we will discuss any possibility of cytokine-induced alteration of esophageal motility in the clinical setting. No COI.

Symposium 32
Gut motility: Understandings from diverse viewpoints

(March 17, 10:25–12:05, Room J)

2S32J-1

The role of interstitial cells in the regulation of gastrointestinal motility

Kito, Yoshihiko(*Department of Pharmacology, Faculty of Medicine, Saga University, Saga, Japan*)

Recent studies have shown that two types of interstitial cells are involved in the regulation of gastrointestinal (GI) motility. The first type is interstitial cells of Cajal (ICC), which are immunopositive to c-Kit. It has been demonstrated that a network of ICC which lies in the myenteric region (ICC-MY) function as pacemaker cells and ICC at the plane of the deep muscular plexus (ICC-DMP) as mediators of enteric motor neurotransmission in the small intestine. ICC-MY generate large, rapidly rising, potential changes (pacemaker potentials), which had two components, a rapid upstroke component that was followed by a plateau component. We found that the upstroke component is initiated by nifedipine-insensitive voltage-dependent Ca^{2+} currents and the plateau component is produced by Ca^{2+}-activated Cl^- currents in the mouse small intestine. The second type of interstitial cells is fibroblast-like cells (FLCs), which are immunopositive to small conductance Ca^{2+}-activated K^+ (SK3) channel and platelet derived growth factor receptor *α* (PDGFR*α*). Since P2Y1 receptors are expressed in FLCs, it is believed that FLCs are responsible for purinergic inhibitory neurotransmission. Although, purines activated apamin-sensitive K^+ currents that were blocked by P2Y1 antagonist in PDGF*α*+ cells isolated from the mouse colon, it is unclear how PDGFR*α*+ cells *in situ* contribute to the regulation of smooth muscle excitability in GI tract. In this symposium, I will show some of the properties of ICC-MY and FLCs recorded from the rabbit small intestine. No COI.

2S32J-2

Depolarization-induced inward current in the interstitial cells of Cajal

Goto, Kazunori(*The Department of Psychiatry, Kyoto University Hospital, Kyoto, Japan*)

To explore the electrophysiological properties of the interstitial cells of Cajal (ICCs), we developed a new preparation by treating the murine small intestine with collagenase. This thin muscle layer preparation contained interstitial cells with and without intercellular contacts around the enteric nerve bundles, and the cluster of smooth muscle cells displayed a rhythmic contraction. We morphologically identified ICCs and conducted patch clamp experiments on the cells. The single c-kit-positive ICCs showed spontaneous and rhythmic potential fluctuations, and a large transient inward current was evoked by depolarization under voltage clump conditions. Once the inward current was triggered, it took a regenerative time course and lasted approximately 500 ms. The current was inactivated by continuous depolarization, and by removal of external Ca2+. The application of acetylcholine prolonged the duration of spontaneous depolarization as well as the depolarization-induced inward current. This inward current showed a reversal potential of around +3mV and was considered to be due to non-selective cation channels. No COI.

2S32J-3

Hyperpolarization-activated cyclic nucleotide channels as a pacemaker channel in colonic interstitial cells of Cajal

Jun Jae Yeoul(*Department of Physiology, College of Medicine, Chosun University, Gwangju, Korea*)

Hyperpolarization-activated cyclic nucleotide (HCN) channels are pacemaker channels that regulate heart rate and neuronal rhythm in spontaneous active cardiac and neuronal cells. Interstitial cells of Cajal (ICCs) are also spontaneous active pacemaker cells in the GI tract. Here, we investigated the existence and the role of HCN channels in pacemaking activity in colonic ICCs by using whole-cell patch clamp, RT-PCR, and Ca^{2+}-imaging in cultured ICCs from mouse colon. SQ-22537 or dedeoxyadenosine (adenylate cyclase inhibitors) decreased the frequency of pacemaker potentials, whereas rolipram (cAMP-specific phosphodiesterase inhibitor) or cell-permeable 8-bromo cAMP increased the frequency of pacemaker potentials. CsCl, ZD-7288, zetabradine, and clonidine (HCN channel inhibitors) suppressed the pacemaker activity. RT-PCR revealed expression of HCN1 channel and HCN3 channel in colonic ICCs. In recording of spontaneous intracellular Ca^{2+} $[Ca^{2+}]_i$ oscillation, rolipram and 8-bromo cAMP increased $[Ca^{2+}]_i$ oscillations, whereas SQ-22537, CsCl, ZD7288, and zetabradine decreased $[Ca^{2+}]_i$ oscillations. These results suggest that HCN annels in colonic ICCs are tonically activated by basal cAMP production and act as pacemaker channels for pacemaking activity. No COI.

2S32J-4

Acceleration of Ileal Pacemaker Activity in Mice Lacking Interleukin 10

Shozib, Bari Habibul[1]; Suzuki, Haruhiko[2]; Iino, Satoshi[3]; Nakayama, Shinsuke[2]([1]*University of Dhaka,Dhaka,Bangladesh.*, [2]*Nagoya University, Nagoya ,Japan*, [3]*Fukui University, Fukui, Japan*)

Interstitial cells of Cajal (ICC) play a pivotal role in gut motility by coordinating the electric activity of cellular members as well as generating pacemaker potentials. Patients with Irritable Bowel Disease (IBD) suffer from gut motility disorders. Thus, our study was to assess whether ICC pacemaking and their coordinated activity is preserved in the ileum of IL-10 deficient mice, a model animal of IBD. Interestingly, in applying micro electrode array (MEA) experiment we revealed that spontaneous pacemaker electric activity is synchronized over the recording area in both WT and IL-10 deficient mice, but oscillations are significantly accelerated in IL-10-deficient mice, despite no significant histological alterations observed in ICC, macrophages and enteric neurons. The ileal tissues used in the present study did not differ macroscopically. Neither ulcers nor bleeding were observed in both WT and IL-10 deficient mice. In order to assess whether the accelerated electrical activity is associated with any histological changes and/or immune cells contained therein, we go through immunohistochemistry but no significant changes were observed in ICC, macrophages and enteric neurons in the ileum of WT and IL-10 deficient mice. Thus we concluded that the research provides evidence for accelerated pacemaker activity in the ileum of IL-10 deficient mice, not accompanied by any significant histological changes. This could be accounted, as an example, by a genetic cross-link between IBD and IBS. No COI.

Symposium 33
New Approaches to Study Cell Volume/ Body Fluid Balance Regulatory System

(March 17, 8:40–10:20, Room K)

2S33K-1

Correlation between the mechanisms for cell volume regulation and for body fluid osmolarity control

Okada, Yasunobu; Sato-Numata, Kaori(*National Institute for Physiological Sciences, Okazaki, Japan*)

Body fluid osmolarity is maintained at a constant level by regulating the extent of exocytotic release of arginine vasopressin (AVP) from osmosensory brain AVP neurons which are constantly interacting with nearby-existing astrocytes. On the other hand, all types of mammalian cells sense changes in ambient osmolarity through altered activities of cell volume-sensing ion channels and/or transporters that are thereafter participating in cell volume regulation (CVR). Therefore, it is highly likely that these volume-sensing or volume-regulatory channels/transporters are involved in osmosensory mechanisms in AVP neurons and nearby-interacting astrocytes. Actually, it has been hypothesized that enhancement of AVP secretion is caused by depolarization induced by activation of stretch-inactivated cation channel (SICC) in AVP neurons, and that suppression of AVP secretion is by hyperpolarization induced by glycine receptors in response to taurine released via volume-sensitive outwardly rectifying anion channels (VSOR) from astrocytes. Thus, our studies should focus on the correlation between the mechanisms for CVR and for regulation of body fluid osmolarity, including reexamination of above-mentioned hypotheses involving neuronal SICC and glial taurine release. No COI.

2S33K-2

Visualization from osmosensitive areas in the brain to vasopressin-containing vesicles by fluorescent proteins in transgenic rats

Ueta, Yoichi(*Department of Physiology, School of Medicine, University of Occupational and Environmental Health, Japan*)

The circumventricular organs (CVOs) including the organum vasculosum of the lamina terminalis (OVLT), the subfornical organ (SFO) and the area postrema (AP) are known to be osmosensitive areas in the central nervous system. Recently, we have generated transgenic rats that express the c-fos-monomeric red fluorescent protein 1 (mRFP1) fusion gene after adequate physiological stimuli such as acute and chronic osmotic challenge. The expression of the c-fos-mRFP1 fusion gene in the OVLT and the SFO were observed after chronic dehydration. However, it is interesting that the fusion gene in the AP was marked increased after rehydration followed by chronic dehydration. We also generated another transgenic rats that express the vasopressin-enhanced green fluorescent protein (eGFP) transgene in the supraoptic (SON) and paraventricular nuclei (PVN). The magnocellular neurosecretory cells in the SON and the PVN contain eGFP fluorescent vesicles. The fluorescent imaging techniques give us new insights to understand central osmosensitive circuits and dynamics in vasopressin-containing vesicles. No COI.

2S33K-3

Xenograft of vasopressin neuron derived from mouse ES cell

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Recently, mouse embryonic stem cell-derived hypothalamus (mES-hypo) reported to develop variety of hypothalamic neuropeptides including vasopressin, neuropeptide Y, AGRP, and tyrosine hydroxylase. The feederless mES line, EB5, quickly aggregates to form embryoid body in serum-free medium in the phospholipid-coated well (SFEBq). Then, it differentiates to hypothalamic progenitor cells in SFEBq when cultured in growth factor-free chemically defined medium. Vasopressinergic neuron is one of the major populations in the mES-hypo, and both histologically and functionally, it is considered identical to the native one: Clusters of parvocellular or magunocellular-like neurons are found in ES-hypo, and they secrete vasopressin to the conditioned medium after various stimulations including potassium, sodium, mannitol and angiotensin II. To evaluate ES-hypo was viable in the central nerve system, the dispersed ES-hypo cells were injected to the cortex region in adult Sprague Dawley rats. Vasopressinergic neuron survived more than 60 days after the graft, and no tumor formation found in the studies more than thirty xenografts so far. The maturity of the neuron in the graft depends on the days after the induction in vitro: when immature cells were injected, they survive but poorly differentiated, and when mature cells were injected, magunocellular-like neuron are found in the graft. These findings suggest ES-hypo may be valuable to deconvolute the physiology of vasopressin system. No COI.

Symposium 34
Local regulation of Ca2+ signaling and cellular response in excitable cells

(March 17, 10:25–12:05, Room K)

2S33K-4

Exploring the role of anion channels in cell volume regulation and intercellular communications in the developing brain

Akita, Tenpei; Fukuda, Atsuo(*Department of Neurophysiology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan*)

Cell volume regulation is an essential function involved in cell shape changes, proliferation, migration and programmed cell death, thus playing important roles in developing tissues. The regulation is attained by regulating the net influx or efflux of solutes and water across the cell membrane, and many different types of ion channel and transporter may participate in the regulation. In the developing brain in embryos, neuronal cell volume is dynamically changed and adjusted, but how and what types of volume-regulated ion channel and transporter control the regulation has yet to be examined. Recently we have checked that in the neurons in ganglionic eminences, from which cortical GABAergic interneurons originate, the current through the volume-sensitive outwardly rectifying (VSOR) anion channel, the major regulator of anion flux during cell volume regulation in many tissues, has typical characteristics of mild outward rectification, inactivation and sensitivity to its inhibitor DCPIB. We are now investigating how the channel regulates neuronal cell volume during brain development. Moreover, we are also examining the possibility of the channel as a pathway for the release of GABA and taurine, the major neurotransmitters in the embryonic brain, since synaptic structures do not develop yet at the embryonic stage. We discuss the importance of fine control of the multimodal roles of anion channels in response to guiding cues to determine the precise location and differentiation of migrating neurons in the developing brain. No COI.

2S34K-1

Regulation and molecular mechanisms of Cav1.2 channel by calmodulin and ATP

Minobe, Etsuko[1]; Han, Dong-Yun[1]; Asmara, Hadhimulya[1]; Feng, Rui[1,2]; Xu, Jianjun[1]; Kameyama, Masaki[1]([1]*Dept. Physiol., Grad. Sch. Med. Dent. Sci., Kagoshima Univ. Kagoshima, Japan,* [2]*Dept. Pharmac. Toxicol., Sch. Pharmac. Sci., China Med. Univ., Shenyang, China*)

Ca^{2+} ions, a widespread signal messenger, enter into the cytoplasm from outside the cell through voltage-gated Ca^{2+} (Cav) channels, ligand-activated cation channels, or are released from SR/ER. In the heart, Cav channels play key roles in pace-maker activity, EC coupling and linkage of electrical activity to metabolic adaptation and gene expressions. Its activity is modified by auxiliary subunits and several regulatory factors. In the inside-out recording, Cav1.2 channel maintained the activity in the presence of calmodulin (CaM) and ATP. CaM is a ubiquitous Ca^{2+} binding protein that mediates Ca^{2+}-dependent regulation of various target proteins. The channel activity increased and then decreased with increasing in CaM or Ca^{2+}, and increased in ATP dependent manner. A semi-quantitative pull-down assay to examine the binding of CaM or ATP to Cav1.2 channel suggested that both CaM and ATP bound to the proximal region of the channel C-terminal tail. Based on our experiments, we propose a model for the regulation of Cav1.2 channels by intracellular Ca^{2+}, CaM and ATP. The model consists of two CaM-binding sites in the channel each for Ca^{2+}-dependent facilitation (CDF) and inactivation (CDI), and one or more ATP-binding site(s) for repriming the channel to be available, independent of the regulation by cAMP-PKA signaling pathway. Our model explains that the balance among Ca^{2+}, CaM and ATP near the channel is critical for its activity. No COI.

2S34K-2

SR counter-ion channels mediated blood pressure regulation

Yamazaki, Daiju[1,2]; Tao, ShengChen[2]; Takeshima, Hiroshi[1,2]
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The TRIC (trimeric intracellular cation) channel subtypes, namely TRIC-A and TRIC-B, which assemble into bullet-shaped homo-trimers to form monovalent cation-selective channels in the sarco/endoplasmic reticulum (SR/ER). Double-knockout mice lacking both the *Tric-a* and *Tric-b* genes show embryonic cardiac failure, and the mutant cardiomyocytes display weak ryanodine receptor (RyR)-mediated Ca^{2+} release. Moreover, mutant skeletal muscle from *Tric-a*$^{-/-}$ mice occasionally exhibits alternan contraction responses, likely due to destabilized RyR-mediated Ca^{2+} release. These defects observed in the knockout mice support our hypothesis that TRIC channel subtypes partly mediate counter-ion movements to facilitate SR/ER Ca^{2+} release.Vascular smooth muscle cells (VSMCs) contain both TRIC subtypes and two Ca^{2+} release mechanisms; incidental opening of RyRs generates local Ca^{2+} sparks to induce hyperpolarization and relaxation, whereas agonist-induced activation of IP_3R produces global Ca^{2+} transients causing contraction. *Tric-a*$^{-/-}$ mice develop hypertension due to insufficient Ca^{2+} sparks in VSMCs. On the other hand, the smooth muscle-sepcific transgenic mice overexpressing TRIC-A channels developed congenital hypotension due to frequent Ca^{2+} sparks in VSMCs. Moreover, in our clinical study, the *TRIC-A* gene polymorphisms in the Japanese population were associated with both hypertension susceptibility and sensitivity to antihypertensive medications. No COI.

2S34K-3

Regulation of nuclear and cytoplasmic Ca^{2+} signaling and its function in cardiomyocytes: role of neuronal Ca^{2+} sensor-1 (NCS-1)

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In cardiomyocytes, cytoplasmic Ca^{2+} transients occur during excitation-contraction (E-C) coupling and hormonal stimulation, leading to muscle contraction and gene expression (excitation-transcription/E-T coupling), respectively. Interestingly, such Ca^{2+} elevations are also detected in organelles such as the nucleus, where E-C coupling is not thought be involved. However, why and how nuclear Ca^{2+} transients occur is not completely understood. Our results suggest that during E-C coupling, nuclear Ca^{2+} is derived from the cytoplasm, possibly through the nuclear pore to buffer cytoplasmic Ca^{2+}, whereas upon receptor stimulation, it is derived from the nuclear envelope Ca^{2+} store, at least in part through the inositol 1,4,5-trisphosphate receptors (IP_3Rs). Since we had reported that a Ca^{2+}-binding protein NCS-1 interacts with IP_3Rs in the heart (Circ. Res. 2011), we examined whether NCS-1 is involved in the nuclear Ca^{2+} signal regulation. We found that stimulation of IP_3R signal by IGF-1 extensively increased NCS-1 levels and strengthened the interaction between NCS-1 and IP_3Rs in all subcellular fractions, including the nucleus, in cardiomyocytes. Furthermore, IGF-1-induced nuclear Ca^{2+} transients was significantly lesser in *Ncs1*$^{-/-}$ myocytes compared to wild-type myocytes, with a concomitant decrease in the levels of hypertrophy-related molecules such as nuclear phospho-CaMKII. These observations suggest a novel mechanism of nuclear Ca^{2+} regulation mediated by NCS-1 and IP_3Rs that may be involved in E-T coupling. No COI.

2S34K-4

Mitochondrial Na-Ca exchanger NCLX-mediated mitochondria-sarcoplasmic reticulum Ca crosstalk and cardiomyocyte automaticity

Takeuchi, Ayako; Matsuoka, Satoshi(*Integr. Physiol. Fac. Med. Sci. Univ. Fukui*)

NCLX, a mitochondrial Na-Ca exchanger, serves as a Ca extrusion system in mitochondria (Palty et al., 2012; Kim et al., 2012). Very recently, we reported that NCLX regulates automaticity of HL-1, a spontaneously beating cardiac cell line originating from mouse atrial myocytes (Takeuchi et al., 2013). We clarified that NCLX functions as a Ca provider to sarcoplasmic reticulum (SR), thereby modulating the automaticity which is driven by "spontaneous Ca leak from SR". However there is few information on the role of NCLX in the intact cardiac pacemaker cells, SA node cells. There are two controversial mechanisms for explaining the automaticity of SA node cells, a "membrane clock" mechanism in which membrane channels determines the automaticity, and a "Ca clock" mechanism in which a "spontaneous Ca leak from SR" determines the automaticity. In order to clarify the contribution of NCLX on the automaticity of SA node cells, we incorporated mitochondrial Ca dynamics into two different SA node cell models, the "Ca clock"-driven model (Maltsev and Lakatta, 2009) and the "membrane clock"-driven model (Himeno et al., 2008). In both models, reduction of NCLX resulted in the marked decrease of SR Ca content. Interestingly, the beating rate was prolonged in the "Ca clock"-driven model, but shortened in the "membrane clock"-driven model, which was attributable to the increase of sustained inward Na current I_{st} via $[Na]_i$ decrease. These results suggest that contribution of NCLX on the pacemaker activity depends on composition of ion channels. No COI.

Symposium 35
Expanding frontiers in appetite research explored by young investigators

(March17, 15:05–17:05, Room B)

2S35B-1

Paraventricular nucleus oxytocin to arcuate nucleus POMC: a novel anorexigenic neuronal pathway in hypothalamus

Maejima, Yuko; Sakuma, Kazuya; Shimomura, Kenju; Yada, Toshihiko(*Jichi Medical University, School of Medicine*)

Recent studies reported that Oxytocin (Oxt) acts as a regulator of energy metabolism. Although the role of Oxt in reducing food intake is well established, the underlying mechanisms are less defined. Recently, we have shown that Oxt neurons in the paraventricular nucleus (PVN) regulate pro-opiomelanocortin (POMC) neurons at least in the brain stem nucleus tractus solitarius (NTS) and thereby induce melanocortin dependent anorexia. Since POMC neurons are also present in the arcuate nucleus (ARC), we investigated the effect of Oxt on ARC POMC neurons. We found that the injection of Oxt into the 3rd ventricle increased the expression of c-Fos in ARC POMC neurons. Oxt receptors have been found in the ARC POMC neurons. To assess the direct effect of Oxt, the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured in single ARC POMC neurons. The application of Oxt increased $[Ca^{2+}]_i$ with reversible inhibition by Oxt receptor blocker. Also, confocal images revealed that Oxt terminals contacted ARC POMC neurons, and intra-ARC retrograde tracer injection showed that the Oxt fibers in ARC were originated from PVN Oxt neurons. Moreover, intra-ARC injection of Oxt decreased food intake. These results indicate that the projection of PVN Oxt neurons to ARC POMC neurons is anorexigenic. Physiological significance of this neural pathway remains unclear. However we show, for the first time, that Oxt acts as an activating factor of the ARC POMC neurons and that the PVN Oxt to ARC POMC pathway can serve as a new melanocortin dependent pathway. No COI.

2S35B-2

Dnmt3a in Sim1 cells is necessary for the normal control of body weight and energy homeostasis

Kohno, Daisuke[1,2,3]; Lee, Syann[3]; Harper, Matthew J[3]; Kim, Ki Woo[3]; Sone, Hideyuki[4]; Fan, Guoping[5]; Elmquist, Joel K[3]
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Global obesity rates are rapidly increasing. How environmental factors cause long-term changes in metabolism remains unclear. The epigenetic status of a gene can be altered in response to environmental changes. To investigate the role of DNA methylation in the regulation of energy balance, we deleted the de novo DNA methyltransferase, DNMT3a, in Sim1 neurons, which is expressed in the forebrain including the PVH. Sim1-specific DNMT3a deletion mice became obese from 7-weeks onwards. The amount of fat was increased in the mice. The mice also showed hyperphagia and glucose-intolerance. Furthermore, the mice developed hyper-LDL-cholesterolemia when fed a high-fat diet. Gene expression profiling revealed that the expression of tyrosine hydroxylase was highly upregulated in the PVH of Sim1-specific DNMT3a deletion mice. This was accompanied by decreased DNA methylation of the tyrosine hydroxylase promoter, which suggests that the gene is a potential target of DNMT3a. These results demonstrate that DNMT3a, a key methylation enzyme, in the PVH neurons is necessary for normal regulation of body weight and energy homeostasis. No COI.

2S35B-3

AMPK in the paraventricular hypothalamus regulates food selection behavior in mice

Okamoto, Shiki; Minokoshi, Yasuhiko(*Division of Endocrinology and Metabolism, National Institute for Physiological Sciences, Okazaki, Japan*)

Hypothalamic AMP-kinase (AMPK) regulates feeding behavior in response to hormonal and nutrient signals. However, the effect of AMPK on food preference remains to be established. We found that refeeding after overnight fasting, which activates AMPK in the PVH, increased the choice of high carbohydrate diet (HCD) but decreased that of high fat diet (HFD) in mice. The effect of fasting was suppressed by expression of shRNA for AMPK alpha1 and 2 in the PVH with lenti virus. In contrast, expression of constitutively-active AMPK (CA-AMPK) in PVH neurons increased HCD selection. We examined the principle neurons in the PVH for the regulation of food selection behavior. Microinjection of CRH into the PVH was found to increase HCD selection, and expression of shRNA for CRH in the PVH blunted the HCD selection in response to fasting. Preferenctial expression of CA-AMPK in CRH neurons also increased the HCD selection. We found that the change in food selection was dependent on the AMPK-induced fatty acid oxidation (FAO) in the PVH. Furthermore, pharmacological activation of AMPK increased cytosolic [Ca2+] in CRH neurons isolated from the PVH, and the effect of AMPK was abolished with the expression of shRNA for AMPK or with the suppresion of FAO. Diet-induced and gnetically obese mice have been shown to prefer HFD. We found that the obese mice decreased AMPK and FAO activity as well as CRH mRNA expression in the PVH.Thus, our results suggest that AMPK-FAO system in CRH neruons in the PVH regulates food selection behavior for HCD and HFD. No COI.

2S35B-4

Role of ghrelin signaling in the ventral midbrain in binge-like sugar overconsumption in mice

Yasoshima, Yasunobu; Yamaguchi, Erina; Nishioka, Haruna; Shimura, Tsuyoshi(*Division of Behavioral Physiology, Graduate School of Human Sciences, Osaka University, Suita, Japan*)

Binge-like sugar overconsumption is suggested to be due to metabolic need and hedonic desire for taste/postingestive reward. Our previous study has showed that hedonic-driven sucrose consumption is enhanced after sucrose bingeing in mice. Other studies showed elevated dopamine release in the nucleus accumbens during sugar bingeing in rats, suggesting that activated brain reward system including the ventral tegmental area (VTA) is involved in the binge-like sugar overconsumption. However, molecular regulation of the VTA activity in the binge-like behavior remains to be solved. To explorer the issue, we examined roles of ghrelin-related signaling in the VTA in binge-like sucrose overconsumption. An intraperitoneal injection of a ghrelin receptor antagonist, D-Lys3-GHRP-6 (DG-6), and bilateral microinfusions of DG-6 into the VTA remarkably reduced hedonic-driven sucrose consumption in trained mice. Next, we compared post-training chow intake elicited by intra-VTA infusions of ghrelin between bingeing and non-bingeing mouse groups. Infusions of ghrelin into the bilateral VTA produced greater chow intake in bingeing group but not in control groups, suggesting that ghrelin receptors in the VTA are sensitized during or after the binge-like behavior. These data suggest that sensitization of ghrelin signaling in the VTA plays a crucial role in hedonic-driven component of binge-like sugar overconsumption. No COI.

2S35B-5

The molecular mechanism of feeding regulation from the viewpoint of anorexia nervosa

Amitani, Haruka; Asakawa, Akihiro; Amitani, Marie; Inui, Akio(*Department of Psychosomatic Internal Medicine, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima, Japan*)

Anorexia nervosa (AN) is a serious disorder affecting adolescents and young adults, and it decreases the quality of life of affected individuals for prolonged periods. In spite of many treatments such as drug therapy, behavioral therapy, cognitive-behavioral therapy and family therapy, AN continues to be a refractory disorder because of its unknown pathogenesis. The mechanisms underlying persistent anorexia are largely unknown, but we reported the relation between AN and feeding regulatory peptides such as PP, CCK, ghrelin, obestatin and nesfatin. Adiponectin is a protein hormone produced almost exclusively in adipose tissue and exists as multiple isoforms in the blood circulation: a low-molecular-weight (LMW) form, a middle-molecular-weight (MMW) form, and a high-molecular-weight (HMW) form. Previous study revealed that HMW had an effect opposite to others on body weight gain. The klotho gene, which encodes a single-pass transmembrane protein expressed primarily in renal tubes, has been identified as a systemic anti-aging hormone. Klotho plays a role in adipocyte maturation and systemic glucose metabolism, and also increases adipocyte differentiation in vitro. Recently, we reported the association of AN with adiponectin and klotho. In this symposium, we review the relationship these feeding regulatory peptides and AN. No COI.

Symposium 36
The functional roles of Parabrachial nucleus as an coordinator between sensory and autonomic system

(March 17, 15:05–17:05, Room C)

2S36C-1

The parabrachial nucleus as a bridge linking nociception and emotional memory

Kato, Fusao(*Dept Neurosci, Jikei Univ Sch Med, Tokyo, Japan*)

The external lateral parabrachial nucleus (elPB) is the most predominant target of the nociception-specific neurons in the superficial layer of the spinal cord. The neurons in the elPB in turn project to the capsular part of the central amygdala (CeC), a subregion of central amygdala involved in nociception-induced emotional responses, regulation of spinal nociception and conditioned fear/threat learning. This pathway is thus well situated as a non-thalamocortical route for sending aversive information to the amygdala. To directly examine this, we pharmacologically inactivated the bilateral elPB during fear acquisition and found a significantly decreased freezing time in response to the retrieving auditory cue, indicating that the elPB actively participates in fear learning (Sato, Watabe et al). As CeC neurons are composed of distinct types of neurons playing distinct roles in fear learning, we examined which type receives the input of elPB origin by selectively stimulating the axons of elPB neurons in the central amygdala with optogenetics approach. Surprisingly, almost all types of CeC neurons, as classified by firing pattern, exhibited monosynaptic excitatory responses to light stimulation, suggesting that parabracial input is a potent global activator of CeC neurons (Sugimura, Takahashi et al). Together with our previous findings of robust synaptic potentiation of the elPB-CeC transmission in chronic pain models (Nakao et al, 2012) and fear-conditioned mice (Watabe et al, 2013), we conclude that the elPB relays nociceptive information to the amygdalar site of convergence/integration/plasticity for aversive memory formation. No COI.

2S36C-2

Roles of the lateral parabrachial nucleus in body temperature regulation

Nakamura, Kazuhiro[1,2]([1]*Career-Path Promotion Unit for Young Life Scientists, Kyoto University, Kyoto, Japan,* [2]*PRESTO, JST, Japan*)

To maintain body temperature, changes in environmental temperature are sensed by thermoreceptors in the skin and the thermosensory information is transmitted to the thermoregulatory center, preoptic area. This feedforward thermosensory signaling is essential to elicit defensive thermoregulatory responses before the changes in environmental temperature affect body core temperature. I have studied this thermosensory pathway and found that this ascending signaling is mediated by two populations of glutamatergic neurons in the lateral parabrachial nucleus (LPB). A neuronal population in the external lateral part of the LPB (LPBel) receives cutaneous cool-sensory signals from the spinal dorsal horn and transmits them to the preoptic area through their direct axonal projection. The other population, which is located in the dorsal part of the LPB (LPBd), mediates the transmission of cutaneous warm-sensory signals from the spinal dorsal horn to the preoptic area. These two populations are located nearby, but formed segregated neuronal groups in the LPB. Inhibition of these LPB neurons eliminates the feedforward thermoregulatory responses to changes in skin temperature. The spinal-LPB-preoptic pathway is essential for eliciting rapid and precise responses for the regulation of body temperature and importantly, constitutes a novel thermosensory neural pathway that is distinct from the spinothalamocortical pathway well-known for perception and discrimination of skin temperature. No COI.

2S36C-3

Information processing in the brainstem neurons defined by genetic tracing of taste representation

Sugita, Makoto; Yamamoto, Kuniyo; Hirono, Chikara; Shiba, Yoshiki(*Department of Physiology and Oral Physiology, Institute of Biomedical & Health Sciences, Hiroshima University, Hiroshima, Japan*)

Bitter and sweet taste stimuli are detected by the families of G protein-coupled receptors, T2Rs and T1Rs, expressed in distinct populations of taste receptor cells. We recently applied a genetic approach to delineate the neuronal circuitries of bitter and sweet tastes by selectively expressing the fluorescent transneuronal tracer, tWGA-DsRed, in either bitter- or sweet-responsive taste receptor cells in mice, and then visualizing the spatial distribution of tWGA-DsRed-labeled neurons in the brain. The locations of tWGA-DsRed-labeled neurons suggested the topographic segregation of taste representation in the solitary tract nuclei and the parabrachial nuclei, where the gustatory neurons are organized with sweet inputs located rostral and with bitter inputs located caudal, except for bitter inputs into the external-lateral and external-medial subdivisions of the parabrachial nuclei. Here we combined genetic tracing with electrophysiological and immunohistochemical approaches to predict the roles of the tracer-labeled neurons in the solitary tract nuclei and the parabrachial nuclei. Analyses of the neuron types and the mechanisms of synaptic transmission of tWGA-DsRed-labeled neurons revealed the heterogeneities of taste-relaying neurons, showing the architectural solution in the brainstem that processes taste information. Our data suggest the neuronal bases underlying interaction between the processing of taste information and the homeostatic control of feeding. No COI.

2S36C-4

Cardiovascular control by the cerebellum via parabrachial nucleus

Nisimaru, Naoko[1,2]([1]*Brain Science Institute, RIKEN, Wako, Japan,* [2]*Dept. of Physiol., Facult. of Med., Oita Univ., Yufu, Japan*)

The cerebellum is generally regarded as a motor center, but it is involved in autonomic functions and also cognitive functions. The cerebellar cortex consists of numerous microzones, each extending 10 mm^2 or so. Each microzone is combined with subcortical structures constituting a microcomplex, that is, a functional unit of the cerebellum. A micrcomplex is equipped with inputs via mossy, climbing and beaded fibers and an output via cerebellar or vestibular nuclear neurons. During past nearly 30 years, five cardiovascular control microzones in the cerebellar cortex have been found: (1) region of lobules I, II and III of anterior vermis, (2) medial region of lobule VII and VIII of posterior vermis, (3) medial uvula, (4) lateral nodulus-uvula and (5) folium p in flocculus. Recently, it has been found that three of these (3–5) projected to the lateral parabrachial nucleus (l-PBN) to constitute microcomplexs (cardiovascular modules). These cerebellar areas receive climbing fiber signals from aortic nerves, and mossy fibers from vagus nerves, nucleus tractus solitalius or pedunculopontine nucleus. l-PBN receives inputs from cardiovascular system and serves as one of the major cardiovascular control centers in the brainstem. Behavioral experiments showed that medial uvula regulate baroreceptor reflex during excise (Bradley et al., 1990) and that the lateral nodulus-uvula maintains blood pressure during positional changes of the body (Nisimaru et al., 1998). We now revealed that the folium p regulates blood flow while animals perform defense behaviors (Nisimaru et al., 2013). No COI.

2S36C-5

Nucleus Parabrachialis is the mode switch as an inspiratory-expiratory system and as a sleep-arousal system

Arata, Akiko(*Div. of Physiome, Dept. of Physiology, Hyogo Collge of Medicine*)

The nucleus parabrachialis complex (NPB) of the pons is known as a respiratory modulating center and the NPB plays a crucial role in the inspiratory off-switch. We aimed to examine how the NPB participates in the inspiratory off-switch using pons-medulla-spinal cord preparations obtained from 0 to 4 days old rats. First, the effects of NPB electrical stimulation on C4 ventral root inspiratory activity were examined. The electrical stimulation of NPB induced inspiratory-expiratory phase switching. Here we describe the respiratory neurons in the dorsal pons and their interacting the respiratory center, central chemoreception and adrenergic modulation. We found several types of respiratory neurons; inspiratory, tonic inspiratory, expiratory and inspiratory-expiratory (I-E) neurons in the NPB using whole cell patch clamp method. The population of I-E neurons was more than 50% of the recorded neurons. I-E neurons might determine respiratory cycle using inspiratory-expiratory phase switching. Orexin is the peptide of maintenance of arousal, and facilitated I-E neuron rhythm. Respiratory cycles faster when Orexin is increased under stress condition. NPB is sending axons to amygdala regarding emotion, and is receiving inputs from respiratory center related to respiratory rhythm. NPB might be involved in hyperventilation syndrome and panic disorder by controlling respiration using inspiratory termination as an active phase-switch under stress condition. The pontine respiratory neurons would play an important role in the balance between stress and autonomic function. No COI.

Symposium 37
Cutting Edge of the TRP Channel Research

(March 17, 15:05–17:00, Room D1)

2S37D1-1

Role of TRPC3 channels in mechano-chemo transduction in hearts

Nishida, Motohiro(*Division of Cardiocirculatory Signaling, Okazaki Institute for Integrative Bioscience (National Institute for Physiological Science), National Institute of Natural Sciences, Aichi, Japan*)

Mechanical stretch during diastolic filling of the heart activates the mechanotransduction signalling pathways that have broad implications for adaptation and maladaptation of the heart against mechanical load. A diastolic stretch of ventricular myocytes reportedly induces local production of reactive oxygen species (ROS) in a process dependent on microtubule-localized nicotineamide adenine dinucleotide phosphate (NADPH) oxidase 2 (Nox2). However, the molecular mechanism underlying Nox2 activation by mechanical stretch is still unclear. We demonstrated that canonical transient receptor potential subfamily 3 (TRPC3) contribute to Nox2-mediated ROS production in rodent hearts. Mechanical stretch of rat neonatal cardiomyocytes increased ROS production accompanying the increase in frequency of Ca^{2+} oscillations. Although either inhibition of TRPC3 or TRPC6 suppressed mechnical stretch-induced Ca2+ oscillation, only TRPC3 protein physically interacts with Nox2 protein, and inhibition of TRPC3, but not TRPC6, abolished mechanical stretch-induced ROS production. Futhermore, pressure overload-induced cardiac fibrosis (but not hypertrophy) as well as ROS production were significantly suppressed in TRPC3-deficient mouse hearts. These results strongly suggest that TRPC3 participates in chronic mechano-chemo transduction in rodent hearts, and will be a strong therapeutic target of heart failure. No COI.

2S37D1-2

Roles of TRPM2 channel in neurological diseases based on neuroinflammatory responses

Nakagawa, Takayuki[1,2]; Shirakawa, Hisashi[1]; Kaneko, Shuji[1]
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Accumulating evidence suggests that neuroinflammation contributes to the etiopathogenesis of neurological diseases, such as neurodegenerative diseases, cerebrovascular disorders, multiple sclerosis and chronic pain. Neuroinflammatory responses in the CNS are mediated by glial cells and CNS-infiltrated peripheral immune cells. TRPM2 channels are expressed abundantly in immune and glial cells, and acts as a reactive oxygen species sensor. Recent studies focus on the roles of TRPM2 in immune and inflammatory responses. In this study, we examined the involvement of TRPM2 in some neurological diseases by using TRPM2-knockout (KO) mice. In transient focal cerebral ischemic mice by middle cerebral artery occlusion, TRPM2-KO reduced cerebral infarct volume, neurological scores and migration of activated macrophages/microglia in ischemic penumbra. In experimental autoimmune encephalomyelitis (EAE) mice, TRPM2-KO reduced EAE clinical symptoms and mechanical allodynia. In peripheral nerve injury-induced neuropathic pain models, TRPM2-KO inhibited mechanical allodynia, which was accompanied with reduced activation of spinal resident microglia and infiltration of macrophages into the spinal cord. In this symposium, I will discuss about the roles of TRPM2 and its mechanisms in neuroinflammation in these neurological diseases. No COI.

2S37D1-3

Functional coupling between Ca^{2+}-sensing receptor and TRPC6 channel in pulmonary hypertension

Yamamura, Aya[1]; Yamamura, Hisao[2]; Yuan, Jason X.-J.[3]([1]*School of Pharmacy, Kinjo Gakuin University, Nagoya, Japan,* [2]*Graduate School of Pharmaceutical Sciences, Nagoya City University, Nagoya, Japan,* [3]*Department of Medicine, University of Illinois at Chicago, Chicago, USA*)

Idiopathic pulmonary arterial hypertension (IPAH) is a rare, progressive, and fatal disease of unknown pathogenesis. Sustained vasoconstriction and vascular remodeling due to pulmonary arterial smooth muscle cell (PASMC) proliferation are key pathogenic events that lead to early morbidity and mortality. These events have been linked to Ca^{2+} mobilization and signaling in PASMCs. We have previously shown that Ca^{2+}-sensing receptor (CaSR) is upregulated in PASMCs from IPAH patients and contributes to enhanced Ca^{2+} response. In this study, we examined whether TRPC channel is involved in enhanced Ca^{2+} influx followed by CaSR activation in IPAH-PASMCs. Application of La^{3+} inhibited the plateau phase of extracellular Ca^{2+}-induced $[Ca^{2+}]_{cyt}$ increase through CaSR activation in IPAH-PASMCs. The mRNA and protein expression levels of TRPC6 in IPAH-PASMCs were greater than in normal PASMCs. Knockdown of TRPC6 channel in IPAH-PASMCs with siRNA attenuated the CaSR-mediated $[Ca^{2+}]_{cyt}$ increase. In contrast, overexpression of TRPC6 channel with CaSR in normal PASMCs mimicked the extracellular Ca^{2+}-induced $[Ca^{2+}]_{cyt}$ increase. TRPC6 current was evoked by the activation of CaSR in IPAH-PASMCs. In conclusion, the extracellular Ca^{2+}-induced $[Ca^{2+}]_{cyt}$ increase due to functional coupling between CaSR and TRPC6 channel is a novel pathogenic mechanism contributing to the augmented Ca^{2+} influx and excessive PASMC proliferation in IPAH patients. No COI.

2S37D1-4

The molecular mechanism of muscle hypertrophy; roles of TRPV1

Takeda, Shinichi(*National Center of Neurology and Psychiatry, Translational Medical Center, Tokyo, Japan*)

Skeletal muscle atrophy occurs in aging and pathological conditions including cancer, diabetes and AIDS. Treatment of atrophy is based on either preventing protein degradation pathways, or activating protein synthesis pathways. Neuronal nitric oxide synthase (nNOS), the enzyme that produces nitric oxide, is mainly localized at the sarcolemma, as a peripheral member of the dystrophin-glycoprotein complex. We previously demonstrated that nNOS was trans located to by to plasm and promoted muscle atrophy by activating FoxO3a pathway (Suzuki N et al,. J. Clin. Invest. 117: 2468-76, 2007). Here, we show that the overload-induced hypertrophy was prevented in nNOS-null mice. Moreover, nNOS was transiently activated within three minutes after the initiation of overload. This activation promoted formation of peroxynitrite, a reaction product of nitric oxide with superoxide, which was derived from NADPH oxidase 4 (NOX4). Nitric oxide and peroxynitrite then activated TRPV1, resulting in an increase of intracellular Ca2+ concentration ([Ca2+]i) that subsequently triggered activation of mammalian target of rapamycin (mTOR). Notably, administration of the TRPV1 agonist, capsaicin, induced hypertrophy without overload, and alleviated the unload- or denervation-induced atrophy. These findings identify nitric oxide, peroxynitrite and [Ca2+]i as the critical mediators that convert a mechanical load into an intracellular signaling pathway, and suggested that TRPV1 could be a novel therapeutic target for treatment of muscle atrophy (Ito N et al,. Nat Med. 19: 101-106, 2013). No COI.

2S37D1-5

Regulation mechanisms of thermosensitive TRP channels

Tominaga, Makoto[1]([1]*Division of Cell Signaling, Okazai Institue for Integrative Bioscience (National Institute for Physiological Sciences), Okazaki, Japan,* [2]*Department of Physiological Sciences, SOKENDAI*)

There are ten thermosensitive TRP channels having distinct temperature thresholds for activation. Are the temperature thresholds changed? It is well known that temperature thresholds for TRPV1 and TRPM8 activation are decreased and increased, respectively, in the presence of minimal amounts of chemical activators (capsaicin and menthol). In addition, we previously reported that temperature threshold for TRPV1 activation can be decreased to the body temperature range upon PKC-dependent phosphorylation, explaining the acute inflammatory pain. We also found that temperature threshold for TRPM8 activation is changed dynamically with changes in PIP2 binding, which could explain the well-known three-bowl experiment. Furthermore, we found that temperature threshold for TRPM2 activation was dramatically reduced by oxidation of the single methionine with hydrogen peroxide, which could explain the temperature-dependent changes in macrophage functions. In terms of mouse TRPA1, we found a splice variant which regulates TRPA1 activity and is involved in the inflammatory and neuropathic pain although temperature-sensitivity was not changed. Thus, functions of thermosensitive TRP channels are regulated in a many different ways. No COI.

Symposium 38
Mechanism and role of inhibitory synaptic plasticity

(March 17, 15:05–17:05, Room E)

2S38E-1

IP_3/Ca^{2+} signaling-dependent regulation of GABAergic synapses

Bannai, Hiroko[1]; Niwa, Fumihiro[2]; Mark W Sherwood[2]; Triller, Antoine[3]; Mikoshiba, Katsuhiko[2]([1]*Dept. Biol. Sci., Grad. Sch. Sci. Nagoya Univ., Nagoya, Japan,* [2]*RIKEN BSI, Saitama, Japan,* [3]*IBENS, Paris, France*)

Regulation of the number of GABA-A receptors ($GABA_AR$) clustering at the inhibitory synapse is a crucial determinant of GABAergic synaptic transmission efficacy, and thus play a key role for learning, memory and their patho-physiology. Ca^{2+} influx from the extracellular space induces the dispersal of $GABA_ARs$. Ca^{2+} release from the intracellular Ca^{2+} store also plays various roles in neurons, however, the impact of Ca^{2+} release on the GABAergic synapse remains to be elucidated. We report here that inositol 1,4,5-trisphosphate (IP_3)-induced Ca^{2+} release (IICR) is responsible for the stabilization of GABAergic synaptic structures in hippocampal neurons. Loss of IICR activity by gene knockout of intracellular Ca^{2+} releasing channel IP_3 receptor type 1 (IP_3R1) and by specific inhibitors resulted in the decrease in $GABA_AR$ and gephyrin cluster size and GABAergic mIPSC amplitude, without reducing the amount of $GABA_AR$ on the cell surface. Furthermore, we found that IICR is required for the reclustering of $GABA_ARs$ after dispersion of clusters evoked by Ca^{2+}-influx. Single particle tracking with quantum dot (QD-SPT) revealed that the loss of IP_3R1 and inhibition of IICR enhanced the lateral diffusion of $GABA_ARs$. The increase of $GABA_AR$ lateral mobility resulting from the loss of IICR was completely prevented by an inhibition of dephosphorylation. Our results suggest that IP_3/Ca^{2+} signaling contributes to the stabilization of synaptic $GABA_AR$ clusters through the regulation of $GABA_AR$ lateral diffusion, possibly through phosphorylation. COI properly decleared.

2S38E-2

Postsynaptic determination of the polarity of long-term modifications at inhibitory synapses in neocortical pyramidal cells

Komatsu, Yukio(*Dept. of Neurosci., Res. Inst. Env. Med., Nagoya Univ., Nagoya, Japan*)

Inhibitory synapses in pyramidal cells can undergo long-term potentiation (LTP) or depression (LTD). Because the role of inhibition is to regulate the initiation of spikes produced by excitatory inputs, the polarity of inhibitory synaptic plasticity may be affected by excitatory inputs arriving at pyramidal cells simultaneously with inhibitory inputs. In this study, we investigated the mechanism determining the polarity. We recorded postsynaptic responses evoked in layer 2/3 pyramidal cells by extracellular stimulation of presynaptic fibers in mouse visual cortical slices. Inhibitory postsynaptic currents (IPSCs) were recorded at -50 mV in the presence of the glutamate receptor antagonist kynurenate. High-frequency stimulation (HFS, 50 Hz, 1 s) was applied in the current clamp mode, while kynurenate was temporarily washed out. HFS induced LTD of IPSCs. When the NMDA receptor (NMDAR) antagonist MK801 was loaded into postsynaptic cells or when the membrane potential was held at -90 mV during HFS, HFS induced LTP instead of LTD. Postsynaptic loading of the Ca^{2+} chelator BAPTA prevented both LTD and LTP, indicating that both modifications require postsynaptic Ca^{2+} increases for their induction and that LTD induction requires postsynaptic NMDAR-mediated Ca^{2+} elevation. Additional pharmacological studies showed that LTP induction required $GABA_B$ receptor-mediated PLC activation and Ca^{2+} release from postsynaptic IP_3-sensitive stores without NMDAR activation. These results suggest that the polarity is determined by the balance of excitatory and inhibitory inputs. No COI.

2S38E-3

Stress-induced (meta)plasticity at hypothalamic GABA synapse during stress

Inoue, Wataru; Baimoukhametova V Dinara; Fuzesi Tamas; Wamsteeker Cusulin I Jaclyn; Koblinger Kathrin; Whelan J Patrik; Pittman J Quentin; Bains S Jaideep[1](*Hotchkiss Brain Institute, University of Calgary*)

Exposure to a stressor sensitizes behavioral and hormonal responses to future stressors. Stress-associated release of noradrenaline enhances the capacity of central synapses to show plasticity (metaplasticity). I will present noradrenaline-dependent metaplasticity at GABA synapses in the paraventricular nucleus of the hypothalamus that controls the hypothalamic-pituitary-adrenal axis. These GABA synapses undergo activity-dependent long-term potentiation (LTPGABA) in acute brain slices, only when slices are prepared immediately after in vivo stress exposure: in slices from naive, non-stressed animals, LTPGABA did not occur. Activation of βadrenergic receptors (β-ARs) during stress is necessary for this stress-dependent gating of LTPGABA. Mechanistically, β-ARs cause a functional upregulation of metabotropic glutamate receptor1 (mGluR1). This allows glutamate spillover, during high frequency stimulation, to induce mGluR1-dependent LTPGABA. This LTPGABA was expressed postsynaptically and manifested as the emergence of new functional synapses. Our findings provide the first demonstration that noradrenaline release during an in vivo challenge alters the information storage capacity of GABA synapses. These changes may be the building blocks of learning and memory that contribute to neuroendocrine adaptations to stress. No COI.

Symposium 39
Current Status of Physiome & Systems Biology: the Efforts in Japan

(March 17, 15:05–17:05, Room F)

2S38E-4

Regulation mechanism and functional role of inhibitory synaptic plasticity in a cerebellar Purkinje neuron

Hirano, Tomoo(*Department of Biophysics, Graduate School of Science, Kyoto University*)

At inhibitory synapses on a Purkinje neuron in the cerebellum, postsynaptic depolarization induces long-term potentiation of GABAergic synaptic transmission, which is called rebound potentiation (RP). We have been studying molecular regulation mechanisms and functional roles of RP. The increase in intracellular Ca^{2+} concentration caused by depolarization induces the enhanced responsiveness of $GABA_A$ receptor ($GABA_AR$) in RP. Binding of $GABA_AR$ associated protein (GABARAP) to $GABA_AR$ regulated by Ca^{2+}/ calmodulin-dependent kinase II (CaMKII) is necessary for RP induction. Whether RP is induced or not is determined by the balance of phosphorylation and de-phosphorylation activities regulated by intracellular Ca^{2+} and by metabotropic GABA and glutamate receptors. We recently found that subunit composition of CaMKII has significant impact on the RP induction. When the relative amount of α- to β-CaMKII is large, RP induction is suppressed. Functional significance of RP has also been studied using transgenic mice, in which a peptide which inhibits binding of GABARAP and $GABA_AR$ is expressed selectively in a Purkinje neuron. The transgenic mice showed abrogated RP and subnormal adaptation of vestibulo-ocular reflex, a type of motor learning. Thus, RP is implicated in motor learning. No COI.

2S39F-1

Multi-scale modeling of the brain and the body

Doya, Kenji; Yoshimoto, Junichiro(*Neurobiology Research Unit, Okinawa Institute of Science and Technology Graduate University*)

It was generally regarded that the major role of skeletal muscle was to produce movement by contracting. However, recent studies reported multiple cases in which metabolism was adjusted by coupling to skeletal muscle contraction, which indicated that skeletal muscles might have other biological roles in addition to the production of movement. For example, muscle contraction strongly stimulates glucose transport from blood into the muscle cells, the effect of which is equivalent to that of insulin. In addition, it has been revealed that skeletal muscle secretes several humoral factors (myokines) for metabolic control. Skeletal muscle was rarely regarded as a target for medication in the past, but recent findings have led to it being focused on as a potential drug target. This presentation will focus on the molecular mechanism of coupling of muscle contraction to metabolic control. No COI.

2S39F-2

Analysis of the toxicity of molecular target drugs

Suzuki, Hiroshi; Honma, Masashi(*Department of Pharmacy, The University of Tokyo Hospital, Faculty of Medicine, The University of Tokyo*)

Systems biology has been most successful in understanding and predicting the dynamics within single cells, such as gene regulatory networks and molecular cascades. The function of the brain, on the other hand, depends critically on specific interactions of a large number of neurons. Furthermore, the real function of the brain can be tested only when it is exposed to complex sensory-motor environments. This necessitates multi-scale modeling, simulation, and analysis of the neural, musculoskeletal, and environmental dynamics. In order to understand how the brain realizes reinforcement learning, or learning by reward and punishment, we are modeling the function of the basal ganglia and the dopaminergic function at multiple levels. In our Kakenhi project (Scientific Research on Innovative Areas: Prediction and Decision Making, www.decisions.jp), we combine theoretical and experimental studies of human/animal behaviors, neural network dynamics, and their molecular control. In the Strategic Research Program for Brain Science (brainprogram.mext.go.jp/missionG), we systematically collect connectome and proteome data and construct multi-scale models of signaling cascade, cellular morphology, and electric activities. Under the Strategic Programs for utilizing RIKEN's K supercomputer (www.kobe.riken.jp/stpr1-life), in order to understand the motor symptoms in Parkinson's disease, we are combining large-scale neural network models of the thalamo-cortico-basal ganglia loop with multi-scale models of the musculoskeletal system. We discuss current achievements and future challenges in multi-scale modeling of the brain and the body. No COI.

2S39F-3

UT Heart, a multi-scale, multi-scale heart simulator to bridge the gap between bench and bedside

Sugiura, Seiryo[1]; Cui , Xiaoke[1]; Okada, Jun-ichi[1]; Washio, Takumi[1]; Yamashita, Hiroshi[1]; Kariya, Taro[1]; Nagai, Ryozo[2]; Kadooka, Yoshimasa[3]; Watanabe, Masahiro[3]; Hirahara, Takao[3]; Yamazaki, Takashi[3], Iwamura, Takashi[3]; Nakagawa, Machiko[3]; Hatanaka, Kohei[3]; Yoneda, Kazunori[3]; Hisada, Toshiaki[1](*[1]3rd Department of Internal Medicine, University of Toyama, [2]Jichi Medical University, [3]Fujitsu Ltd*)

Many of the molecular target drugs are associated with their severe and selective toxicity. One of the mechanisms for the development of toxicity is related to their off-target effects. In this presentation, we will discuss on the mechanism of toxicity of sunitinib, and will further discuss on the methods to predict the toxicity of molecular target drugs. Although sunitinib is used for the treatment of renal carcinoma, its administration often results in the development of adverse effects in several tissues including heart, liver and thyroid. Decrease in platelet count is also a sever side effect. We compared the magnitude of inhibition of tyrosine kinases in the body between sunitinib and less toxic sorafenib at their therapeutic concentrations. It was found that phosphorylase kinase gamma subunits 1 and 2 (PHKG 1/2) are extensively inhibited by sunitinib, but not by sorafenib. Systems-biological analysis revealed that the inhibition of PHKG 1/2 results in the decrease in the cellular glutathione (GSH) levels. In vitro studies demonstrated that the reduction of the cellular GSH potentiates the intrinsic toxicity of sunitinib. In vivo studies in mice demonstrated that the recovery of GSH by the administration of anti-oxidants results in the decreased toxicity of sunitinib, without affecting its anti-tumor effects. These results suggest that the pharmacodynamics/systems-biological analysis is useful in the prediction of mechanism-based toxicity of molecular target drugs. COI properly declared.

2S39F-4

Reproducing neuronal images of visual circuits induced by natural scenes in silico

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We have developed a multi-scale, multi-physics heart simulator aiming at a wide range of applications. This simulator is based on the finite element method and the molecular models of excitation-contraction coupling process are implemented in each element (20 million elements for the electrophysiological analysis, one million elements for mechanical analysis). Excitation initiated at the pace-maker site propagates from atria to ventricle and induces synchronized contraction and relaxation to cause physiological blood flow in the heart chamber. We have also created the conduction system, valves and coronary circulation. Such multi-scale, multi-physics nature of the model enables us to examine the impact of molecular or cellular abnormalities on the macroscopic findings observed at the bedside. Examples of applications in basic and clinical sciences will be presented. COI properly declared.

Symposium 40

Recent advancements in our understanding of central pattern generators for rhythmic movements: a phylogenetically preserved circuit from non-vertebrates to mammals

(March 17, 15:05–17:05, Room G)

2S40G-1

Structural and physiological analysis for a central pattern generator controlling swimming locomotion of the ascidian larva

Horie, Takeo[1,2]; Ohkura, Masamichi[3]; Kusakabe, Takehiro G[4]; Nakai, Junichi[3]; Nakagawa, Masashi[5]([1]*Shimoda Marine Research Center, University of Tsukuba,* [2]*JST, PREST,* [3]*Saitama University, Brain Science Institute,* [4]*Department of Biology, Konan University,* [5]*Graduate School of life Science, University of Hyogo*)

It is essential in vision research to reveal the representation of the natural images in neuronal circuits and understand underlying circuit properties. However, the natural scenes are far more complex and dynamic compared with images used in physiological experiments, e.g., oriented slits and moving gratings. Furthermore, the images projected on the retina are continuously influenced by eye movements. In this regards, animal experiments are confronted with unsurmountable technical difficulties to study natural vision in realistic environments. One possible approach to overcome this problem is virtually reproducing the neuronal activities, namely neuronal images, with physiological models in silico under natural visual environments. This approach allows one to visualize how natural scenes are mapped on the neuronal circuits and elucidate the relation between the natural scenes and their neuronal representations. For this purpose, a simulation platform with the following features is required: real-time reproduction of neuronal images for natural scenes, a configurable model structure that performs parallel processing similar to the visual circuits, and compact hardware to be installed on a mockup system that can mimic eye movements. Here, we developed a high-speed, compact and configurable simulation platform to study early vision in natural environments. The platform consists of built-in analog circuits mimicking the essential circuit structure of outer retina and reconfigurable circuits that simulate the neuronal images of inner retina and primary visual cortex. Computations that employed image data sampled at a high frame rate (200 frames/s) achieved real-time reproduction of neuronal images in response to natural scenes under realistic condition. No COI.

2S40G-2

Normal locomotion speed requires pre-motor inhibitory interneurons in Drosophila larva

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Speed of locomotion is significant in animal life. While spatio-temporal activity pattern of motor neurons is controlled by the central nervous system, the identity of interneurons (INs) and the mechanisms of the speed control remain unclear. We use Drosophila larva as a model animal to dissect neural circuits for locomotion. Larvae progress in space by propagation of muscular contraction from the posterior to the anterior segments. To identify INs regulating the larval locomotion, we conducted a genetic screening with Ca imaging and identified a class of INs, termed PMSIs. By anatomical and optogenetic analyses, we found that PMSIs are glutamatergic, inhibitory, premotor, local and segmental INs. When the activity of PMSIs is silenced, the speed of locomotion is reduced, indicating that PMSIs play crucial roles in generating innate speed of locomotion.By dual-color Ca imaging, we found PMSIs and motor neurons in the same segment become active at a similar timing, suggesting that PMSIs shape the temporal pattern of motor neuronal firing through local inhibition. To test this possibility, we acutely blocked the activity of PMSIs by optogenetics, while extracellularly recording motor nerve burst activity. We found that blocking PMSIs activity prolongs the duration of burst of motor nerve activity. These results suggest that PMSIs control the speed of locomotion by regulating, through direct inhibition, the duration of motor neuronal burst in each segment. No COI.

2S40G-3

Functional analysis of locomotor circuits in the spinal cord and brainstem in zebrafish

Higashijima, Shin-ichi; Kimura, Yukiko; Satou, Chie(*National Institutes of Natural Sciences, Okazaki Institute for Integrative Bioscience*)

Neuronal circuits in the spinal cord and brainstem play an important role for producing locomotion in vertebrates. Investigation of locomotor circuits in amniotes, however, is not trivial due to enormous complexity of their neuronal circuits. Zebrafish locomotor circuits are much simpler with less number of distinct classes of neurons, making it more feasible to address this issue. We have set to define the morphology and functional properties of neurons that express a particular transcription factor. In this symposium, we focus on our studies in chx10 positive neurons in the brainstem. We first analyzed the function of Chx10 neurons in locomotion by using of channelrhodopsin (ChR). ChR was expressed in Chx10 neurons using a Gal4-UAS system. Photo-stimulation of the hindbrain of Chx10:Gal4; UAS:ChR transgenic zebrafish reliably elicited swimming, indicating that activation of Chx10 neurons are sufficient to evoke swimming. Then, we performed electrophysiological recordings to ask whether hindbrain Chx10 neurons were active during fictive swimming. We found that some of the Chx10 neurons were active during fictive swimming. Finally, to confirm necessity of the activity of Chx10 neurons for swimming, we used halorhodopsin (Halo) and archaerhodopsin-3 (Arch) for optogenetic inhibition. Both Chx10:Gal4/UAS:Halo and Chx10:Gal4/UAS:Arch fish stopped spontaneous swimming upon green light-application to the hindbrain. These results indicate Chx10 neurons in the hindbrain play indispensable roles to generate swimming. No COI.

2S40G-4

The role of excitatory neurons in the locomotor circuit in mammals: What we can learn from the α-chimaerin knockout mouse

Nishimaru, Hiroshi(*Fac. Medicine, Univ. Tsukuba, Tsukuba, Japan*)

Neuronal circuits in the spinal cord (SC) generate the basic coordinated rhythmic limb movements for walking in mammals. These circuits are controlled by supraspinal inputs such as the motor cortex. During the development of these motor circuits, the receptor tyrosine kinase EphA4 is expressed in the corticospinal tract (CST) axons and in a subpopulation of ipsilaterally-projecting spinal neurons. It has been shown that a-chimaerin (Chn1) plays a crucial role in the downstream signaling of EphA4 and global Chn1 knockout (Chn1-KO) mouse shows abnormal hopping gait as well as aberrant midline-crossing of both of these axons in the SC (Iwasato et al. 2007). Since these axons are suggested to be responsible for the abnormal gait we examined the locomotor circuit in mice which lack Chn1 in the excitatory neurons (ENs) in the telencephalon including the CST neurons (Emx1-Chn1-KO mouse) and in mice which lack Chn1 in vesicular glutamate transporter type 2 (VGLUT2)-positive ENs including the majority of the excitatory spinal neurons (VGLUT2-Chn1-KO mouse). Interestingly, Emx1-Chn1-KO mice showed normal alternating gait while VGLUT2-Chn1-KO mice showed both hopping and alternating gaits. Electrophysiological studies using isolated SC preparations *in vitro* revealed that the spinal network of these mice is capable of generating locomotor patterns similar to those observed *in vivo*. These results indicate that aberrantly-crossed axons of VGLUT2-positive ENs are partly responsible for the generation of the hopping gait while those of CST neurons are less likely to be involved in such abnormal phenotype. No COI.

Symposium 41
Recent progress in cardiovascular research-morphological and physiological approach
[Collaboration Symposium with The Japanese Association of Anatomists]

(March 17, 15:05–17:05, Room H1)

2S41H1-1
PGE-EP4 signaling is a key regulator of cardiovascular stiffness and elasticity

Yokoyama, Utako(*Cardiovascular Research Inst., Yokohama City Univ., Yokohama, Japan*)

Collagen and elastic fiber formation are responsible for structural integrity of cardiovascular system. Since prostaglandin E (PGE) receptor EP4 is abundantly expressed in the developmental and diseased arteries and in the heart, our study has been focusing on the role of PGE-EP4 signaling in the regulation of cardiovascular extracellular matrices. During fetal period, placenta-derived PGE_2 activates EP4 and EP4-c-Src-PLC-signaling pathway inhibits elastogenesis through degrading lysyl oxidase protein in the ductus arteriosus which is a fetal bypass artery between the aorta and pulmonary artery. In arterial pathological condition, i.e. aortic aneurysm and atherosclerosis, PGE_2 production is locally increased and enhances interleukin-6 production and matrix metalloprotease 2 activity, resulting in degradation of collagen and elastic fibers. In addition, we recently found that EP4 is a primarily PGE receptor in cardiac fibroblasts and that endogenous PGE_2-EP4 signaling has a protective role against cardiac fibrosis possibly through inhibiting connective tissue growth factor (CTGF)-mediated activation of myofibroblasts. Stimulation of EP4 decreased α-smooth muscle actin, a myofibroblast marker, and CTGF protein expression in rat cardiac fibroblasts. In contrast, in cardiac fibroblasts of $EP4^{+/-}$ mice, CTGF expression was significantly higher compared to those of wild-type mice. Systemic administration of angiotensin II-induced cardiac fibrosis was greater in $EP4^{+/-}$ than in wild-type mice. Regulation of EP4 signaling may be a new therapeutic strategy to prevent excessive cardiac fibrosis which leads to heart failure. COI properly declared.

2S41H1-2
Endothelial class II PI3K-C2α controls vasucular barrier integrity through regulating membrane trafficking.

Yoshioka, Kazuaki[1]; Takuwa, Noriko[1,2]; Okamoto, Yasuo[1]; Takuwa, Yoh[1]([1]*Department of Physiology, Kanazawa University School of Medicine, Ishikawa, Japan,* [2]*Department of Health and Medical Sciences, Ishikawa Prefectural Nursing University, Ishikawa, Japan*)

Vascular barrier function, which is structurally supported mainly by the adherens junction (AJ) comprising VE-cadherin, maintains low vascular permeability in healthy vasculatures. The assembly of the AJ is tightly controlled by intracellular signaling molecules including Rho GTPases. Phosphatidylinositol (PI) 3-kinase (PI3K) family regulates diverse cellular functions; while class I PI3Ks and class III Vps34 are well-characterized, the physiological roles of PI3K class II, which comprises C2α, C2β and C2γ and produces PI(3)P, remain largely unknown. We generated C2α KO mice, which were embryonic lethal due to severe defects in angiogenesis. $C2\alpha^{+/-}$ mice and inducible endothelial cell (EC)-specific C2α-deleted mice exhibited vascular barrier dysfunction: C2α-KO mice were much more sensitive to challenge with an anaphylaxis mediator platelet-activating factor with increased lethality and chronic infusion of angiotensin II with the formation of dissecting aneurysms. In EC, siRNA-mediated C2α knockdown induced decreased $PI(3)P^+$-endosomes, impaired endosomal trafficking, and defective delivery of VE-cadherin to AJ. C2α knockdown also impeded cell signaling including VEGF receptor-2 and S1P1 receptor internalization and Rho activation on the endosomes. Thus, our data disclose the novel crucial functions of PI3K-C2α in barrier integrity and vascular formation and represents a new therapeutic target for vascular diseases. No COI.

2S41H1-3
Visualization of secretory dynamics of tissue-type plasminogen activator and cell surfase-associated fibrinolytic activity on vascular endothelial cells

Suzuki, Yuko; Sano, Hideto; Brzoska, Tomasz; Urano, Tetsumei(*Department of Medical Physiology, Hamamatsu University School of Medicine, Hamamatsu, Japan*)

Vascular endothelial cells (VECs) contribute to keep the patency of vasculature through anti-coagulatory and pro-fibrinolytic activities. Tissue-type plasminogen activator (tPA) secreted from VECs as an active form, directly enhances fibrinolytic activity. Recently, we succeeded to visualize its secretory dynamics in GFP-tagged tPA (tPA-GFP) expressing VECs using total internal reflection fluorescence microscopy. tPA-GFP appeared to have a unique secretory dynamics and to remain on the cell surface after exocytosis from its secretory granules. Studies using mutants of tPA-GFP suggested that the binding to the cell surface could be altered either by modifying its molecular structure via catalytically inactivation, fibrin-associated domain deletion, replacement in glycosylation sites, or by modifying the interaction with its specific inhibitor. The retained active tPA was shown to enhance the cell surface fibrinolytic potential to activate plasminogen into plasmin as follows. Fluorescent-labeled plasminogen appeared to accumulate on cell surface at tPA-GFP retained spots as well as pericellular/matrix adhesive area in lysine-binding sites- and tPA/plasmin activity-dependent manner. Fibrin network formed on VECs was effectively dissolved when tPA-GFP, but not catalytically inactive tPA-GFP, was expressed. Our results provide new insights into the mechanism maintaining high fibrinolytic activity on the VEC surface. No COI.

2S41H1-4

Origin of cardiovascular cells and heart development controlled by VEGF-Flk1 signaling.

Ema, Masatsugu[1]; Otsu, Ayaka[2]; Azami, Takuya[2]; Hirashima, Masanori[3]; Koshiba-Takeuchi, Kazuko[4]; Takeuchi, Jun[4]; Shibuya, Masabumi[5]; Rossant, Janet[6]; Nishie, Tomomi[2]([1]*Shiga University of Medical Science, RCALS,* [2]*Tsukuba Univ., Fac. of Med., Dept. of Anat. and Embryol.,* [3]*Kobe Univ., Grad. Sch. of Med., Dept. of Vascular Biology,* [4]*Tokyo Univ., Inst. of Mol. and Cell. Biosci., Div., of Cardiovascular Regeneration,* [5]*Jobu University*)

During the development, vascular development relies on VEGF and its receptors such as Flk1 and Flt1. Previous data indicate that hemato-cardiovascular cells such as hematopoietic cells, endothelial cells, cardiomyocytes and smooth muscle cells are generated from Flk1-positive cells during mouse and human ES (Embryonic Stem) cell differentiation in vitro. However, developmental process in vivo remains unclear. Our lineage tracing experiment indicates that most of cardiomyocytes and endothelial cells, if not all, originate from Flk1 positive cells, while smooth muscle cells at E11.5 originate from Flk1 negative population, in sharp contrast with that of in vitro data. Furthermore, we find that the VEGF-Flk1-Flt1 signaling is involved in heart development. Previous studies revealed that hematopoietic and endothelial cells are not generated in the absence of Flk1 gene, while hematopoietic and endothelial cells increase in the absence of Flt1 gene. Interestingly, we find that cardiomyocyte markers such as Nkx2.5, Tbx5, Isl1 and Mlc2a are up-regulated in the absence of Flk1. On the other hand, cardiomyocyte markers such as Nkx2.5, Tbx5, Isl1 and Mlc2a are down-regulated in the absence of Flt1. Thus, VEGF-Flk1-Flt1 pathway is involved in normal heart development. No COI.

Symposium 42
The Challenge of the Center for Promotion of Gender Equality
[Symposium for Comittee for Gender Equality]

(March 17, 15:05–17:05, Room J)

2S41H1-5

Functional mechanisms of cardiomyocyte plasticity during heart regeneration

Takeuchi, Jun K.([1]*Div. of Cardiovas. Res., IMCS, the University of Tokyo,* [2]*Grad. Sch. of Science., the Univ. of Tokyo,* [3]*JST PRESTO*)

Transcription factors have specific expression to produce heart cells from pluripotent stem cells, and to keep their functions in the adult. However, these factors are insufficient as master regulators of cardiac lineages form pluripotent stem cells, and maintenance for a healthy heart. To address these questions, we generated tissue-specific gene profiles to stimulate cardiac fate. Fortunately, we have reported that Baf60c, a member of SWI/SNF-BAF family acts as a key molecule to promote cardiac cells from mesodermal cells with cardiac Tanscription Factors; TFs, Tbx5 and Gata4 in vivo (Takeuchi & Bruneau, Nature 2009; Takeuchi et al., Nat. Commun. 2011). In addition, this factor strongly regulates chromatin conformation of BAF complexes and Histone modification on these TFs promoters by ChIP analysis. BAF complexes act as key factors to keep cardiomyocyte survival in adult heart as well as its induction in embryonic stage. BAF overexpressed heart protect heart failure after myocardial infarction via promoting several-gene expression such as cell-growth factors, cardiac transcription factors, angiogenesis as well as repression of inflammation signals. These results indicate that stage specific factors with chromatin remodelers promote cardiac cell fate and keep its life. No COI.

2S42J-1

The Challenge of the Center for Promotion of Gender Equality, Kagoshima University

Masuda, Mina(*Department of Anesthesiology and Critical Care Medicine Kagoshima University Graduate School of Medical and Dental Sciences*)

2S42J-2

Personalized work-life balance—You can put bread on your table—

Uchiyama, Asako(*Shin Nippon Biomedical Laboratories, Ltd.*)

2S42J-3

「単身赴任＋子育て＋親の援助を年数回」での研究生活

Nakasako Izumi, Hiroko(*Dept. Pharmacol., Fclt. Med., Toho Univ., Tokyo, Japan*)

2S42J-4

子育て夫婦ポスドク生活と、単身赴任二重生活（親の援助有り）を振り返って

Miyakawa, Naohisa(*Department of Ultrastructural Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry*)

2S42J-5

大規模アンケート調査（科学技術系専門職の男女共同参画実態調査）から見る安定した研究者ライフとは

Sekino, Yuko[1]; Sugiura, Midori[2]([1]*National Institute of Health Sciences,* [2]*Aichi Gakusen University*)

Symposium 43
ATP as a regulatory factor: New biological function regulated via interaction with membrane proteins

(March 17, 15:05–17:05, Room K)

2S43K-1

The Na^+/H^+ exchanger NHE1 as an ATP-binding protein: regulation via lipid-interacting domain

Wakabayashi, Shigeo; Shimada-Shimizu, Naoko; Hisamitsu, Takashi; Nakamura-Nishitani, Tomoe Y(*Dept. of Mol. Physiol.,Natl. Cer. Cardiovas. Ctr., Osaka, Japan*)

While many biological reactions such as ion-pumping require the energy from hydrolysis of ATP, little is known about the role of ATP as a direct regulatory factor for membrane ion transporters. We here report that ATP directly interacts with the plasma membrane Na^+/H^+ exchanger NHE1. NHE1 does not require energy input from ATP and has no consensus ATP-binding motif, but curiously the physiological NHE1 activity is abolished by cellular ATP depletion. Using the purified complex protein of NHE1 and its subunit CHP1 from SF9 insect cells, we examined a photo-affinity labeling reaction with 8-azido-ATP[γ]-biotin. UV irradiation promoted the incorporation of 8-azido-ATP into NHE1, but not into CHP1, which was inhibited by non-labeled ATP at IC_{50} of 2.2 mM, close to the ATP concentration activating NHE1. ATP was found to bind to the membrane-proximal cytoplasmic region (G542-P598) called the lipid-interacting domain (LID) that had also previously been identified as an ATP-sensitive region for NHE1 regulation. These findings suggest that NHE1 is an ATP-binding transporter. Interestingly, staurosporine derivatives were found to bind to the LID in competition with ATP and strongly inhibited the NHE1 activation in response to various stimuli. These results suggest that the LID functions as a molecular switch that dictates the activation state of NHE1 in response to various stimuli. Thus, we propose that ATP is a primarily important molecule in NHE1 regulation. No COI.

2S43K-2

K_{ATP} channel mutation that causes hyperinsulinism in early life and progresses to glucose intolerance in adults.

Shimomrua, Kenju[1]; Maejima, Yuko[1]; Ashcroft, Frances[2]; Yada, Toshihiko[1]([1]*Department of Physiology, Division of Integrative Physiology,* [2]*Department of Physiology Anatomy and Genetics, Oxford University, Oxford, England*)

Loss-of-function mutations in the K_{ATP} channel genes KCNJ11 (Kir6.2) and ABCC8 (SUR1) cause neonatal hyperinsulinism in humans. Dominantly inherited mutations cause less severe disease, which may progress to glucose intolerance and diabetes in later life. One of these mutations is E1506K mutation in SUR1 subunit. The mouse expressing SUR1-E1506K in SUR1 was generated. In these mutant mice, K_{ATP} channel inhibition by MgATP was enhanced, due to impaired channel activation by MgADP. Mutant beta-cells showed decreased K_{ATP} channel activity and firing of action potential in glucose-free solution. E1506K mutated mice exhibited enhanced insulin secretion and lowered fasting blood glucose within 8 weeks of birth. At 6 months of age, in contrast, these mice showed reduced insulin secretion and impaired glucose tolerance. The reduced insulin secretion correlated with lower insulin content; insulin content increased with age in wild-type mice but not in E1506K mice. There was no difference in the number and size of islets or beta-cells. These results in the mouse model suggest that the gradual development of glucose intolerance in patients with the SUR1-E1506K mutation might result from impaired insulin secretion primarily due to the failure of insulin content to increase with age. No COI.

2S43K-3

Regulation of L-type Cav1.2 Ca^{2+} channels by ATP

Kameyama, Masaki[1]; Feng, Rui[1,2]; Liu, Shunyuan[1,2]; Minobe, Etsuko[1]; Xu, Jianjun[1]; Kameyama, Asako[1]; Hao, Liying[2] ([1]*Dept. Physiol., Grad. Sch. Med. Dent. Sci., Kagoshima Univ.,* [2]*Dept. Pharm. Toxicol., Sch. Pharm., China Med. Univ.*)

L-type Cav1.2 class Ca^{2+} channels (LTCC) is regulated by number of cytoplasmic signaling systems and factors, such as protein phosphorylation, Ca^{2+}/calmodulin (CaM) and oxidation/reduction. It is known since 1980s that activity of LTCC is also dependent on ATP. However, the underlying mechanism has not been established to date. Although ATP-dependent change in the balance of phosphorylation/dephosphorylation state of LTCC is implied, a mechanism independent of phosphorylation has also been suggested. This idea has been supported by inside-out patch experiments, in which activity of LTCC requires ATP (EC_{50} ~0.5 mM) together with CaM. Since AMP-PNP, a non-hydrolyzable analog of ATP, was partly substitutable for ATP, we hypothesized that ATP might bind directly with the LTCC proteins and regulate its activity. 2′/3′-*o*-(2-aminoethyl-carmamoyl)-ATP-biotin ([EDA-ATP]-biotin; biotin conjugated at ribose), but not γ-[(6-aminohexyl)]-ATP-biotin ([6AH-ATP]-biotin, biotin conjugated at γ-phosphate), mimicked the ATP effect, implying that the phosphate group of ATP might be involved in the binding of ATP. Photo-affinity labeling of LTCC protein with 8-azido-[EDA-ATP]-biotin *in vitro* revealed that the reagent labeled N-terminal and proximal C-terminal tail of the α1-subunit of LTCC. These results support the idea that ATP binds directly with LTCC protein and regulates channel activity. This mechanism may be relevant to the suppression of LTCC activity during hypoxia of cells. No COI.

2S43K-4

Mechanism of ligand recognition and activation of ATP-gated cation channels

Hattori, Motoyuki[1,2]([1]*Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, Tokyo Japan,* [2]*JST PRESTO, Tokyo, JAPAN*)

ATP is known as the vital energy source involved in energy metabolism, biosynthetic reactions and active transport. It is also an essential extracellular signaling molecule that activates two distinct families of ATP receptors: ionotropic P2X and G-protein-coupled P2Y receptors. P2X receptors are trimeric non-selective cation channels involved in various physiological processes including synaptic transmission, taste, neuronal pain and inflammation. A recent crystal structure of zebrafish P2X4 receptor in an apo, closed state, revealed the chalice-shaped trimeric architecture of P2X receptors (Kawate et.al. Nature 2009). However, due to the lack of a crystal structure in complex with ATP, the agonist binding site and the mechanism of channel activation remained unclear. I will present the agonist-bound structure of zebrafish P2X4 receptor and discuss the mechanism of ligand binding and activation of P2X receptors (Hattori & Gouaux Nature 2012). No COI.

Symposium 45
Disorders of the autonomic nervous system in the menopause and underlying mechanisms

[Collaboration Symposium with Japan Society of Neurovegetative Research]

(March 18, 10:05–12:05, Room C)

3S45C-1

Involvement of sex steroids in the regulation of the central autonomic nervous system

Ueyama, Takashi(*Department of Anatomy and Cell Biology, Wakayama Medical University School of Medicine, Wakayama, Japan*)

Receptors for sex steroids such as estrogen and androgen (ERα, ERβ and AR) are widely expressed in the brain, where sex steroids modulate central nervous function. Limbic systems (amygdala, lateral septum, infralimbic, insular, ventromedial temporal cortical regions), and several hypothalamic and brainstem nuclei have been identified as the central sites that regulate stress-induced sympathetic nervous activation. All amygdaloid subnuclei receive psychological information from other limbic regions, while the lateral and central subnuclei receive sensory and immune information from parabrachial nucleus and medical geniculate nucleus. Output to the hypothalamus mainly originates from the medial amygdala, while output to the bed nucleus of the stria terminalis originates from the central amygdala and the medial amygdala. As ERα, ERβ and AR are highly expressed in the medial amygdala, sex steroids modulate the autonomic nervous activities. Estrogen supplementation attenuated stress-induced cardiovascular changes. It also attenuated the stress-induced increase of c-Fos in the lateral septum, medial amygdaloid nucleus, paraventricular hypothalamic nucleus, dorsomedial hypothalamic nucleus, laterodorsal tegmental nucleus and locus coeruleus. It also down-regulated *c-fos* mRNA expression in the adrenal gland and the heart, suggesting an increase of estrogen attenuated the stress-induced hypothalamo-sympathoadrenal outflow from the central nervous system to the target organs. No COI.

3S45C-2

The role of estrogen in thermoregulation: assessment from animal to human studies.

NAGASHIMA, KEI([1]*Body Tem and Fluid Lab, Fac Human Sci, Tokorozawa, Japan,* [2]*IABS, Waseda Univ., Tokorozawa, Japan*)

Estrogen has many roles besides those for puberty and reproduction. In the present symposium, I introduce our studies investigating the role of estrogen in thermoregulation. **Human Study** In 8 women (age, 20–22 y), metabolic rate, body core (T_{core}) and skin temperature (T_{skin}, skin blood flow, and thermal sensation were assessed during cooling of room temperature from 29°C to 23.5°C. The measurements were repeated twice in the follicular and luteral phases. T_{core} was higher in the luteral phase. T_{skin} and blood flow decreased and metabolic rate increased with the reduction of room temperature, but there were no differences between the phases. Thermal sensation of cold was stronger in the luteral phase. The results suggest that sex hormones may affect thermal sensation in humans, although its influence on autonomic responses is small. **Animal Study** To assess the difference in thermal sensation between genders, and its role of estrogen, we estimated thermal preference. Normal mele and female mice, and ovariectomized mice with and without estrogen replacement were used (5 ea). Thermal preference was assessed by placing the mice in a behavioral box, of which bottom was covered with 5 Pertier boards and the temperature was randomly changed (28°C, 31°C, 34°C, 37°C, and 40°C). Female mice liked hotter temperature compared to male mice (40°C and 34°C); however, there was no difference among normal female and ovariectomized mice. Gender may has an influence on thermal preference; however, estrogen is not involved in the mechanism. No COI.

3S45C-3

Estrogen-induced anorexia is dependent on light environment

Takamata, Akira; Mabuchi, Kaori; Nishimura, Yuri; Morimoto, Keiko
(*Department of Environmental Health, Nara Women's University, Nara, Japan*)

Estrogen has anti-obesity and anorexigenic effects. The prevalence of obesity in women increases after menopause, which elevates a risk for metabolic and cardiovascular diseases. In animals, ovariectomy increases food intake and body weight gain, and estrogen replacement can reverse these changes, strongly suggesting that estrogen plays a critical role in controlling food intake and body weight in females. We found that estrogen replacement attenuated food intake and increased c-Fos expression in the suprachiasmatic nucleus (SCN) specifically during the light phase in ovariectomized rats. We also found that removal of light exposure during the light phase increased food intake and decreased c-Fos expression in the SCN specifically during the subjective day in estradiol-replaced ovariectomised rats but not in estrogen deficit rats, indicating that the anorexigenic effect and enhanced neuronal activity of the SCN induced by estradiol replacement are abolished by removal of light stimulation during the light phase. These suggests that the disrupted circadian rhythm of ingestive behavior is possibly involved in the mechanism for the estrogen deficiency-induced hyperphagia, and that light stimulation during the subjective day is necessary to elicit the anorexigenic effect of estrogen. No COI.

Symposium 46
Risk stratification of lethal ventricular arrhythmias: from bench to bedside

(March 17, 9:00–11:00, Room D1)

3S45C-4

Estrogen replacement suppresses hypertensive responses to psychological stress and intermittent hypoxia in ovariectomized rats

Morimoto, Keiko[1]; Tazumi, Shoko[1]; Omoto, Sayo[1]; Takamata, Akira[1]; Kudo, Risa[2]; Hatake, Katsuhiko[2]; Nagai, Hisashi[3]; Yoshida, Ken-ichi[3]([1]*Dept. Environmental Health, Facult. Human Life and Environmental Sci., Nara Women's Univ., Nara, Japan,* [2]*Dept. Forensic Med., Nara Medical Univ., Nara, Japan,* [3]*Dept. Forensic Med. Grad. Sch. Med. Tokyo Univ., Tokyo, Japan*)

Hypertension is a major cardiovascular risk factor. The prevalence of hypertension in women increases after the average age of menopause. Estrogen has been postulated to involve in blood pressure (BP) regulation. We examined whether estrogen replacement in ovariectomized rats affects the BP elevation induced by acute psychological stress or chronic intermittent hypoxia (IH). Female Wistar rats aged 9 wk were ovariectomized and implanted with radiotelemetry devices for BP and heart rate measurements. After 4 wk, the rats were assigned either to a placebo-treated (Pla) group or a group treated with 17β-estradiol (E2). Rats were exposed to cage-switch stress for 60 min. The stress-induced pressor response was attenuated in the E2 group compared with the Pla group. Another set of Pla and E2 groups underwent IH exposure for 2 wk (90 s at 4 % O_2 every 3 min, 8 h day-1 during the light phase). Estrogen replacement could not prevent the elevation of BP in IH-phase, but inhibited the increase of BP in non-IH-phase at 1-wk IH exposure. Further, we investigated the mechanisms accounting for the suppressing effect of estrogen on pressor response to the cage-switch stress or hypertension induced by IH. Our findings suggest that renin-angiotensin system or endothelium-dependent vasodilation was involved in the effect of estrogen. No COI.

3S46D1-1

Underlying mechanisms in acquired long QT syndrome

Itoh, Hideki[1]; Crotti, Lia[2]; Schwartz, Peter J[2]; Hayashi, Kenshi[3]; Nakajima, Tadashi[4]; Ohno, Seiko[1]; Makiyama, Takeru[5]; Yamagishi, Masakazu[3]; Imoto, Keiji[6]; Pascale, Guicheney[7]; Hoire, Minoru[1]([1]*Department of Cardiovascular and Respiratory Medicine, Shiga University of Medical Science, Shiga, Japan,* [2]*Department of Molecular Medicine, University of Pavia, Pavia, Italy,* [3]*Division of Cardiovascular Medicine, Kanazawa University Graduate School of Medical Science, Kanazawa, Japan,* [4]*Department of Medicine and Biological Science, Gunma University Graduate School of Medicine, Maebashi, Japan,* [5]*The Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan,* [6]*Department of Information Physiology, National Institute for Physiological Sciences, Okazaki, Japan,* [7]*Faculte de Medecine Pierre et Marie Curie, Paris, France*)

While about half of congenital long QT syndrome (cLQTS) cases are associated with cardiac ion channels gene mutations, the genetic background of long QT syndrome (aLQTS) manifested by acquired factors remains to be clarified.From 6 centers in Japan, Italy and France, this study consisted of 168 aLQTS probands. In aLQTS, the mean QTc intervalwas 450±41ms at baseline,significantly longer than in noncarriers (402±44ms, $p<0.0001$) and shorter than in cLQTS (475±48 ms, $p<0.0001$). Genetic analyses revealed 51 mutations in aLQTS and these mutations were detected regardless the races and gender. When we simulated QT interval in the pseudo-ECG with the dynamic ORd model, the simulated QT interval in aLQTS was milder than cLQTS. aLQTS has collapsed reporalization reserve and 30% have mutations associated with a forme fruste of cLQTS. No COI.

3S46D1-2

Is Ca^{2+}/calmodulin-dependent protein kinase II involved in ventricular fibrillation degeneration?

Tsuji, Yukiomi; Makita, Naomasa(*Department of Molecular Physiology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan*)

Ventricular fibrillation (VF) is the most common cause of sudden cardiac death. The trigger like a premature ventricular contraction initiates ventricular tachyarrhythmia, which degenerates into VF. However, molecular basis underlying the progressive process leading to VF is poorly understood. Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII) has emerged as a pro-arrhythmic signaling molecule. CaMKII activation initiated by Ca^{2+}/calmodulin is sustained by autophosphorylation and oxidation. CaMKII can participate in arrhythmia signaling by effects on ion channel proteins, intracellular Ca^{2+} uptake and release, regulation of cell death, and by activation of hypertrophic signaling pathways. The actions of CaMKII on voltage-gated Ca^{2+} and Na^{+} channels and Ca^{2+} releasing ryanodine receptor channels are important components for arrhythmia-initiating afterdepolarizations. CaMKII also promotes the ventricular remodeling progression by activating transcription factors for hypertrophy, inflammation and apoptosis, producing a substrate for reentry. Studies using a variety of models of cardiac diseases consistently show that CaMKII-inhibition provides antiarrhythmic benefits. We have recently created a rabbit model of VF storm that features repetitive defibrillator firing for recurrent VF and demonstrated the connection between VF storm and CaMKII in this model. Here, we focus on potential roles of CaMKII on transition from premature ventricular contraction into VF, based on recently-emerging experimental findings by us and others. No COI.

3S46D1-3

A simulation study of ventricular arrhythmias generated from the Purkinje network with gap junction mutation

Inada, Shin[1]; Harrel, Daniel Toshio[2]; Haraguchi, Ryo[1]; Ashihara, Takashi[3]; Makita, Naomasa[2]; Nakazawa, Kazuo[1,3]
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The Purkinje fiber network is the part of the cardiac conduction systems that ensures coordinated contraction of the ventricles, but can also be an origin of ventricular arrhythmias such as ventricular fibrillation. Recently, a mutation in connexin40 (Cx40), a gap junction isoform predominantly expressed in atrium and His-Purkinje system, was identified in patients with progressive cardiac conduction defect associated with lethal ventricular arrhythmias. However, it remains unknown how this gap junction dysfunction of the Purkinje fiber results in generating arrhythmias. To resolve this issue, we investigated the relationship between the excitation conduction property of the Purkinje fiber and the initiation of reentrant beats using computer simulations. We constructed an anatomical models of the rabbit Purkinje network with multiple conduction pathways between the His bundle and ventricle. To simulate Cx40 mutation, the gap junction conductance was reduced down to 5 %. Under conditions of reduced gap junction conductance, conduction blocks occurred at the intersections between Purkinje fibers that result in establishment of reentrant circuits. Our simulation results suggest that reduction in gap junction conductance in the Purkinje network may be sufficient to generate reentrant ventricular arrhythmias without structural abnormalities. No COI.

3S46D1-4

The application of induced pluripotent stem cells-derived cardiomyocytes in drug safety testing and disease modeling

Makiyama, Takeru(*Department of Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, Japan*)

Human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) are a promising new tool in drug safety testing because we can study the 'human cardiomyocytes'. Studies using multi-electrode array system with beating hips-CMs or Ca transient recording with cell sheets have been reported to be useful to investigate the antiarrhythmic drug response of iPS-CMs. In addition, disease modeling by iPS-CMs from patients with an inherited cardiac diseases are expected to elucidate the disease causing mechanisms and be a helpful in vitro system to investigate the new pharmacotherapies. However, there are several limitations in iPS-CMs. They have spontaneous activity and anomalous behavior of action potential parameters resulting from mainly increased If and decreased IK1. Recently, we reported the ultrastructural maturation process of iPS-CMs during a long time culture. We also discuss about the electrophysiological maturation process in this presentation. In addition, we generated disease-specific iPS cells from a patient with catecholaminergic polymorphic ventricular tachycardia (CPVT). The differentiated cardiomyocytes had an increased susceptibility to catecholamine induced diastolic calcium waves and ryanodine suppressed them. It is suggested that this iPS cell-based model of CPVT is useful for investigating new therapeutic approach. We also present our recent progress in other cardiac disease modeling (long-QT syndrome and cardiomyopathy). No COI.

Symposium 47
Attractiveness of exercise physiology based on molecular biological research

(March 17, 9:00–11:00, Room E)

3S47E-1

Molecular exercise physiology of skeletal muscle—Basic research and its practical applications—

Takemasa, Tohru(*Faculty of Health and Sport Sciences, University of Tsukuba*)

"Exercise physiology" is study for elucidating how a body responds and is changed by exercise stimulation. Skeletal muscle is extremely plastic tissue that changes its quantitative and qualitative characteristics by exercise. Skeletal muscle increases its mass by resistance training and increases its aerobic capacity by endurance exercise, whereas decreases its mass by unloading or aging. In our laboratory, in order to elucidate the molecular mechanism for above-mentioned phenomena, we applied overload/unload in mice skeletal muscle and analyze it by molecular biological methods. I would like to introduce one of the results and plan for future studies in this symposium.
Gene therapy is becoming an important technology for medical treatment, and further progress is anticipated with further improvements in biotechnology. On the other hand, in the field of sports, anti-doping authorities fear that "gene doping", which is abuse of gene therapy, will become a means by which athletes can improve their performance. To investigate the feasibility of developing a method for detection of gene doping in power-athletes, we devised an experimental model system. We tried to detect gene doping with this model, and I will discuss the limit of gene doping detection at this point. No COI.

3S47E-2

Epigenetics for the preservation of characteristics in skeletal muscle

Kawano, Fuminori(*Graduate School of Medicine, Osaka University*)

Histone modifications, such as methylation and acetylation, play a critical role for the transcriptional onset of most genes via the conformational change of chromatins. The main purpose of the present study was to identify the major histone modification involved in the induction of skeletal muscle characteristics. In first, four fast-specific genes with minor expression in rat soleus and four slow-specific genes with minor expression in rat plantaris were selected from all transcripts as the targets by microarray analysis. Responses of the expression of these genes to functional overloading by transection of tendons of synergists were determined in plantaris of adult rats. Overloading caused the increase in the number of slow fibers, hypertrophy in slow fibers and the enhanced expression of four slow-specific genes. No changes were observed in fast genes. Further, the enhanced expression of fast genes in plantaris was noted in neonatal rats at 2-day after birth, although the expression of slow genes started increasing after the beginning of voluntary locomotion in the late stage of lactation period. These data indicated the different manner in the gain of muscle type-specific genes. Next, Modification of histone was checked using ChIP-qPCR. Transcriptionally active marks, H3K4me3 and H3 acetylation, were noted in the transcription start site of fast genes in plantaris, whereas the expression of slow genes was not related to the epigenetic marks in soleus. These findings suggested that genes with different epigenetic marks showed the different responses to the physiological stimuli. No COI.

3S47E-3

Development of biological strategy for preventing skeletal muscle wasting with exercise.

Miyazaki, Mitsunori(*Health Sciences University of Hokkaido, Graduate School of Rehabilitation Sciences*)

The maintenance of skeletal muscle mass is critical for long-term health and quality of life, because muscle mass wasting induced by disuse, aging or catabolic diseases is highly associated with functional impairment and disability, which then leads to the loss of independence and increased risk of morbidity/mortality. Despite the significance of skeletal muscle loss and weakness as inevitable concomitants with catabolic conditions, the coordinated strategy for preventing muscle wasting has been under development. At this time, only proven and effective intervention to counteract to muscle mass wasting is the physical exercise (particularly resistance exercise in combination with adequate nutrients supplementation). It has been generally accepted that the net balance between protein synthesis and degradation is a critical determinant of the regulation of skeletal muscle mass. Recent studies indicate that the skeletal muscle protein metabolism (protein synthesis and protein degradation) is regulated largely through environmental cues including physical exercise. The aim of the present session is to summarize and discuss, 1) recent progress in the understanding of cellular mechanisms in anabolic (protein synthesis) and catabolic (protein degradation) signaling pathways that govern the regulation of skeletal muscle mass, 2) altered capacity in the protein metabolism in response to physical exercise. It is quite possible that precise understanding of cellular mechanisms that govern protein metabolism in skeletal muscle will develop more effective therapeutic interventions to prevent the loss of muscle with aging and disease. No COI.

3S47E-4

Coupling of contraction to metabolic control in skeletal muscle

Fujii, Nobuharu(*Dept. Health Promotion Sciences, Grad. Sch. Human Health Sciences, Tokyo Metropolitan University*)

It was generally regarded that the major role of skeletal muscle was to produce movement by contracting. However, recent studies reported multiple cases in which metabolism was adjusted by coupling to skeletal muscle contraction, which indicated that skeletal muscles might have other biological roles in addition to the production of movement. For example, muscle contraction strongly stimulates glucose transport from blood into the muscle cells, the effect of which is equivalent to that of insulin. In addition, it has been revealed that skeletal muscle secretes several humoral factors (myokines) for metabolic control. Skeletal muscle was rarely regarded as a target for medication in the past, but recent findings have led to it being focused on as a potential drug target. This presentation will focus on the molecular mechanism of coupling of muscle contraction to metabolic control. No COI.

Symposium 48
Cutting-edge researches of membrane proteins—Towards molecular mechanisms and physiological functions

(March 18, 10:05–12:05, Room F)

3S48F-1

Activation-signal transmission in the trimeric $P2X_2$ receptor channel upon voltage- and [ATP]- dependent gating

Kubo, Yoshihiro[1]; Keceli, Batu(*Div Biophys and Neurobiol, Natl Inst Physiol Sci, Okazaki, Japan*)

ATP receptor channel $P2X_2$ is a trimer composed of three subunits. We previously reported that the activation of $P2X_2$ is voltage and [ATP] dependent in spite of the absence of a canonical voltage sensor. It has been known that $P2X_2$ can be activated by the binding of two ATP molecules to the trimer. The aim of this study is to know how the activation signal by the binding of two ATP to the trimeric $P2X_2$ is transmitted to the pore opening. Towards this aim, we introduced mutations at different levels, i.e. ATP binding site (K308A), linker region on β-14 (D315A) and pore domain (T339S), by controlling the number of mutations in the tandem trimeric constructs (TTC). We observed the followings. (1) Two or three, but not one, K308A mutation in the trimer affected the activation of $P2X_2$. (2) Two or three, but not one, D315A mutation induced two different gating modes. (3) T339S mutation, which induced constitutive activity at all membrane potentials, showed gradual changes from WT with the increase in the number of T339S, suggesting independent contribution of three subunits at the pore level. (4) We introduced one K308A mutation and one D315A mutation on the same (cis) or different (trans) subunit. Their phenotypes were clearly different one another. (5) In the case of T339S and K308A (or D315A), the phenotype of cis and trans mutants were similar. Taken together, it was suggested that the ATP binding signal is directly transmitted to the corresponding β-14 strand down to the level of D315 and then it spreads to all three subunits equally at the pore level. No COI.

3S48F-2

Molecular mechanisms of voltage-gated proton channel, VSOP/Hv1

Okamura, Yasushi[1]; Fujiwara, Yuichiro[1]; Kawanabe, Akira[1]; Sakata, Souhei[2]; Kurokawa, Tatsuki[1]([1]*Laboratory of Integrative Physiology, Osaka University, Suita, Japan,* [2]*Inst. Academic Initiatives, Suita, Japan*)

Proton plays key roles in homeostasis and numerous physiological functions including metabolism, respiration, digestion, bone remodeling, and renal excretion and is also for critical for microenvironment for pathogen infection, inflammation and cancer progression. VSOP/Hv1 is the proton-selective ion channel that is conserved from algae to human. It is dimeric and each protomer has four transmembrane segments with homology to the voltage sensor domain of voltage-gated ion channels. The fourth transmembrane segment, S4, contains multiple arginines as the signature pattern of voltage sensor. VSOP/Hv1 also contains the cytoplasmic stretch of the coiled-coil region that is essential for dimerization. Channel activities are innate to protomers of VSOP/Hv1, and dimer channels exhibit cooperative gating with steeper voltage sensitivity than protomer channel. Given that the minimum unit of proton channel is ascribed to the portion consisting only of about 150 amino acids, VSOP/Hv1 is the smallest cation channel among so far known in mammals. Despite recent intensive studies, no atomic structural information has been available, and it remains yet unclear how molecular mechanisms of VSOP/Hv1 are related to those of other voltage-sensor-regulated channels and phosphatase. In this symposium, we will introduce recent findings of structure and functions of VSOP/Hv1 and discuss on how sophisticated properties such as voltage sensing, proton permeation and pH sensing can be achieved in this simple protein architecture. No COI.

3S48F-3

TCA suppress the transduction channel activity in the olfactory transduction cascade

Takeuchi, Hiroko; Kurahashi, Takashi(*Graduate School of Frontier Biosciences, Osaka University*)

We examined the effect of off-flavors generated in foods/beverages on the olfactory receptor cells. The off-flavor substances induce exogenous unpleasant smells even at a very low concentration (ppt level). Although not yet scientifically demonstrated, it has also been pointed out that off-flavors reduce the pleasant flavors contained in foods/beverages. One of the most powerful off-flavors is 2,4,6-trichloroanisole (TCA), that is especially known for inducing the cork taint in wines. In the present study, we show with human psychophysical tests that TCA actually reduces flavors of food (banana) and beverage (wine) with very low concentration. In parallel, it was shown that TCA suppressed cyclic nucleotide-gated (CNG) channels potently, when examined on newt olfactory sensory cilia. TCA suppressed CNG channels even with at-to-molar (aM) level. To explain such super-efficiency, the TCA effect showed the time-integration and slow recovery from the current suppression, presumably representing the integration of the substance with the hydrophobic site of the membrane. The findings will be useful for the basic sciences and the industry of foods and beverages. No COI.

3S48F-4

Calcium Homeostasis Modulator (CALHM): A novel ion channel family encoding voltage-gated ATP release ion channels involved in non-synaptic neurotransmission from taste cells

Taruno, Akiyuki[1]; Marunaka, Yoshinori[1];
Foskett, J Kevin[2]([1]*Department of Molecular Cell Physiology, Kyoto Prefectural University of Medicine, Kyoto, Japan,* [2]*Department of Physiology, University of Pennsylvania, Philadelphia, PA, USA*)

It has been a long-standing enigma how taste cells devoid of synapses transmit taste information to the nervous system. Recognition of sweet, bitter and umami tastes requires the non-vesicular release from taste cells of ATP, which has been suggested as a neurotransmitter to activate afferent neural pathways. We recently demonstrated that CALHM1, which has a wide ion-conducting pore (~1.4nm in diameter), was a novel voltage-gated ATP-permeable ion channel and served as a *bona fide* conduit for ATP release from sweet-, umami- and bitter-sensing type II taste cells. *Calhm1* is expressed in taste buds exclusively in type II cells and its product has structural and functional similarities with connexins and pannexins, two families of channel protein candidates for ATP release by type II cells. *Calhm1* knockout in mice leads to loss of perception of sweet, umami and bitter compounds and to impaired gustatory nerve responses to these tastants. These new studies validate the concept of ATP as the primary neurotransmitter from type II cells to gustatory neurons. Furthermore, they identify voltage-gated ATP release through CALHM1 ion channel as an essential molecular mechanism of non-vesicular ATP release in taste buds. We discuss these new findings, as well as unresolved issues in peripheral taste ATP signaling that may involve yet-unknown subunits in the ATP release machinery. No COI.

Symposium 49
Mechanisms on distributive shock and other blood pressure control failure
[Collaboration Symposium with The Japan Shock Society]

(March 18, 9:00–11:00, Room G)

3S49G-1

Central blood pressure regulation in salt-sensitive hypertension

Tandai-Hiruma, Megumi; Kemuriyama, Takehito;
Nishida, Yasuhiro(*The Department of Physiology, National Defense Medical College, Saitama, Japan*)

Blood pressure is the driving force of the circulation and integrates functionally organs throughout the body. Besides the local regulation of peripheral organs, central sympathetic nervous system also involves in long-term control of blood pressure and the pathogenesis of hypertension. In the present study, we will focus on sympathoinhibitory functions of central neuronal nitric oxide synthase (nNOS) neurons in salt-sensitive hypertension. Inhibition of central nNOS by acute intracerebroventricular (i.c.v.) infusion of S-methyl-L-thiocitrulline (SMTC) significantly enhances peripheral sympathetic activity in both Dahl salt-sensitive (DSS) normotensive and hypertensive rats. The rise of activity was more prominent and brain stem nNOS activity is significantly greater in hypertensive DSS rats. Next, Hypertension was normalized by nifedipine in DSS rats on high-salt diet, resulting in restoring the number of nNOS neurons in the brain stem. Furthermore, central nNOS was inhibited for a long-term by chronic i.c.v. infusion of SMTC and the mean arterial pressure was assessed in DSS rats using a radiotelemetry system, resulting in worsen hypertension. Our data suggest that central nNOS neurons may be one of suppressors of the sympathetic system even in normotension, and be enhanced by hypertension rather than high-salt diet in salt-sensitive hypertension to relieve high blood pressure. However, this compensatory response may be insufficient to overcome sympathetic hyperactivity due to increased brain rennin-angiotensin system and abnormalities associated with peripheral organs. No COI.

3S49G-2

Sphingosine-1-phosphate receptor-2 protects against anaphylactic shock through inhibiting eNOS

Okamoto, Yasuo[1]; Cui, Hong[1]; Yoshioka, Kazuaki[1];
Takuwa, Noriko[1,2]; Zhao, Juanjuan[1]; Takuwa, Yoh[1]([1]*Dept. of Physiol., Kanazawa Univ. Sch. Med. Ishikawa, Japan,* [2]*Dept. of Health & Med. Sci., Ishikawa Pref. Nursing Univ. Ishikawa, Japan*)

Sphingosine-1-phosphate (S1P) plays an important role of vascular and immune systems via a family of G protein-coupled receptors ($S1P_1$-$S1P_5$). $S1P_1$ contributes to maintenance of vascular barrier integrity. However, the role of $S1P_2$ in barrier integrity is unknown. In mouse anaphylaxis models of antigen challenge and platelet-activating factor (PAF) injection, $S1P_2$ deletion augmented vascular leak due to barrier disruption, hypotension and lethality. Nitric oxide (NO) is implicated in barrier disruption. In $S1P_2$-null mice, PAF-induced activation of endothelial NO synthase (eNOS) and Akt, an activating kinase of eNOS, in aorta and lung was enhanced compared with wild-type (WT) mice. Consistently, PAF-induced increase in the cyclic GMP level in aorta was enhanced in $S1P_2$-null mice. Either pharmacological eNOS blockade or genetic eNOS deletion protected $S1P_2$-null mice from aggravation of anaphylaxis after antigen challenge and PAF injection. Endothelial cells (EC) isolated from $S1P_2$-null mice (KO-EC) exhibited greater stimulation of Akt and eNOS with enhanced NO production by either S1P or PAF, compared with WT-EC. Moreover, KO-EC showed more severe disassembly of adherens junctions with augmented *S*-nitrosylation of β-catenin by PAF, which was restored by pharmacological eNOS blockade. These results indicate that $S1P_2$ inhibits eNOS stimulation and thereby improves anaphylaxis, suggesting potential usefulness of $S1P_2$ agonists as a novel therapeutic drug for anaphylaxis. No COI.

3S49G-3

The Role of Autophagy in Septic Shock

Watanabe, Eizo[1,2]; Hatano, Masahiko[2]; Takahashi, Waka[1]; Hotchkiss, Richard S[3]; Hirasawa, Hiroyuki[1] (*[1]Department of Emergency and Critical Care Medicine, Graduate School of Medicine, Chiba University, [2]Biomedical Research Center, Chiba University*)

It is not well understood whether the process of autophagy is accelerated or blocked, and whether it is beneficial or harmful to the immune defense mechanism over a time course during septic shock. The aim of our study was to determine both the kinetics and the role of autophagy in septic shock. Electron microscopy (EM) was performed on liver samples obtained from both an observational clinical cohort of severely septic patients and control patients without any incidents of sepsis. EM demonstrated increased autophagic vacuoles in septic patients compared to non-septic patients. Membrane alterations (membrane vacuoles, invagination into adjacent organelles and myelin figure-like changes) occur in a subpopulation of mitochondria in severe sepsis, but other hepatocyte organelles showed no consistent ultrastructural injury. We examined autophagy flux in mice with surgical sepsis (via cecal ligation and puncture (CLP) with C57BL/6N mice and GFP-LC3 transgenic mice), using western blotting, immunofluorescence, and EM. Autophagy is induced in several organs in the early phase of sepsis, and that the entire process of autophagy, from early envelopment of damaged cytosolic elements to fusion of autophagosomes with lysosomes, is activated. Also, inhibition of autophagy process by chloroquine administration after CLP resulted in liver injury and higher mortality, indicating that autophagy may play a protective role in septic animals. The developments of both real time authophagy monitoring method and the specific modulators of authophagy will be critical to the successful introduction of pro-autophagic therapies to septic shock. No COI.

3S49G-4

Interferon 15 reverses age-related T cell inactivation by enhancing interferon-gamma production and STAT 5 phosphorylation in aged mice.

Inoue, Shigeaki[1]; Komori, Yukako[2]; Inokuchi, Sadaki[1]; Hozumi, Katsuto[3]; Sato, Takehito[3] (*[1]Tokai University, School of Medicine, Department of Emergency and Critical Care Medicine, [2] Tokai University, Institute of Innovative Science and Technology*)

Background: Aging is associated with impaired immune responses to pathogens and vaccines. Interleukin 15 (IL-15) is a pluripotent antiapoptotic cytokine that promotes lymphocytes activation and proliferation. We examined whether IL-15 increases T-cell activation by modulating cytokine production and signal transduction of T cells. Method: Splenocytes from young (6 to 8 weeks) and aged (20 to 22 months) C57B6 mice were stimulated overnight using an anti-CD3 antibody with or without recombinant mouse IL-15. We performed flow cytometric analysis for T-cell activation identified by CD25 expression and the intracellular expression of phosphorylated STAT5, which is one of key molecules of IL-15 signal transduction. We also measured interferon-gamma levels in the supernatants. Results: In vitro stimulation of splenocytes showed that compared to young mice, aged mice showed impaired T-cell activation (68% reduction in both CD4+ and CD8+ T cells, $p<0.01$). IL-15 reversed this impaired activation with increasing STAT5 phosphorylation in CD4+ and CD8+T cells from aged mice. IL-15 reversed this impaired CD8+T cells activation in aged mice and increased interferon-gamma levels dose dependently. Conclusion: IL-15 reverses age-related T cell inactivation by enhancing interferon-gamma production and STAT 5 phosphorylation in aged mice. No COI.

3S49G-5

Neutrophil Extracellular Traps under critical condition

Hamaguchi, Shigeto[1]; Hirose, Tomoya[2]; Masafumi, Seki[1]; Naoya, Matsumoto[2]; Yukihiro, Akeda[3]; Norihisa, Yamamoto[1]; Osamu, Tasaki[4]; Takeshi, Shimazu[2]; Kazunori, Tomono[1] (*[1]Division of Infection Control and Prevention, Osaka University Graduate School of Medicine, Osaka, Japan, [2]Department of Traumatology and Acute Critical Medicine, Osaka University Graduate School of Medicine, Osaka, Japan*)

Neutrophil extracellular traps (NETs) are structures composed of DNA and granular proteins. NETs are thought to be a part of innate immunity and it rapidly traps and kills pathogen. Although the formation of NETs has been observed during infection, their role in vivo is still unclear. In our previous studies, we evaluated NETs in the blood of patients with systemic inflammatory response syndrome (SIRS) and various clinical conditions by immunohistochemical staining of blood smears collected from patients in the intensive care unit. We also reported the dynamic alteration of the expression of NETs in sputum collected from patients with acute respiratory infection.NETs are thought to reflect some part of disease progression under critical conditions. Quantification of NETs might have a potential as a novel inflammatory biomarker. We will discuss about NETs function under various disease conditions including literature review. No COI.

Symposium 50
Beyond the front line of neural optical imaging

(March 18, 10:05–12:05, Room H1)

3S50H1-1

Voltage-sensitive dye imaging of neural activities in the embryonic central nervous system

Sato, Katsushige[1]; Sato, Yoko[2]([1]*Dept Hlth & Nutr Sci, Fac Human Hlth, Komazawa Women's Univ, Tokyo, Japan,* [2]*Dept Hlth & Nutr, Coll Human Enviro Studies, Kanto-Gakuin Univ, Yokohama, Japan*)

Investigating the developmental organization of the embryonic nervous system has been one of the major challenges in neuroscience. Despite their significance, functional studies of the vertebrate embryonic CNS have been hampered, since conventional electrophysiological means have some technical limitations. First, early embryonic neurons are small and fragile, and the application of microelectrodes is often difficult. Second, the simultaneous recording of electrical activity from multiple sites is limited, and as a consequence, spatio-temporal patterns of neural network responses cannot be assessed. The advent of optical techniques using voltage-sensitive dyes has enabled the non-invasive monitoring of electrical activity in living cells and also facilitated the simultaneous recording of neural responses from multiple regions. Using optical recording techniques, it is now possible to follow the functional organization of the embryonic nervous system and to image the spatio-temporal dynamics of the neural network's formation. Here, we present recent progress in optical studies on the embryonic nervous system with special emphasis on the spontaneous depolarization wave, which demonstrates the utility of fast voltage-sensitive dye imaging as a powerful tool for elucidating the functional organization of the embryonic CNS. No COI.

3S50H1-2

Functional organization of the auditory cortex revealed by application of in vivo voltage-sensitive dye imaging

Song, Wen-Jie(*Dept of Sensory and Cognitive Physiology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan*)

Optical imaging using voltage-sensitive fluorescent dyes can reveal cortical activity in an area of tens of square millimeters, with a spatial resolution in the tens of micrometers and a time resolution less than one millisecond. In this talk, I will present two examples showing how the high spatial and temporal resolution of this technique can be exploited to advance our understanding of the auditory cortex and the auditory thalamus. One example shows the power of the imaging technique in revealing a fundamental rule governing spatial representation of sound frequency in the primary auditory cortex. The other will show how the technique can be used for determining cortical positions with high precision, for the purpose of guiding multiple tracer injections. Retrograde labeling of cells in the medial geniculate body revealed new principles of the auditory thalamocortical organization. No COI.

3S50H1-3

Surround modulation in cortical orientation map revealed by optical imaging based on intrinsic signals

Wang, Gang; Okamura, Jun-ya(*Dept. of Information Science and Biomedical Engineering, Kagoshima University*)

We applied optical imaging based on intrinsic signals to investigate the modulation of the surround stimulus presence and its dependence on the receptive field eccentricity in cat visual cortex. Presentation of center?surround stimuli at the center of gaze resulted in no clear surround modulation, no significant modulation on the orientation map was observed in the response magnitude and spatial pattern of response. However, at 10 deg eccentricity in more peripheral parts of the visual field, significant modulation was observed in its corresponding cortical area when the center-surround stimuli were presented. Modulation was observed both in the response magnitude and in the spatial pattern of the response. Surround orientation perpendicular to the orientation of a center patch grating showed the largest modulation in the response magnitude. The modulation became weaker as the orientation difference between the center and surround gratings became smaller. These results demonstrate the difference in information processing between the cortical area corresponding to the center of gaze and that corresponding to the peripheral part of visual field, and suggest a relative specialization for visual information processing in peripheral representations of cortical areas. No COI.

3S50H1-4

Higher visual functios revealed by flavoprotein fluorescence imaging in mice

Shibuki, Katsuei(*Department of Neurophysiology, Brain Research Institute, Niigata University, Niigata, Japan*)

Neural activities, coupled with energy metabolism, can be imaged as activity-dependent changes in endogenous green fluorescence derived from mitochondrial flavoproteins. This method is especially useful for visualizing higher cortical functions in the intact mouse brain through the transparent skull. Mice navigate nearby space using their vision and whiskers. Therefore, young mice must learn to integrate these heterogeneous inputs in perceptual space. We found that cortical responses were depressed in the primary visual cortex of young mice after wearing a monocular prism goggle. This depression was uniformly observed in the primary visual cortex, and was eliminated by whisker trimming or lesions in the posterior parietal cortex. However, ocular dominance plasticity was not affected by these manipulations. Compensatory visual map shifts of responses elicited via the eye that had worn the prism were also observed. As a result, cortical responses elicited via each eye were clearly separated when a visual stimulus was placed in front of mice, and ocular-dominance column-like structures were formed. Comparison of response areas before and after prism wearing indicated that the map shifts were produced by depression with spatial eccentricity. The cortical depression and the resulting visual map shifts based on whisker-guided cues may serve as a simple model to investigate cellular and molecular mechanisms underlying higher visual functions in the mammalian brain, and transcranial flavoprotein fluorescence imaging is a useful tool for investigating higher visual functions in mice. No COI.

Symposium 51
Morphological analysis of 3D reconstructed image using serial block-face electron microscope

(March 18, 9:00–11:00, Room J)

3S51J-1
Serial neuronal EM images using TEM, FIB/SEM or DiK-SEM

Kubota, Yoshiyuki(*Div. Cerebral Circuitry, NIPS, Okazaki, Japan*)

The serial electron microscopic (EM) neuronal images capturing methods using conventional TEM, Focus Ion Beam (FIB)/SEM, or diamond knife cutting type (DiK)-SEM are introduced. We capture images from serial ultrathin sections using conventional TEM and reconstruct neuronal profiles, such as dendrites, spines and soma with 3D reconstruction software. It is a time consuming labor work, and can be done only by skilled worker. It was desired for long years to develop EM system to capture the serial EM images automatically. Recently the new EM systems are available: FIB/SEM or DiK-SEM. Both EMs are based on SEM that captures images on block surface. FIB/SEM uses very fine focus ion beam to mill the block surface as thin as 2 nm in z-step. DiK-SEM uses diamond knife on ultra microtome in SEM chamber to cut the entire block surface serialy. The cutting section thickness is usually 40 - 50 nm. The advantages of the DiK-SEM method are to obtain larger size image and faster imaging time than the FIB/SEM. The max image size of DiK-SEM is 100 μm × 100 μm, and that of FIB/SEM is 20 μm × 20 μm. The DiK-SEM can obtain 120 serial images/hour, and FIB/SEM 15 -20 serial images/hour. The DiK-SEM requires highly metal stained tissue, on the other hand, the conventional stained tissue for TEM can be used for FIB/SEM, because of different SEM detectors, EsB or BSE. The different nature of the blocks makes the image quality different. Image of the DiK-SEM is with higher contrast due to lack of mid-tone electron density than that of FIB/SEM. As introduced above, the each methods has superiority and inferiority. Ideally we use them depending on the research project purpose. No COI.

3S51J-2
3D entire membrane organization of the mitochondria revealed by high resolution FIB/SEM tomography method

Ohta, Keisuke; Nakamura, Keiichiro(*Department Anatomy, Kurume University school of Medicine*)

Chemically fixed rat hepatocyte was examined using high-resolution focused ion beam/scanning electron microscope (FIB/SEM) tomography that revealed the entire membrane organization of mitochondria including its cristae structure. Understanding of cristae organization in the mitochondria was a difficult subject for even in the serial sections TEM (ssTEM) method because the distance between cristae (30nm) is usually smaller than the thickness of ultrathin section. Electron tomography method has a high enough spatial resolution to observe each cristae, but the observation area is too small to observe the entire mitochondria. The FIB/SEM tomography we used is a recent advanced powerful 3D reconstruction method that has smaller spatial resolution than the ssTEM, and is able to observe larger volume than the electron tomography method. In this method, 3D volume is reconstructed from serial images obtained by fully automated cycles of surface milling of specimen by gallium ion-beam and imaging by SEM from the newly created flat surface of the specimen within the FIB/SEM machinery. This cycle was repeated 650 times at a10nm milling pitch. The final reconstruction (216μm^3) contained dozens of mitochondria and had high enough resolution to analyze each crista in a mitochondrion. This reconstruction shows that the inner membrane of mitochondria invaginated to form cristae through narrow bridges and tubular openings. The tubular opening then extends to sheet-like cristae. Most of the cristae have multiple tubular junctions to the inner membrane throughout the whole of the mitochondrion. No COI.

3S51J-3
SBF-SEM for ultrastructural analyses of physiology and pathology of the nervous system

Ohno, Nobuhiko[1,2]; Lu, Haiyan[3]; Ransohoff, Richard M[3]; Trapp, Bruce D[3]([1]*Department of Anatomy and Molecular Histology, University of Yamanashi, Chuo, Japan,* [2]*National Institute for Physiological Sciences, Okazaki, Japan,* [3]*Department of Neurosciences, Cleveland Clinic, Cleveland, USA*)

Serial Block Face-Scanning Electron Microscopy (SBF-SEM) facilitates relatively rapid acquisition of serial electron microscopic images from areas as large as tens to hundreds micrometer square. This acquisition is achieved by repeated milling of tissue blocks by built-in ultramicrotome and subsequent observation of the block surface with SEM. This approach makes precise 3-dimentional ultrastructural analyses of cells and organelles easier and faster compared with conventional observation of serial ultrathin sections with transmission electron microscopy. Such ultrastructural analyses have provided evidences that mitochondrial behavior in myelinated axons was modulated by axonal electrical activity and Ca^{2+} to support the energy demand of saltatory nerve conduction. Furthermore, SBF-SEM was combined with pre-embedding immunohistochemistry to examine morphology and functions of monocyte-derived and microglia-derived macrophages in inflammatory demyelinating lesions of spinal cord. The results suggested that the two populations of macrophages coexist in the lesions but have distinct nuclear morphology, and monocyte-derived macrophages play pivotal roles in demyelination at disease onset. By introducing these applications, this presentation will discuss the characteristics of the 3-dimentional ultrastructural analyses using SBF-SEM. No COI.

3S51J-4

Correlative three-dimensional reconstruction for confocal laser-scanning microcopy and FIB/SEM in the Double immunohistochemical staining

Sonomura, Takahiro[1]; Furuta, Takahiro[2]; Nakatani, Ikuko[3]; Yamamoto, Yo[3]; Honma, Satoru[1]; Kaneko, Takeshi[2]([1]*Anatomy II, Kanazawa Medical University, Uchinada, Japan,* [2]*Dep. of Morph. Brain Sci.,Grad. Sch. of Med., Kyoto Univ., Kyoto, Japan,* [3]*Hitachi High-Tech Science Corporation, Chiba, Japan*)

Serial block-face scanning electron microscopy (SFB-SEM) has made easy to acquire image stacks of the transmission electron microscopy (TEM) in the mesoscale, which is taken with the confocal laser-scanning microcopy (CF-LSM). We tried immunocytochemistry for FIB-SEM and correlated this immunoreactivity with that in CF-LSM. Dendrites of neurons in the rat neostriatum were visualized using a recombinant viral vector. Moreover, the thalamostriatal afferent terminals were immunolabeled with Cy5 fluorescence for vesicular glutamate transporter 2 (VGluT2). After detection of the sites of terminals apposed to the dendrites by using CF-LSM, GFP and VGluT2 immunoreactivities were further developed for EM by using immunogold/silver enhancement and immunoperoxidase/diaminobenzidine (DAB) methods, respectively. We showed that conventional immuno-cytochemical staining for TEM was applicable to FIB-SEM. Furthermore, several synaptic contacts, which were thought to exist on the basis of CF-LSM findings, were confirmed with FIB-SEM, revealing the usefulness of the combined method of CF-LSM and FIB-SEM. COI properly declared.

Symposium 52
Essential Brain and Physiological Function
[FAOPS Joint Symposium]

(March18, 10:15–12:05, Room K)

3S51J-5

Analysis of Neural Circuit using Automated Tape Ultra Microtome;ATUM

Iwasaki, Hirohide; Okabe, Shigeo(*Department of Cellular Neurobiology, Graduate School of Medicine, The University of Tokyo*)

The brain consists of a huge number of neurons and glial cells, and neurons connect each other via synapses to generate neuronal circuits. In order to understand how the brain works at the circuit level, it is important to figure out the complete connections of neurons in neuronal circuits. However, there are some difficulties to draw the complete neuronal map and one of the major problems is that synapses are so tiny that it is essential to use electron microscopy to observe. For the three-dimensional reconstruction of neuronal circuits at the synapse level, it is necessary to collect continuous series of ultrathin sections successfully. ATUM (Automated Tape Ultra Microtome) is the equipment attached to the ulramicrotome to collect ultrathin sections on the plastic tape. By comparing to other equipments for the similar purpose, such as FIB-SEM or SBF-SEM, one of the advantages to use ATUM is that sections remain on the tape after observation and we can observe the same sections for many times by using several methods. In this symposium, we will introduce the ATUM and will show some data from sections collected by ATUM by using both electron microscopy and light microscopy. COI properly declared.

3S52K-1

Sympathetic-respiratory coupling and the development of hypertension

Allen, Andrew M; Menuet, Clement(*Department of Physiology, University of Melbourne, Parkville, Australia*)

That breathing patterns regulate cardiovascular function has been understood for a very long time and this knowledge forms a basis for many meditative practices. Several years ago we demonstrated that an altered interaction between the central respiratory generator and circuits generating sympathetic vasomotor activity to the cardiovascular system occurs from very early postnatal periods in the Spontaneously Hypertensive (SH) rat ? many weeks before the development of altered blood pressure. This increased respiratory-sympathetic coupling was responsible for the elevated sympathetic activity and Traube-Herring waves observed in the SH rat. Using a variety of lentiviral transduction approaches to enable cell-specific activation or inhibition of neurons with particular chemical phenotypes in adults, we have shown that this altered respiratory-sympathetic coupling in the SH rat involves the catecholaminergic C1 neurons of the rostral ventrolateral medulla. Activation of these cells enhances sympathetic-respiratory coupling, whilst inhibition selectively reduces the inspiratory-related peak of sympathetic activity. We propose that these data shed light on a mechanism by which altered breathing patterns affect cardiovascular function and suggest a potential approach for ameliorating the elevated sympathetic nerve activity that occurs in some pre-hypertensive humans. No COI.

3S52K-2

Odor-induced analgesia in mouse

Kashiwadani, Hideki[1]; Tashiro, Shogo[1,2]; Kanmura, Yuichi[2]; Kuwaki, Tomoyuki[1]([1]*Department of Physiology, Graduate School of Medical and Dental Sciences, Kagoshima University,* [2]*Department of Anesthesiology, Graduate School of Medical and Dental Sciences, Kagoshima University*)

Pain is essential for animals including human to detect the actual or potential tissue damages. However the excessive pain often induces the immobilization to animals and sometimes prevents the appropriate behaviors. Therefore the gain control of the pain is one of the essential functions in our brain. For the gain control, animals have developed the intrinsic neuronal circuits for analgesia. In folk remedy, odorous compounds were often prescribed to drive the intrinsic analgesic circuits. However, it has not yet known whether "the odor", or the sense of smell, could really induce the analgesia.To address the question, we performed the hot plate test and the formalin test under odor exposure. Among several odor molecules examined, the exposure of an odor molecule ("Odorant X") showed significant analgesic effects in wild type mice. The analgesic effects were not observed in olfactory-deprived mice, indicating that the olfactory input evoked by the odorant X induced the analgesia. Furthermore, the odor-induced analgesia was disappeared in mice lacking orexinergic neurons in hypothalamus, suggesting that the intrinsic analgesic circuits including hypothalamic orexinergic neurons mediate the odor-induced analgesia. In conclusion, we found the odor-induced analgesia mediated by hypothalamic orexinergic neurons in mouse. Our findings suggest that odor input could modify the pain processing by driving the intrinsic analgesic circuits including hypothalamic orexinergic neurons. No COI.

3S52K-3

Hypothalamic orexin-synthesizing neurons have a physiological role in emotional hyperthermia

Mohammed, Mazher[1]; Ootsuka, Youichirou[1]; Yanagisawa, Masashi[2]; Blessing, William W[1]([1]*Centre for Neuroscience, School of Medicine, Flinders University, Adelaide SA, Australia,* [2]*Department of Molecular Genetics and HHMI, University of Texas Southwestern Medical Center, Dallas, USA.*)

When animals encounter salient, potentially threatening environments, body temperature increases and thermoregulatory cutaneous blood flow decreases, a response that can be described as emotional hyperthermia. The hypothalamic orexin-synthesizing neurons influence a number of physiological and behavioral processes, including body temperature and brown adipose tissue (BAT) thermogenesis [1,2]. Restraint-induced hyperthermia is reduced in orexin-deficient mice [3]. We hypothesized that orexin-synthesizing neurons contribute to emotional hyperthermia by induction of BAT thermogenesis and cutaneous vasoconstriction. We used the orexin-ataxin3 transgenic rats in which orexin neurons are postnatally destroyed. A caged intruder rat was introduced into the home cage of a resident male Sprague-Dawley rat. Intruder-evoked increases in BAT thermogenesis and the associated tail artery vasoconstriction were reduced in transgenic rats. These results add to the growing body of evidence that orexin neurons participate in the coordination of the animal's response to environmental threats. [1]Inutsuka et al., 2013; [2]Tupone et al., J Neurosci 2011; [3] Zhang et al., J Physiol 2010. No COI.

3S52K-4

Preoptic-raphé connections for thermoregulatory and febrile cutaneous vasoconstriction

Tanaka, Mutsumi[1,2]; McKinley, Michael J.[1]; McAllen, Robin M.[1]([1]*Florey Institute of Neuroscience and Mental Health, University of Melbourne, Australia,* [2]*Japan Automobile Research Institute*)

Body temperature is maintained by the balance between heat production and heat dissipation. In rats, the tail circulation is a major organ of heat dissipation, and is controlled by sympathetic vasoconstrictor nerves that are activated during cold exposure and experimental fever induced by prostaglandin E2 (PGE2). The preoptic area is the key structure for body temperature regulation and the febrile action of PGE2. It has been considered that under warm conditions preoptic neurons directly inhibit tail sympathetic premotor neurons located in the medullary raph? [1], and that cold signals and PGE2 inhibit those preoptic neurons, causing tail vasoconstriction by disinhibition [1].
This inhibitory restraint originates from two distinct preoptic regions; a rostromedial region (RMPO) surrounding the organum vasculosum of the lamina terminalis and the median preoptic nucleus, and a second region centred ~1mm caudolaterally (CLPO) [2]. We recently showed that, in parallel with the inhibitory pathway, an excitatory pathway from the RMPO to the medullary raphé mediates tail vasoconstrictor responses to cold skin [3] and to experimental fever [4]. Our findings suggest that both inhibitory and excitatory preoptic-raph? descending drives regulate tail vasoconstriction in concert. No COI.

Symposium 53
Mind-body interaction: How does brain know visceral condition?

(March 18, 14:00–16:00, Room B)

3S53B-1

A cerebral cortical region that responds to amino acid injection into the hepatic portal vein

Sekiguchi, Masayuki[1]; Koppensteiner, Peter[1]; Odagiri, Saori[1]; Hatanaka, Yusuke[1]; Yamada, Daisuke[1]; Yamada, Tetsuya[2]; Katagiri, Higeki[2]; Wada, Keiji[1]([1]*Department of Degenerative Neurological Diseases, National Institute of Neuroscience, National Center of Neurology and Psychiatry, Tokyo, Japan,* [2]*Department of Diabetes and Metabolism, Tohoku University Hospital, Sendai, Japan*)

Information about visceral activities (gastrointestinal, respiratory, and cardiovascular) is localized in the insular cortex via vagus nerve afferents. However, it is unknown whether there is a cortical region that responds to the hepatic branch of the vagus nerve. This branch innervates the hepatic portal vein, bile duct, etc. The spontaneous activity of this branch is changed by injection of amino acids into the portal vein. In the present study, activity mapping was carried out in arc (activity-regulated cytoskeleton -associated protein) -Venus mice. Arc is an immediate-early gene and expressed in response to potent neuronal activity. In arc-Venus mice, the fluorescent protein Venus is expressed mainly in cortical regions under the control of the arc promoter. Portal-vein injection of amino acids known to enhance vagus activity increased the number of Venus-positive cells specifically in the secondary somatosensory cortex (S2) (saline-injection as control). Correspondingly, injection of amino acids that reduce vagus activity led to a decrease of Venus-positive cells in S2. Vagotomy abolished the action of amino acids upon Venus expression in S2. These results suggest that S2 includes neurons that potently respond to the injection of amino acids into the portal vein via the vagus nerve. No COI.

3S53B-2

Inter-organ neural network mediate body weight regulation

Yamada, Tetsuya; Tsukita, Sohei; Katagiri, Hideki(*Division of Metabolism and Diabetes, Tohoku University Graduate School of Medicine, Sendai, Japan*)

Despite remarkable advancements in obesity research over the past decade, the mechanisms underlying obesity are still not fully understood. In recent years, it has been revealed that numerous metabolic interactions between organs, which are organized by the brain, function as a negative-feedback mechanism and are involved in maintaining body weight homeostasis against excess energy intake. On the other hand, we recently discovered a new inter-organ neural network, from the liver, possibly representing a positive-feedback mechanism. Under conditions of excessive energy intake, changes in glucose metabolism occur in the liver with increased expression of hepatic glucokinase and the induction of neuronal signal transmission via the afferent vagus nerve. These signals, received by the medulla, result in the inactivation of sympathetic innervation of brown adipose tissue (BAT). This leads to suppression of thermogenesis in BAT and thereby favors obesity development. Furthermore, the efficacy of the liver-to-BAT interaction differs among mouse strains and these differences may contribute to determining the obesity predispositions of various strains. In conclusion, this novel inter-organ neuronal relay system functions to suppress energy expenditure when energy intake is increased. During periods when sufficient food was not always available, this system worked in favor of survival. However, in the current age of plenty, it is assumed to work as a mechanism flipping a metabolic switch toward obesity. No COI.

3S53B-3

Clinical neuromodulation by vagal stimulation: vagus nerve stimulation for epilepsy

Kawai, Kensuke[1]; Kawai, Kensuke[1]; Takahashi, Hirokazu[2]; Usami, Kenichi[3]([1]*Department of Neurosurgery, NTT Medical Center Tokyo, Tokyo, Japan,* [2]*Research Center for Advanced Science and Technology, The University of Tokyo, Tokyo, Japan,* [3]*Department of Neurosurgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan*)

Vagus nerve stimulation (VNS) is an established treatment of epilepsy and depression. It chronically stimulates the left cervical vagus nerve using an implanted device, and decreases frequency and severity of epileptic seizure. We introduce clinically utilized VNS for epilepsy and present our data on the mechanisms of action with review of literature. Clinical VNS in Japan was approved and reimbursed in 2010. 385 patients were enrolled for 3 years in the Japanese postmarketing survey. Preliminary data demonstrated the efficacy and safety similar to the previous reports from the United States. Patient rates with a seizure reduction greater than 50% after 3, 6 and 12 months were 37, 47 and 60%, respectively. Our electrophysiological study demonstrated that the upward neural transmission of the vagus nerve actually occurs in human clinical VNS. Our experimental study demonstrated that VNS modulates the cortices in the way that it increases the phase locking in a nonepileptic state but decreases it in an epileptic state, thus exerting the homeostatic influence on the brain. Although biological effect and mechanisms of clinical action of VNS are still largely unknown, accumulating evidence suggests that the upward neural signals modulate the broad areas of cerebral cortex via various pathways including noradrenergic, serotonergic and cholinergic systems. No COI.

3S53B-4

Energetics of the synaptic transmission in the nucleus of the solitary tract

Nagase, Masashi; Watabe, Ayako M; Kato, Fusao(*Dept. Neurosci., Jikei Univ. Sch. Med., Tokyo, Japan*)

The synaptic transmission between the primary afferents and the second-order neurons in the nucleus of the solitary tract (NTS) is the first site in the brain where the CNS obtains the direct information regarding the on-going state of the internal environment. Such information, especially that on the energetic and metabolic state of the body, is crucial not only for maintaining internal organ functions but also for assuring energy supply to the brain. It is therefore imperative that the integrity of this NTS synapse should be robustly maintained even in the situations with reduced energy supply, such as hypoxia and hypoglycemia. As lactate supplied by astrocytes, which are rich in glycogen unlike neurons, can support the high requirement for ATP of the neurons, we examined the role of astrocyte-to-neuron lactate transport in the NTS synaptic transmission. We found that 1) inhibition of monocarboxylate transporters (MCTs), key molecules in astrocyte-to-neuron lactate transport, inhibited excitatory synaptic transmission, 2) this effect of MCT inhibition was more potent at reduced intracellular ATP, which was counteracted by intracellular addition of lactate and 3) extracellular addition of lactate rescued aglycemia-induced suppression of synaptic transmission in a MCT-dependent manner. Interestingly, MCT did not affect basal membrane potential in NTS neurons, unlike in the cerebellum. We conclude that the integrity of the NTS synapse is maintained largely by lactate transfer from astrocytes, thus allowing robust transfer of the visceral information to the brain. No COI.

Symposium 54
Involvement of TRP channels in respiratory regulation

(March 18, 14:00–16:00, Room C)

3S54C-1

Transient receptor potential channels and synaptic activities

Kumamoto, Eiichi; Fujita, Tsugumi; Jiang, Chang-Yu; Xu, Zhi-Hao; Ohtsubo, Sena; Matsushita, Akitomo(*Dept. Physiol., Saga Med. Sch., Saga, Japan*)

Transient receptor potential (TRP) channels located in the peripheral terminals of primary-afferent neurons receive various chemical and thermal stimuli given to the periphery; the information is transmitted to the CNS as an action potential. TRPs are also expressed in the central terminals of the neurons and involved in modulating the transmission to the CNS. Many of the properties of the TRPs have been investigated in the cell body of the primary-afferent neuron while the central TRPs have not yet been examined thoroughly. We examined the actions of various TRP agonists on synaptic transmission in lamina II neurons of adult rat spinal cord slices by using the blind whole-cell patch-clamp technique. The agonists increased the frequency of spontaneous EPSC with a minimal increase in the amplitude in a manner resistant to a Na^+-channel blocker tetrodotoxin; this was accompanied by an inward or outward current at -70 mV. The facilitatory actions were inhibited by capsazepine or HC-030031. Primary-afferent evoked EPSCs were inhibited in peak amplitude by TRP agonists. On the other hand, spontaneous inhibitory transmissions were potentiated by some TRPA1 but not TRPV1 agonists in a manner sensitive to tetrodotoxin. These results indicate that TRP agonists activate TRPV1 or TRPA1 to facilitate the spontaneous release of L-glutamate onto lamina II neurons. These activations were different in pharmacology from those in the cell body of primary-afferent neuron. Central TRPs were suggested to be different in property from peripheral ones. No COI.

3S54C-2

TRPA1 contributes to arousal and respiratory activation to mild hypoxia

Kuwaki, Tomoyuki(*Department of Physiology, Kagoshima University Graduate School of Medical and Dental Sciences*)

TRPA1 channel, a member of the transient receptor potential super family, is expressed in a subset of sensory neurons in the trigeminal, nodose, and dorsal root ganglia. At the vagal afferent nerve terminals in the lower airway, TRPA1 detects oxygen concentration of the inspired gas and triggers respiratory acceleration or slowing depending on hypoxia or hyperoxia. We hypothesized that TRPA1 would also contribute to arousal from sleep and respiratory activation, as a defense mechanism to hypoxia. To test our hypothesis, we measured arousal time and respiratory volume and frequency in TRPA1 knock out mice (TRPA1-KO) and wild-type mice (WT) with indwelling EEG and EMG electrodes. During natural sleeping, the continuous flushing air into the body plethymographic chamber was switched to a hypoxic gas mixture. Oxygen concentration in the chamber was gradually declined to 10% by ~40s and kept for another 2min at 10%. The time to arousal from natural sleep in response to hypoxia in TRPA1-KO was extremely longer than that in WT mice. Notably, WT mice awoke before oxygen concentration reached 10% whereas TRPA1-KO did not. The increase of respiratory minute ventilation, just after arousal from sleep, was attenuated in TRPA1-KO than in WT mice. We conclude that TRPA1 is important for arousal and respiratory activation in response to mild hypoxia. TRPA1, probably located in the nasal cavity, seems to be a front line sensor for a subtle change in the inspired gas oxygen. No COI.

3S54C-3

Putative mechanisms underlying pH sensitivity of the brainstem astrocytes

Marina, Nephtali(*Neuroscience, Physiology & Pharmacology, University College London, London, United Kingdom*)

Ion channels of the transient receptor potential (TRP) family are key chemosensory ion channels in the respiratory system. However, the role of TRP channels in the control of respiratory chemosensitivity has never been described. Astrocytes residing in the ventral aspect of the medulla oblongata respond to acidification with elevations in intracellular Ca2+ and ATP release which propagates Ca2+ excitation among neighbouring astrocytes and activates neurones of the ventral respiratory column triggering adaptive increases in breathing. Here, we performed a pharmacological survey aiming to determine the mechanisms underlying pH sensitivity of astrocytes which reside near the ventral surface of the medulla oblongata (VMS). Acidification-evoked [Ca2+]i responses in VMS astrocytes were unaffected by blockade of TRPA1 or TRPV channels (HC030031, AMG9810, ruthenium red). However, significant reductions in [Ca2+]i responses triggered by a decrease in extracellular pH from 7.4 to 7.2 were observed after application of Na+/Ca2+-exchange inhibitor SN-6, Na+/HCO3- cotransport inhibitor S0859 and anion exchange inhibitor DIDS. Similarly, hypercapnic acidosis-evoked [Ca2+]i responses in VMS astrocytes were reduced in the presence of either S0859 or DIDS. Application of S0859 to the VMS in anaesthetized, vagotomized and mechanically ventilated rats, reduced respiratory response triggered by systemic hypercapnia (10% inspired CO2) by ~50%. No COI.

3S54C-4

Role of Transient Receptor Potential (TRP) channels in respiratory motor pattern generation

Koizumi, Hidehiko(*NINDS, National Institutes of Health (NIH), Bethesda, USA*)

The pre-Botzinger complex (pBC) is a key brainstem microcircuit that generates and transmits rhythmic respiratory activity. TRPM channels have been proposed to contribute to respiratory rhythm generation in pBC network by mediating calcium-activated non-selective cationic currents (ICAN). TRPC channels have also been suggested to be functionally important. However, TRP channel-based mechanisms, plausible alternative to persistent sodium channel-based mechanisms, are not completely understood, and there is continuing controversy about the roles of TRP channels in respiratory rhythm/pattern generation. We first examined expression of TRPM/C channels in pBC inspiratory neurons identified in rhythmically active in vitro brainstem slice preparations by combining patch-clamp recording and single-cell multiplex RT-PCR. We detected TRPM4 and TRPC3 mRNA expression primarily in excitatory neurons. Only a small percentage of inhibitory neurons expressed TRPC3 mRNA. To further define the contributions of TRPM/C channels to the functional behavior of pBC network, we tested perturbations of the frequency and amplitude of hypoglossal inspiratory activity following bath application of pharmacological blockers of TRPM4, TRPC3 and ICAN in brainstem slice preparations. In all cases we found significant reductions in the amplitude of inspiratory activity without any significant perturbations of the frequency. These results suggest that while TRPM4/TRPC3/ICAN contributes to the formation of inspiratory drive, this function is independent of rhythm generation mechanisms at least in vitro. No COI.

Symposium 55
What lies between physiology and pathophysiology
[Collaboration Symposium with Japanese Society of Pathophysiology]

(March 18, 14:00–16:00, Room D1)

3S54C-5

Neuronal mechanisms of effects of TRP channel related substances on respiratory center neurons

Onimaru, Hiroshi(*Department of Physiology, Showa University School of Medicine, Tokyo, Japan*)

It is not well understood whether chemicals that are known as transient receptor potential (TRP) channel agonists or antagonists exert any effects on the medullary respiratory center. Recently, we have examined effects of transient receptor potential (TRP) channel-related substances on respiratory rhythm generation in the brainstem-spinal cord preparation from newborn rats. These substances are divided into roughly two groups that induced excitatory effects (e.g. TRPV1 agonist, capsaicin; TRPA1 agonist, cinnamaldehyde) or inhibitory effects (TRPM8 agonist, menthol; TRPV3? agonist, carvacrol and eugenol). One of characteristics of capsaicin effects was an induction of strong desensitization that might be produced by extracellular calcium-independent mechanisms. We presumed that capsaicin might induce the release of intracellular stored Ca2+ from the endoplasmic reticulum. Cinnamaldehyde induced long-lasting facilitation of respiratory rhythm for more than 2 hrs after washed out. In contrast, carvacrol and eugenol induced inhibition of respiratory rhythm followed by pronounced shortening of pre-inspiratory and inspiratory burst duration, suggesting that eugenol or carvacrol inhibited cellular (and/or network) mechanisms that are essential for maintenance of burst duration of respiratory neurons. Effects of these compounds may illuminate new aspects of cellular mechanisms of respiratory rhythm generation. In this presentation, I will show recent results regarding effects of these TRP channel-related substances and discuss the cellular mechanisms. No COI.

3S55D1-1

Prostaglandin E2 Receptor EP4 Signaling in smooth muscle cells regulates arterial elasticity

Ichikawa, Yasuhiro; Yokoyama, Utako; Ishikawa, Yoshihiro
(*Cardiovascular Research Institute, Yokohama City University, Yokohama, Japan*)

Chronic inflammation is known to contribute advancing aortic aneurysm (AA) in which arterial elasticity was impaired. In addition to the role of inflammatory cells, using human tissues, we have demonstrated that the PGE2 receptor EP4 may be involved in chronic inflammation in AA. Therefore, we further examined whether PGE2-EP4 signaling in SMCs decreases elasticity in the aorta. We generated mice with vascular smooth muscle-specific overexpression of human EP4 (hEP4) using the Cre-loxP system (EP4TG). hEP4 mRNA was over-expressed in the aorta of EP4TG (n=8, respectively). Vasodilative effect of EP4 agonist (10 μM) was greater in the aorta of EP4TG than non-transgenic mice (non-TG) by wire myograph (25.0±2.9 vs. 9.6±2.0% of phenylephrine-induced contraction, $p<0.01$, n=4–6). Pressure-induced dilation (20 to 220mmHg) was decreased in EP4TG than in non-TG (0.84±0.04-fold, n=5–6, $p<0.05$), although under basal condition, aortic diameter and elasticity were not different between EP4TG and non-TG. Furthermore, after angiotensin II (ATII) infusion (3.0μg/kg/min for 28 days), the aortae of EP4TG were deformed and enlarged to a greater degree than that of non-TG (1.1±0.02-fold, n=5-6, $p<0.05$). MMP-2 activity of EP4TG is higher than that of non-TG by gelatin zymography (1.7±0.07-fold, n=5, $p<0.05$) under basal conditions. After ATII treatment, enhancement of MMP-9 activation was greater in EP4TG than in non-TG (1.6±0.19-fold, n=5, $p<0.05$). These data suggest that EP4 signaling in SMCs decreases aortic elasticity by MMP activation. No COI.

3S55D1-2

Regulation and roles of TRPM7 kinase activity in pathophysiology

Matsushita, Masayuki(*Department of Molecular and Cellular Physiology, University of the Ryukyus, Okinawa, Japan*)

The mammalian transient receptor potential (TRP) superfamily of cation channels is divided into six subfamilies based on sequence homology TRPC, TRPV, TRPM, TRPA, TRPP and TRPML. These ion channels can be activated by a diverse range of chemical and physical stimuli. Physical stimuli include temperature, membrane potential changes and osmotic stress, and some of the more well known chemical stimuli include capsaicin (TRPV1), menthol (TRPM8) and acrolein (TRPA1). Among TRP families, TRPM7 is unique molecule composed of the domain with the ion channel structure and the kinase activity. As for the role of the kinase activity, it is still unclear though the intensive work has been performed. To clarify the physiology function of TRPM7 and the role of the kinase activity, we made TRPM7 genetically modified mouse by one amino acid substitution in ATP binding site in kinase domain. This mutant mouse grew normally, while kinase domain deletion mouse was embryonic lethal. Interestingly, TRPM7 kinase dead mutant mouse show metabolic abnormality. The novel metabolic role of TRPM7 kinase activity will be useful for establishment of physiological role of TRPM7 channel. No COI.

Symposium 56
Mechanism of acupuncture for musculoskeletal disorders

[Collaboration Symposium with The Japan Society of Acupuncture and Moxibustion]

(March 18, 14:00–16:00, Room E)

3S55D1-3

Sleep hygiene and problems in health

Fujiki, Nobuhiro(*Department of Ergonomics, Institute of Industrial health, Ecological Science University of Occupational and Environmental Health, JAPAN*)

It is important for us to maintain a high quality of sleep so that we could obtain our best mental and physical performance in our daily life. Insufficient sleep and/or dyssynchrony between internal clock and external light/dark cycle acutely cause daytime sleepiness and chronically induce health problems. The daytime sleepiness could result in a reduction of the working efficiency and it might causes economic loss. Moreover, it also could be a fatal risk such as traffic accident due to their low level of vigilance. Recent report also suggested that lack of sleep might be a risk factor for chronic health problems such as metabolic syndrome and depression. It is thus really important to understand the importance of the sleep hygiene and to investigate how the inadequate sleep and/or inappropriate rhythm of sleep cause the chronic health problems. For this investigation, some of common sleep disorders such as an obstructive sleep apnea syndrome and a circadian rhythm sleep disorder, shift work type would be the targets as these diseases are known to be the risks for the chronic health problems. No COI.

3S56E-1

Effect of acupuncture treatment for skeletal muscle disorders in the field of sports

Miyamoto, Toshikazu([1]*Doctoral Program in Sports Medicine, Comprehensive Human Sciences Department, University of Tsukuba, Tsukuba, Japan,* [2]*Acupuncture and Physical Therapy Teacher Training School, Tokyo, Japan*)

Acupuncture is a treatment that you want to penetrate in vivo using acupuncture needle such as stainless steel, expecting a biological reaction. Acupuncture is that it can stimulate the tissue skin, subcutaneous tissues, muscles, and nerves. We are performing acupuncture to the athletes for the purpose of the treatment and prevention of sports injuries. We have also examined the effect of acupuncture stimulation on the repair of muscle strain and disuse muscle atrophy targeting mice.

1. Effect of acupuncture treatment for sports injuries and disability
Many of athletes whom we treated are lumbago and muscle strain. In our treatment, we give the insertion of acupuncture on muscle pain part, and are doing a low-frequency electro acupuncture.

2. Effect of tack needles for muscle pain and muscle fatigue
Tack needles are about 0.6 mm in length and can be applied to athletes while they are playing. We will show you the effect of the tack needles by a double-blind randomized trials. One is about muscle pains and muscle fatigue after the marathon, and the other is about muscle fatigue and muscle hardness of long distance runners in the long-term training camp.

3. Effect of electro acupuncture for muscle damage, muscle atrophy
We introduce the histological study of the muscle damage mouse model, and also show the effect of electro acupuncture on disuse muscle atrophy of using mice hind limb suspension. No COI.

3S56E-2

Molecular and cellular mechanisms underlying muscle plasticity

Ono, Yusuke(*Department of Stem Cell Biology, Nagasaki University Graduate School of Biomedical Sciences*)

Muscle stem cells, called satellite cells, are located between the basal lamina and the sarcolemma of myofibres and play important roles in muscle plasticity such as hypertrophy and regeneration. In adult muscle, satellite cells exist as mitotically quiescent state. Satellite cells are activated rapidly in response to stimulation such as muscle injury and proliferate extensively to give rise to their progeny. Following proliferation, the majority of satellite cells undergo myogenic differentiation to produce new myonuclei, whereas a minority population returns to a quiescent state to self-renew for maintenance of a stem cell pool. Understanding how the fate decisions such as self-renewal, proliferation and differentiation are regulated in satellite cells is central to understanding how skeletal muscle regulates the plasticity. By using satellite-cell specific conditional knockout mouse models, we are undertaking a series of experiments to understand signalling networks that regulate satellite cell fate decisions with a particular emphasis on planar cell polarity (PCP) and Notch pathways. We are also investigating on how satellite cells are a functionally heterogeneous population both between muscles, and within a single myofibre, throughout body. Here, we will discuss emerging findings of the molecular and cellular basis underlying muscle plasticity. No COI.

3S56E-3

Molecular mechanism: Electroacupuncture induces satellite cell activation

Takaoka, Yutaka[1,2]([1]*Division of Medical Informatics and Bioinformatics, Kobe University Hospital, Kobe, Japan,* [2]*Life Science Research Center, Kobe Tokiwa University, kobe, Japan*)

We have been investigating the molecular mechanism of Electroacupuncture (EA) and have obtained its molecular evidence in skeletal muscle [1-2]. We found the molecular evidence that EA suppressed the expression of the myostatin gene, which is an endogenous inhibitor of muscle growth and a reducer of AKT/mTOR/p70S6K signaling, and EA induced a proliferative reaction of muscle satellite cells (stem cells). These results led us to the possibility that the EA prevent muscle atrophy.

We then elucidated the effect of EA on disuse muscle atrophy by using hindlimb-suspended (HS) mice which were treated by EA [3-4]. We found that EA/HS mice maintained a soleus muscle mass that was not significantly different from that of control mice. Also, the diameters of myofibers in mice had the same tendency. Repeated EA treatment suppressed gene expression of myostatin and three ubiquitin ligase genes in EA/HS mice but induced expression of these genes in HS mice. These findings suggest the molecular mechanism of inhibiting the disuse muscle atrophy by EA through the AKT/mTOR/p70S6K signaling pathway. No COI.

[1] Takaoka Y, et al. Physiol Genomics 2007; 30: 102–110.
[2] Ohta M, et al. eCAM 2009; doi:10.1093/ecam/nep121
[3] Ikemune S, et al. J Jap Soc of Acup and Moxi 2010; 60(4): 707–715.
[4] Ikemune S, et al. J Jap Soc of Balneo, Climato and Phys Med 2011; 74(2): 103–111.

3S56E-4

Recent advancements in controversial topics of skeletal muscle research viewed with a special interest in fatigue

Takemori, Shigeru(*Department of Molecular Physiology, Jikei University School of Medicine, Tokyo, Japan*)

Skeletal muscle, build with a beautifully aligned liquid crystalline structure of sarcomere, is essential for us animals to realize force generation and motion. With these unique features, skeletal muscle has been attracting scientific interest intensively for a long time. Symbolized by an 1:1 neuro-muscular transmission and a straight forward excitation-contraction coupling mechanism, skeletal muscle is seemingly a simple and obedient tissue under the control of central nervous system. However, it is still eliciting several confusing arguments in the field of muscle research. Among the arguments, fatigue has been one of the most controversial issues. There certainly is various aspects of fatigue; muscle fatigue, fatigue in neuro-musclular transmission, fatigue in task performance, mentally induced subjective sense of fatigue, and so on. However, these different aspects has been often confusingly discussed as an identical concept. This is probably because we somehow project our sense of fatigue onto our skeletal muscle. For instance, we feel some dull sense in our proximal muscles when got deadly tired with mental stress. This suggests that there is an intimate (direct or indirect) cross-talks between our skeletal muscle and central nervous system, and skeletal muscle is not a mere obedient motor. Recently, skeletal muscle is realized as a significant source of various signals affecting the activity of whole body. With a special interest in fatigue, recent advancements in several controversial topics of skeletal muscle research will be reviewed and discussed. No COI.

Symposium 57
Mechanosensitive regulation of biological function: update
[FAOPS Joint Symposium—Japan/Korea]

(March 18, 14:00–16:00, Room G)

3S57G-1

Reno-pathogenic mutations and PKG-mediated phosphorylation alter TRPC6 mechanosensitivity.

Inoue, Ryuji; Ichikawa, Jun; Nakagawa, Midori; Kurahara, Lin; Hu, Yaopeng(*Department of Physiology, Fukuoka University School of Medicine*)

TRPC6 is a major cardiovascular TRP isoform and susceptible to mechanical stresses in both direct and indirect ways. In renal physiology, mutations in this gene are partly causative for focal segmental glomerulosclerois (FSGS), culminating in an excessive Ca2+ influx into podocytes through receptor-mediated pathways. In this study, we focused on three FSGS mutations identified near the ankyrin repeats of TRPC6 N-terminus (P111Q, M131T, N142S,) and investigated how these mutations affect receptor as well as mechanical responses of the channel. Through functional evaluation of these mutants, it turned out; (1)all mutants showed enhanced receptor responses; (2)mechanical responses almost completely diminished except for exaggeration in M131T mutation, which was abolished by actin depolymerization with cytochalasin D; (3)physical interaction of the mutants with actin was significantly changed; (4)coexpression of a slit diaphragm protein podocin enhanced, whereas PKG-mediated phosphorylation on N-terminal Thr69 attenuated both receptor and mechanical responses of TRPC6 channel and its interaction with actin. These results suggest that physical interaction with actin cytoskeleton is essential for the mechanosensitivity of TRPC6 channel which is significantly affected by FSGS mutations or phosphorylated status of Thr69 near the N-terminal ankyrin domains. This mechanism may not only explain abnormally enhanced Ca2+ mobilization in FSGS patients' podocytes but also hints a new therapeutic strategy that may help prevent the progression of the disease. No COI

3S57G-2

A mechanosensitive ion channel, TRPV2 expressed in gastric myenteric plexus contributes to gastric adaptive relaxation and emptying in mice

Mihara, Hiroshi[1]; Tominaga, Makoto[2]; Sugiyama, Toshiro[1](*[1]3rd internal medicine University of Toyama, [2]Cell Signaling OIIB*)

Aim: Gastric adaptive relaxation (GAR) reduces meal-induced intragastric pressure. NO released from inhibitory motor neurons has an important role in the process. Gastric emptying (GE) is increased when GAR is impaired or enhanced. However, the molecular mechanism is poorly understood. TRPV2 detects mechanical stimuli and chemicals including probenecid. We showed TRPV2 expression in the mouse intestininal inhibitory motor neurons and its involvement in intestinal relaxation. This study evaluated TRPV2 distribution in mouse gastric myenteric plexus and the physiological role in GAR and GE. Methods: C57BL/6, TRPV2KO (V2KO) mice were used. RT-PCR and immunohistochemistry detected TRPV2 mRNA and protein, respectively. GAR was determined with an isolated stomach using a pressure transducer. GE in vivo was determined using phenol red meal with/without TRPV2 activators and/or a TRPV2 inhibitor, tranilast. Results: TRPV2 mRNA was detected in WT stomach, but not in V2KO. TRPV2 protein was detected in 86.5% of inhibitory motor neurons throughout the stomach, but not in V2KO. GAR was significantly enhanced by pretreatment with probenecid and the enhancement was inhibited with tranilast, TTX or L-NAME. GE was significantly enhanced with TRPV2 agonists and the probenecid-induced enhancement was inhibited with tranilast. GE with tranilast only or in V2KO was also significantly enhanced. Conclusion: TRPV2 is expressed in mouse gastric inhibitory motor neurons and may play roles in GAR and GE. No COI.

3S57G-3

Mechano-electric interaction and arrhythmia

Iribe, Gentaro(*Cardiovascular Physiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences*)

Mechanical stimulus modulates electrophysiological properties of the heart (mechanoelectric feedback: MEF). For instance, a blunt precordial impact on the heart may cause fatal arrhythmia which is known as commotio cordis. Mechanically induced ectopic beats are believed to be caused by inward current via cation-selective stretch-activated channels (SACs). On the other hand, sarcolemmal stretch-activated K+ channel, for instance, stretch-activated BKCa (SAKCA) channel causes outward current, which may balance out the arrhythmogenic effects of SACs. Mechanical stimuli are transmitted to whole cell area including ion channels in any intracellular membrane structure via cytoskeleton. Therefore, intracellular organelles may show mechanosensitive responses to play a role in MEF as well as sarcolemmal mechanosensitive channels. We have recently revealed that myocardial stretch increases mitochondrial reactive oxygen species production, which causes stretch-induced increase in Ca2+ spark, spontaneous Ca2+ release from ryanodine receptors on sarcoplasmic reticulum (SR). SR calcium release causes Ca2+ extrusion via Na+/Ca2+ exchanger which causes inward current and may contribute to initiate ectopic action potential. Mechanically induced ectopic beats are the integrated result of these responses from various mechanosensitive components. No COI.

3S57G-4

Mechanosensitive regulation of vascular tone; comparison between systemic and pulmonary arteries

Kim, Sung Joon; Yoo, Hae Young ; Kim, Hae Jin(*Department of Biomedical Sciences, Ischemic/Hypoxic Disease Institute, Seoul National University College of Medicine, Seoul, KOREA*)

Vascular tone is directly or indirectly affected by variety of mechanical stimuli such as shear stress and wall tension. Small arteries in vivo are partially contracted, the extent of which is affected by luminal pressure (Plum) of the vessel. Such contraction in systemic artery is called myogenic response (MR) that is critical for maintaining relatively constant regional blood flow despite fluctuation of perfusion pressure. Mechanosensitive nonselective cation channels (NSCms), protein kinase C (PKC), and Rho kinase (ROCK) are suggested as underlying mechanisms for MR. In Part I, we compare the variability of MRs according to the arterial types (cerebral artery (CA), deep femoral artery (DFA) and mesenteric artery (MA)) in terms of their pressure-sensitivity and underlying mechanisms that are recruited according to Plum. In Part II, mechanosensitive regulation of pulmonary artery (PA) would be demonstrated regarding the NSCms in PA myocytes and mechanosensitive recruitment of NO synthase (NOS) putatively expressed in PA smooth muscle layer. Albeit the expression of NSCms in PA myocytes, the isolated PAs do not show myogenic responses but showed decreased tone by repetitive pressure increase. With partial contraction by applying 3 nM U46619 (TXA2 agonist) in endothelium denuded PAs, additional contraction by NOS inhibitor [nitro-L-arginine methyl ester (L-NAME)] was feeble. However, with combined increase in wall tension equivalent to 10 to 30 mmHg of Plum, L-NAME induced robust contraction of PAs. A partial depolarizing condition (20 mM KCl) combined with 3 nM U46619 induced similar sensitivity to L-NAME. RT-PCR and immunohistochemistry analysis show faint expression of eNOS in PA smooth muscle cells. The supposed activation of eNOS by TXA2 and increased wall tension might contribute to the relatively low peripheral resistance of pulmonary circulation and pulmonary arterial pressure in vivo. No COI.

Symposium 58
Optical approaches toward the understanding of physiological functions

(March 18, 14:00–16:00, Room H1)

3S58H1-1

Developing high-performance indicators for live cell imaging

Horikawa, Kazuki(*Institute of Health Biosciences, The University of Tokushima Graduate School, Japan*)

In the physiological analysis, there is an increasing demand for reliable and quantitative imaging. Here, fluorescent and chemiluminescent indicators that visualize the dynamics of molecules, cells and tissues are essential. Although a variety of functional indicators have been developed, analysis under the "purely physiological condition" is still challenging. This often comes from the non-negligible difference between experimental and physiological condition we are focusing on. For example, live Ca^{2+} imaging is performed by delivering very strong stimuli at the level cells would never experience under the natural codition. In such a case, cells show the "death-cry" like response that can be easily detected due to their exceptionally large amplitude of Ca^{2+} concentration change. On the other hand, cells in vivo accomplish the signal transduction with vanishingly small amplitude for which ultrasensitive indicators are needed for its detection. One of our goals is to develop a series of high-performance indicators which can be used for imaging even under the "purely physiological condition". In this talk, I will introduce our simple and systematic approach to obtain high-performance indicators with improved dynamic range and sensitivity, being the most important parameter for successful detection of subtle physiological responses. With proof-of-concept demonstration for Ca^{2+}, cAMP and cGMP indicators, I would like to show the effectiveness of our strategy in developing the indicator for other physiological phenomena such as the inflammatory response in the immune system. No COI.

3S58H1-2

Large-scale imaging of circadian rhythm in the mammalian master clock

Enoki, Ryosuke[1,2,3,7]; Mieda, Michihiro[4]; Ono, Daisuke[1]; Kuroda, Shigeru[5]; Mazahir, Hasan[6]; Honma, Sato[2]; Honma, Ken-ichi[2]([1]*Hokkaido Univ, Grad Sch of Med, Photonic Bioimaging, Sapporo, Japan,* [2]*Hokkaido Univ, Grad Sch of Med, Dep of Chronomed, Sapporo, Japan,* [3]*Hokkaido Univ, Grad Sch of Med, Dep of Cell Physiol, Sapporo, Japan,* [4]*Kanazawa Univ, Faculty of Medicine, Dep of Mol Neurosci & Integrative Physiol, Kanazawa, Japan,* [5]*Hokkaido Univ, RIES, Sapporo, Japan,* [6]*Charite-Universitatsmedizin, NeuroCure Cluster of Excellence, Berlin, Germany,* [7]*JST PREST, Chiyoda, Japan*)

The circadian pacemaker in the hypothalamic suprachiasmatic nucleus (SCN) is a hierarchical multi-oscillator system in which neuronal networks play crucial roles in expressing coherent rhythms in physiology and behavior. Using a large-scale imaging method with genetically-encoded calcium sensors, we visualized intracellular calcium from the entire SCN neuronal network in culture (J.Neurosci Methods, 2012). We found circadian calcium rhythms at a single-cell level in the SCN, which were topologically specific patterns in the ventral region than the dorsal (PNAS, 2012). The robustness of the rhythm was reduced but persisted even after blocking the neuronal firing with tetrodotoxin (TTX). TTX dissociated the circadian calcium rhythms between the dorsal and ventral SCN. In addition, we developed a dual-color fluorescence imaging method and successfully visualized expression patterns of *Per1*:EGFP and R-GECO (calcium) in the SCN at single cell resolution. Our rhythm analysis showed the distinct spatio-temporal patterns of *Per1* and calcium in the SCN. No COI.

3S58H1-3

non-invasive in vivo NIR fluorescence imaging using iRFP Transgenic mice and iRFP-flox mice

Miwa, Yoshihiro; Sakaguchi, Shota; Sugiyama, Yuka; Tanaka, Junko(*Molecular Pharmacology, Faculty of medicine, University of Tsukuba, Tsukuba, Japan*)

Three dimensional (3D) fluorescence optical tomography of living mice is a necessary technique for noninvasive monitoring of the time course of disease conditions or changes of biological states precisely avoiding surgical effects. It is well known that Near-Infrared (NIR) light of 650-900 nm can propagate by multiple scattering through several centimeters of tissues, because of the low absorbance of tissue chromophores such as oxy- and deoxy-hemoglobin, melanin and fat. However, the directionality of light propagation is randomized in tissues, image rendering requires tomographic reconstruction. Because regular diet contains many kinds of fluorescent compounds, the fluorescence of food is serious impediment of optical tomography. Non-alfalfa diet and low-fluorescence diet are commercially available, however, non-alfalfa diet still fluoresces in NIR. The feeding of low-fluorescent diet led rapid loss of weight of mice and finally resulted death of all mice by three weeks. Therefore, we analyzed the fluorescence spectrum of all components of formula food, and based on these data, we developed new low-fluorescent diet. In 2011, Filonov et al. reported the novel NIR fluorescent protein, iRFP that can autonomously maturate without addition of exogenous biliverdin. Thus iRFP would be suitable for in vivo imaging as a NIR fluorescent probe. Therefore, we attempted to detect the fluorescences from iRFP and ICG separately. For further biological application, we have tried to establish iRFP-transgenic mice and iRFP-flox knock in mice. No COI.

3S58H1-4

Visualization of signaling pathways by FRET and its clinical application

Ohba, Yusuke(*Dept. Cell Physiol., Hokkaido Univ. Grad. Sch. Med., Sapporo, Japan*)

The development biosensors utilizing fluorescent proteins (FPs), including those base on the principle of Förster resonance energy transfer (FRET), has revealed spatiotemporal dynamic of cell signaling. Here we introduce our current trial of clinical application of bioimaging techniques with chronic myeloid leukemia (CML) as a model. CML is a myeloproliferative disease characterized by the emergence of Philadelphia chromosome, which results in the expression of the causative protein BCR-ABL. The specific tyrosine kinase inhibitor of BCR-ABL imatinib mesylate (IM) has radically innovated the treatment of this disease, with a 5-year survival rate of approximately 80%. Nowadays, CML has thus become a disease controllable by oral medication; however, continuous medication is required to suppress the disease progression. Furthermore, there remain concerns that substantial patients display IM resistance both before and during the treatment. To overcome these issues, we have developed the FRET-based biosensor Pickles (named after phosphorylation indicator of CrkL en substrate) using the BCR-ABL substrate CrkL. Pickles was able to evaluate drug efficacy with higher sensitivity and identify smaller population (< 1%) of drug-resistant cells, compared to any previously established methods. Furthermore, it was possible to not only determine the current effectiveness of IM, but also predict future emergence of drug resistance. In this symposium, we will overview the current progress in the development of this method and would like to discuss its potential to overcome drug resistance. No COI.

Symposium 59
Rehabilitation from the aspect of nutrition and visceral function

(March 18, 14:00–16:00, Room K)

3S58H1-5

Spatial and temporal dynamics of ensemble and single-neuron activity in the mouse motor cortex during a voluntary movement

Matsuzaki, Masanori[1]([1]*Division of Brain Circuits, National Institite for Basic Biology, Okazaki, Japan,* [2]*SOKENDAI*)

Two-photon imaging is a powerful tool used to examine molecular and cellular functions in living tissues. In particular, calcium imaging can quantitatively measure neuronal activity i.e. action potential firing. We conducted two-photon calcium imaging of mouse motor cortex during a self-initiated lever-pull task. In this task, head-restrained mice had to pull a lever for ~600 ms to receive a water drop, and then had to wait for more than 3 s to pull it again. In the imaging session after training, we found several types of task-related cells in the mouse motor cortical areas. For example, cells whose peak activities occurred during lever pulls and cells whose peak activities occurred after the end of lever pulls. By predicting lever movement trajectories from ensemble and individual activities, we found spatiotemporal dynamics of the motor cortical activity. We also conducted two-photon calcium imaging in the primary motor cortex during 14 training sessions of the self-initiated lever-pull task. Motor cortical activity dynamically changed at both ensemble and individual-neuron levels over sessions. No COI.

3S59K-1

Responses of glucose output from the liver to somatic afferent stimulation

Kurosawa, Mieko[1]; Shimoju, Rie[1,2]([1]*Center Med. Sci., Intl. Univ. Health & Welfare, Otawara, Japan,* [2]*Dept. Physical Ther., Intl. Univ. Health & Welfare, Otawara, Japan*)

Somatic afferent excitation by physical therapy treatment can produce various effects on motor and autonomic functions. We have investigated the reflex responses of various autonomic functions to somatic (muscle or skin) afferent stimulation in animals whose emotional influences are eliminated with use of anesthesia. In the present symposium I will show our studies on the responses of glucose output from the liver to muscle stimulation in anesthetized rats. Electrical stimulation (10 mA, 20 Hz) was delivered to the unilateral anterior tibial muscle for 10 min. The muscle stimulation increased the hepatic glucose output (HGO), leading increases in the plasma glucose. The HGO response was disappeared after section of the somatic nerves innervating the anterior tibial muscle, and after treatment with phenoxybenzamine and propranolol, an alpha and a beta adrenoceptor blockades. On the other hand, the response was augmented after treatment with atropine, a muscarinic receptor blockade. These results indicate that the response of HGO to muscle stimulation is mediated via the somatic nerves as an afferent limb, and the sympathetic nerves as an efferent limb, and that at the same time the response is reflexly inhibited via the parasympathetic nerves. Finally, I will also show that the same muscle stimulation increases the insulin sensitivity of blood glucose in the animal model of type II diabetes via excitation of the somatic afferent nerves. No COI.

3S59K-2

Resistance exercise-induced endocrine activation and it mechanism

Ishii, Naokata(*Department of Life Science, Graduate School of Arts and Sciences, University of Tokyo, Tokyo, Japan*)

EExercises stimulate secretions of a variety of hormones in an exercise intensity-dependent fashion. In particular, a number of studies have shown that resistance exercise causes marked increases in blood concentrations of anabolic hormones such as growth hormone (GH) and testosterone. However, this effect is not simply dependent on exercise intensity, but strongly depends on the other exercise variables, e.g., exercise volume and rest period between sets (Kraemer, 1995), suggesting that multiple processes are involved in its mechanism. We have shown that resistance exercise with restricted muscular blood flow (RRB) causes dramatic increases in blood GH and noradrenalin, even at low exercise intensity (Takarada et al., 2000). In addition, a low-intensity resistance exercise with slow movement and tonic force generation (LST; Tanimoto & Ishii, 2006) causes larger increases in blood GH, free testosterone and noradrenalin (Tanimoto et al., 2005; Goto et al., 2008) than after normal high intensity exercise. Both RRB and LST are characterized by sustained decline of muscle oxygenation level and regional increases in metabolic sub-products such as lactate. Thus we have investigated the effects of direct electric stimulation of muscle with and without restriction of muscular blood flow on the secretion of GH. Electric stimulation of muscle caused significant increases in blood GH and lactate only when combined with restriction of muscular blood flow, suggesting that chemoreception of metabolites within muscle plays an important part in the stimulation of GH secretion (Inagaki et al., 2011). No COI.

3S59K-3

Combined effects of interval walking training with nutrient supplement intake on physical fitness and improvement of life-style related diseases in middle aged and older subjects.

Nose, Hiroshi[1,2]; Masuki, Shizue[1]; Morita, Atsumi[1]; Okazaki, Kazunobu[1]; Kamijo, Yoshi-ichiro[1]([1]*Dept. of Sports Med. Sci., Shinshu Univ. Grad. Sch. of Med.,* [2]*Jukunen Tai-ikudaigaku Research Center*)

We have developed a health promotion program for middle aged and elder people, Jukunen Taiikudaiiku Program, to examine the physical and mental effects of high-intensity interval walking training (IWT) on 5,400 subjects for these 10 years. Since a prescription of IWT can be conducted by using an IT network system, the participants in the program were able to receive the prescription even if they lived remote from trainers, enabling them to perform IWT at their favored places and times, and also at low cost. We found that IWT for 4 months increased physical fitness by 10–20% and decreased the indices of life-style related diseases by 10–20%. In addition, we examined the effects of a mixture of protein and carbohydrate intake immediately after daily aerobic exercise training including IWT and found that the supplements enhanced the increases in plasma albumin content and thigh muscle strength. Further, we examined the effects of 5-aminolevulinic acid intake during IWT and found that it improved the work efficiency during exercise and increased an IWT achievement. The acid is known to be contained in many kinds of foods and the sole initial material of the heme biosynthesis. These results suggest that nutritional supplement intake accelerates the effects of aerobic training on physical fitness and the improvement of life-style related diseases. No COI.

* Current affiliation: Urban Health and Sports, Osaka City University.

3S59K-4

Nutrition and rehabilitation

Wakabayashi, Hidetaka(*Department of Rehabilitation Medicine, Yokohama City University Medical Center, Yokohama, Japan*)

Malnutrition often occurs in patients with disability. The prevalence of malnutrition in geriatric rehabilitation was higher than hospital (50.5% vs. 38.7%) according to MNA classification. As nutritional status is associated with rehabilitation outcome, a combination of both rehabilitation and nutrition care management may be associated with a better outcome. This concept is defined rehabilitation nutrition. Rehabilitation nutrition is to assess with the International Classification of Functioning, Disability and Health including nutrition status and to practice nutrition care manegement to demonstrate functions, activities, and participation to the fullest. It is not enough for patients with disability to coordinate only rehabilitation or clinical nutrition. Sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and strength. Primary sarcopenia is considered to be age-related when no other cause is evident, other than ageing itself. Secondary sarcopenia should be considered when other causes are evident, such as activity-related sarcopenia, disease-related sarcopenia, or nutrition-related sarcopenia. Activity-related sarcopenia can result from bed rest, deconditioning, or zero-gravity conditions. Disease-related sarcopenia is associated with invasion (acute inflammatory diseases), cachexia (cancer, advanced organ failure, collagen diseases, etc.), and neuromuscular disease. Nutrition-related sarcopenia results from inadequate dietary intake of energy and/or protein. Treatment including rehabilitation and nutrition care management is different according to the causes of sarcopenia. No COI.