# Symposia

# Symposium 1

Expanding frontiers in weight control research explored by young investigators.

March 24 (Thu), 15:00 – 16:30, Room G

## S01-1

### Catecholamines facilitate fuel expenditure and protect against obesity via the network of gut-brain axis in transcriptional factor Skn-1-deficient mice

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Taste and nutrient stimuli detected by the gastrointestinal tract are transmitted to the brain to regulate food intake and energy metabolism, referred to as "gut-brain" axis. Here we report that brush cells, as well as specific types of taste cells, are eliminated in the gastrointestinal tract of *Skn-1* knockout (KO) mice. Despite unaltered food intake, *Skn-1* KO mice gain reduced body weight with lower body fat percentage due to higher energy expenditure. Lipid metabolism, such as lipid degradation and β-oxidation of fatty acid, and energy expenditure in skeletal muscle are augmented in *Skn-1* KO mice. Finally, 24-hr urinary excretion of catecholamines is elevated, accompanied by decreased secretion of insulin after gastric glucose gavage in *Skn-1* KO mice. These results suggest the existence of novel pathways originating from brush cells and taste cells in the gastrointestinal tract to peripheral tissues including the adrenal glands via the brain to maintain energy homeostasis. (COI:Properly Declared)

## S01-2

### Impact of a brown rice-derived bioactive product on feeding regulation and fuel metabolism

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Our clinical study has shown that brown rice improves obesity and glucose dysmetabolism in middle-aged men (Br J Nutr 111:310, 2014). However, the underlying molecular mechanism remains unclear. We discovered that γ-oryzanol, a major and unique bioactive component in brown rice, substantially decreases hypothalamic endoplasmic reticulum (ER) stress in high fat diet (HFD)-fed mice, thereby attenuating preference for the animal fat (Diabetes 61:3084, 2012). We recently demonstrated the metabolically-beneficial impact of γ-oryzanol on dysfunction of pancreatic islet (Endocrinology 156:1242, 2015, Br J Pharmacol 172:4519, 2015). HFD-induced ER stress in β-cells aggravates glucose-stimulated insulin secretion (GSIS), leading to apoptosis and diabetes. Based on this notion, we found that γ-oryzanol reduced ER stress in islets from HFD-fed mice. Furthermore, γ-oryzanol enhanced GSIS via suppression of local dopamine D2 receptor (D2R) signaling in murine isolated islets. We demonstrated that D2R is confined to β-cells and decreases cAMP levels, thereby decreasing GSIS. In islets from HFD-fed mice, expression levels of D2R signaling molecules were significantly increased, which reciprocally decreased by γ-oryzanol. Recent our data suggest that γ-oryzanol also exerts beneficial effect on D2R signaling in the brain. My presentation will update our research on γ-oryzanol in a variety of aspects. (COI:No)

## S01-3

### A Novel GPCR-Regulated Neuronal Signaling Pathway Triggers Sustained Orexigenic Effects

Nakajima Kenichiro[1,2]、Cui Zhenzhong[2]、Li Chia[2]、Fu Ou[1]、Krashes Michael[2]、Wess Jurgen[2]

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G protein-coupled receptors (GPCRs) regulate the activity of virtually all physiological functions including food intake and the control of body weight. Agouti-related peptide (AgRP)-expressing neurons located in the arcuate nucleus (ARC) of the hypothalamus play a key role in triggering appetite. AgRP neurons release three chemically and functionally different orexigenic molecules: GABA, Neuropeptide Y (NPY), and AgRP. The activity of AgRP neurons is modulated not only by synaptic ion channels but also by GPCRs. In the present study, we examined whether receptor-mediated activation of Gs in AgRP neurons modulates food intake. To address this issue, we selectively expressed a Gs-coupled designer GPCR (GsD) in AgRP neurons. Importantly, GsD cannot be activated by endogenous ligands but only by an exogenously administered drug, clozapine-N-oxide (CNO), an otherwise pharmacologically inert compound. A Cre-dependent adeno-associated virus containing the GsD sequence was injected into the ARC of AgRP-ires-Cre knockin mice. A single i.c.v. injection of CNO led to a robust increase in food intake that continued for several days, associated with a significant weight gain. Interestingly, this phenotype was not observed when CNO was co-injected with an anti-AgRP antibody. By contrast, inhibition of NPY receptor or GABA signaling had little effect on GsD-mediated stimulation of food intake. These results suggest the existence of an orexigenic Gs-AgRP pathway in AgRP neurons which promotes chronic food intake (COI:No).

## S01-4

### NMDA receptor co-agonist D-serine regulates food preference

Sasaki Tsutomu

*(Lab Metab Signal, IMCR, Gunma Univ, Maebashi, Japan)*

D-serine is physiologically important for modulating excitatory glutamatergic neurotransmission as a co-agonist of synaptic N-methyl D-aspartate (NMDA) receptor. NMDA signaling has been implicated in the control of food intake. However, the role of D-serine on appetite regulation is unknown. To clarify the effects of D-serine on appetite, we investigated the effect of oral D-serine ingestion on food intake in three different feeding paradigms using three different strains of male mice. The effect of D-serine was also tested in leptin signaling-deficient db/db mice and sensory-deafferented (capsaicin-treated) mice. The expression of orexigenic neuropeptides in the hypothalamus were compared in fast/re-fed experiments. Conditioned taste aversion for high-fat diet (HFD) was tested in the D-serine-treated mice. Under the one-food access paradigm, some of the D-serine-treated mice showed starvation, but not when fed normal chow. HFD feeding with D-serine ingestion did not cause aversion. Under the two-food choice paradigm, D-serine suppressed the intake of high-preference food but not normal chow. D-serine also effectively suppressed HFD intake but not normal chow in db/db mice and sensory-deafferented mice. In addition, D-serine suppressed normal chow intake after 24-h fasting despite higher orexigenic gene expression in the hypothalamus. D-serine failed to suppress HFD intake in the presence of L-701,324, the selective and full antagonist at the glycine-binding site of the NMDA receptor. Therefore, D-serine suppresses the intake of high-preference food through co-agonism toward NMDA receptors. (COI:No)

# Symposium 2

Multi-dimensional approaches toward the understanding of sleep-wake regulation in the brain

March 22 (Tue), 9:00 – 10:30, Room B

## S02-1

### Comprehensive cell analysis of whole organ/body for the organism-level systems biology

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The multicellular organism is composed of various types of cells inside, where they connect with each other and work in a coordinated manner. The mechanism of an organism-level biological function, including the sleep-wake behavioral rhythm, is implemented in such a multicellular circuitry or a multicellular system. In this talk, we will introduce 1) a comprehensive cell and cell circuit analysis pipeline and 2) a high-throughput mouse genetics as the fundamental technologies for *the organism-level systems biology*, a research scheme to identify and analyze cellular components and their relations of the responsible system in the cell and cell circuit layer. The first technology was termed CUBIC (Clear, Unobstructed Brain/Body Imaging Cocktails and Computational analysis), which includes an efficient and reproducible whole organ and body clearing method, a rapid imaging with light-sheet fluorescence microscopy and computational image informatics. The second technology enabled the production and phenotype analysis of new mouse strains within one generation. These technologies thus can be applicable to the wide range of life science and medical researchs, and will facilitate our understanding of the sleep-controlling system. (COI:No)

## S02-2

### Identification of a sleep regulatory circuit and implications for the function of REM sleep

Hayashi Yu[1,2]、Kashiwagi Mitsuaki[1]、Yasuda Kosuke[3]、Ando Reiko[3]、Kanuka Mika[1]、Sakai Kazuya[4]、Itohara Shigeyoshi[3]

*([1]WPI-IIIS, Univ. of Tsukuba, Tsukuba, Japan、[2]JST PRESTO, Kawaguchi, Japan、[3]Brain Science Institute, RIKEN、[4]INSERM, Lyon, France)*

Mammalian sleep comprises rapid eye movement (REM) sleep and non-REM (NREM) sleep. To functionally isolate from the complex mixture of neurons populating the brainstem pons those involved in switching between REM and NREM sleep, we chemogenetically manipulated neurons of a specific embryonic cell lineage in mice. We identified excitatory glutamatergic neurons that inhibit REM sleep and promote NREM sleep. These neurons shared a common developmental origin with neurons promoting wakefulness; both derived from a pool of proneural hindbrain cells expressing Atoh1 at embryonic day 10.5.We also identified inhibitory GABAergic neurons that act downstream to inhibit REM sleep. Artificial reduction or prolongation of REM sleep in turn affected slow-wave activity during subsequent NREM sleep, implicating REM sleep in the regulation of NREM sleep. (COI:No)

## S02-3

### Intracellular coupling mechanisms revealed by simultaneous multi-functional recordings in the suprachiasmatic nucleus

Enoki Ryosuke[1,2]、Ono Daisuke[1]、Honma Sato[2]、Honma Ken-ichi[2]

*([1]Photonic Bio, Hokkaido Univ Grad Sch of Med、[2]Dep of Chronomed, Hokkaido Univ Grad School of Med、[3]PRESTO)*

Mammalian master clock, the hypothalamic suprachiasmatic nucleus (SCN), control daily rhythms in physiology and behavior. At cellular level, the molecular machinery for circadian rhythm generation is hypothesized to be an interlocked transcriptional/translational feedback loop (the core loop) involving several clock genes and their protein products. Intracellular calcium is regarded as an important intracellular signal from the core loop to output functions such as neuronal firings and transmitter release, and vice versa. However, the inter-relationships between them remain largely unknown. In the present study, we performed simultaneous recordings of clock gene expression (with PER2::LUC bioluminescence), intracellular calcium (with GCaMP6s fluorescence), and spontaneous firing (with multi-electrode array dish) in identical locations in the cultured SCN slices from wild-type and Cryptochrome (Cry) 1 / Cry2 double defficient (Cry1,2-/-) SCN. By analyzing parameters of the three rhythms and the phase relationship among them, we found that the Cryptochromes are essential not only for robustness and stability of the three rhythms, but also for intracellular coupling of the core loop, calcium, and spontaneous firing. We also found that network coherence of the calcium rhythm, but not PER2 rhythm, was decreased in Cry1,2-/- SCN. We propose the cellular model in which the neural network in the SCN reinforces the coherence of cellular circadian rhythms in clock gene expression via calcium signaling pathways. (COI:No)

## S02-4

### Genetic dissection of neural mechanisms underlying the central circadian pacemaker

Mieda Michihiro

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The suprachiasmatic nucleus (SCN) is the primary circadian pacemaker in mammals and entrains to the environmental light/dark cycle. It is composed of multiple types of GABAergic neurons, and interneuronal communications among these neurons is essential for normal function of the SCN. However, mechanisms underlying the SCN neuronal network remain unknown.

We considered that neuron type-specific genetic manipulations within the SCN would provide crucial information for the principle of the SCN network. As a first step, we generated mice in which *Bmal1*, an essential clock component, is deleted specifically in GABAergic neurons producing arginine vasopressin (AVP neurons), one of the primary neuronal types in the SCN. These mice showed marked lengthening in the free-running period and activity time of behavior rhythms. *Bmal1*-dependent oscillators of AVP neurons may modulate the coupling of the SCN network, eventually coupling morning and evening behavioral rhythms, by regulating expression of multiple factors important for the network property of these neurons. To further examine the contribution of AVP neurons to the circadian pacemaking of the SCN, we next manipulated the period of cellular clocks specifically in these neurons by selective deletion or overexpression of *casein kinase 1 delta* (*CK1δ*). The deletion lengthened the free-running period of circadian behavior while overexpression shortened it. Collectively, AVP neurons of the SCN may actively regulate the circadian pacemaking. (COI:No)

# Symposium 3

Challenge to"Biophysiological Interphase", the microspace between bulky extracellular solution and plasma membrane, by interdisciplinary approaches

March 23 (Wed), 15:00 – 16:30, Room I

## S03-1

### Fluorescent nanoparticle for sialic acid imaging of biophysiological interphase

Takai Madoka

*(Dept Engineering, The University of Tokyo, Tokyo, Japan)*

We designed biocompatible bioimaging probes, which consist of lectins and fluorescence conjugated polymeric nanoparticles, to specifically monitor the expression level of sialic acids of biophysiological interphase. We fabricated the fluorescence conjugated biocompatible polymer for imaging of living cells, and prepared the nanoparticle with poly(lactic acid) as a core materials. Major constituents of the nanoparticle are lectins and 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer with rhodamine derivative. The lectin-tagged fluorescent polymeric nanoparticles was approximately 35 nm in diameter. Through cellular experiments, we successfully detected sialic acid over expression on cancerous cells, MCF-7 with high specificity. The specificity comes from the biocompatible MPC polymer, which can resist the biological materials such as protein and cells.These fluorescent polymeric nanoparticles can be useful as a potential bioimaging probe for detecting diseased cells. (COI:No)

## S03-2

### Sialylation of H,K-ATPase β-subunit positively regulates the proton pump activity in the biophysiological interphase of gastric parietal cells

Sakai Hideki[1]、Fujii Takuto[1]、Kushiro Keiichiro[2]、Takai Madoka[2]

*([1]Dept Pharm Physiol, Grad Sch Med Pharm Sci, Univ Toyama, Toyama, Japan、[2]Dept Bioengineering, Sch Eng, Univ Tokyo, Tokyo, Japan)*

Gastric proton pump (H,K-ATPase) consists of two subunits, a catalytic α-subunit (αHK) and a glycosylated β-subunit (βHK). So far, it has not been known whether individual carbohydrate residues of the *N*-glycosylation of βHK affect the function of H,K-ATPase in gastric parietal cells. We therefore investigated properties of sialic acids of βHK using the H,K-ATPase-expressing samples. In hog renal proximal tubular LLC-PK1 cells stably expressing αHK and βHK, staining with lectin-tagged fluorescent polymeric nanoparticles showed that βHK is sialylated. Sialylation of βHK was also found in the membrane sample of the cells by Western blotting using an anti-βHK antibody. Sialic acids of βHK could be cleaved by neuraminidase and acidic solution (pH 5). Enzyme activity of H,K-ATPase was significantly decreased by cleavage of sialic acids of βHK. Interestingly, sialylation of βHK was observed in the samples prepared from the gastric mucosa of famotidine-treated rats but not in those of histamine-treated rats, suggesting that βHK is sialylated in resting phase but not in stimulated phase. Enzyme activity of H,K-ATPase in the samples of famotidine-treated rats was significantly higher than that of histamine-treated rats. The effects of famotidine were weakened by neuraminidase. Our results suggest that sialylation of βHK may positively regulate enzyme activity of αHK at the biophysiological interphase of gastric parietal cells. (COI:No)

## S03-3

### Development of Scanning Ion Conductance Microscopy for Non-label Biophysiological Interphase Chemical Imaging

Takahashi Yasufumi[1,2]、Zhou Yuanshu[4]、Ida Hiroki[3]、Shiku Hitoshi[3]、Sakai Hideki[5]、Matsue Tomokazu[3,4]

*([1]Division of Electrical Eng. and Computer Sci., Kanazawa Univ., Kanazawa 920-1192, Japan、[2]JST PREST、[3]Graduate School of Environmental Studies, Tohoku University、[4]Advanced Institute for Materials Research, Tohoku University、[5]Department of Pharmaceutical physiology, University of Toyama)*

Due to the diffraction limit of optical microscope, nano-meter scale imaging is not able to conduct for conventional microscope. Scanning probe microscopy is a branch of microscopy that obtains images of surfaces using a probe that scans the sample surface. However, deformation of the soft and responsive cell by the probe represents a substantial problem. Scanning ion conductance microscopy (SICM), which uses a nanopipette as a scanning probe to detect an ionic current as feedback signal, is reported as an effective tool for non-contact topographical imaging of live cells. The magnitude of ionic current changes with the pipette-sample distance and the approach character depends on the nanopipette aperture. We have developed hybrid system of SICM and scanning electrochemical microscopy (SECM) for evalulating the local cell membrane permiability. In this presentation, we report the SICM topography images of gastric surface mucous cell lines (GSM06), which produce periodic acid-Schiff and concanavalin A positive glycoproteins. To visualize the damage process of the mucosal layer, we added ethanol to GSM06 cells and imaged the topography change using SICM. We also performed topography and electrochemical simultaneous imaging using SICM-SECM to identify the mucosal layer without labelling. (COI:No)

## S03-4

### Surface specific vibrational spectroscopy to study the interfacial water structure at biophysiological interface

Noguchi Hidenori、Uosaki Kohei

*(National Institute for Materials Science, Internationl Center for Materials Nanoarchitectonics, Tsukuba, Japan)*

Biological molecules are physiologically inactive without water. While many aspects of structure and dynamics of bulk water can be regarded as reasonably understood at present, the same is not true for the water which is found in interfacial or restricted environments, such as surface of biological molecules.Since the first report of Shen and co-workers, sum frequency generation (SFG) spectroscopy has proved to be a powerful spectroscopic method to investigate the structure of water at interface. SFG is a second-order non-linear optical technique that has unique advantages for probing the vibrational spectrum of molecules adsorbed at a surface. Here, I would like to talk about two studies utilized SFG spectroscopy to (1) investigate the structure of polymer electrolyte brush under various environment and (2) to find the relation between the structure of interfacial water at polymer brush surface and the protein adsorption behavior studied by quartz crystal micro-balance (QCM). (COI:No)

# Symposium 4

A novel adenosine analogue COA-Cl as a pro-angiogenic and neurotrophic agent

March 23 (Wed), 9:00 – 10:30, Room J

## S04-1

### Promising capabilities of an adenosine analogue, COA-Cl

Tsukamoto Ikuko、Takata Maki、Konishi Ryoji
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COA-Cl is a newly synthesized nucleoside analogue with the molecular weight of 284. It is a water soluble (< 20mM) and chemically stable compound having various physiological activities.
*Angiogenic activities* COA-Cl significantly stimulates tube formation in human umbilical endothelial cells (HUVEC) co-cultured with normal human dermal fibroblasts (NHDF). Its potency at 100μM was stronger than the positive control, 10ng/mL of vascular endothelial growth factor (VEGF). In HUVEC, the angiogenic responses induced by COA-Cl were mediated by a MAP kinase cascade comprising MEK and ERK1/2, but were independent of VEGF receptor. In NHDF, COA-Cl promoted the synthesis and secretion of VEGF.
*Neurotrophic/neuroprotective activities* COA-Cl increased neurite outgrowth and activity of acetylcholine esterase, a marker enzyme for the differentiation of PC12 cells. In addition to ERK1/2, COA-Cl promoted the phosphorylation of tyrosine hydroxylase. These potencies are similar to those of an endgeneous neurotrophic compound, nerve growth factor (NGF). COA-Cl was revealed to promote VEGF like and NGF like effects. Both effects were also confirmed *in vivo*. Therefore, COA-Cl can be a capable candidate for a medicinal drug for ischemic and/or neuronal disorders. And more, the application to regenerative medicine is also promising. *Xeno free* materials, such as culture media, growth factors etc. are eagerly desired in regenerative medicine recently. COA-Cl is indeed the compound that can be used as a *xeno free* alternative trophic factor. (COI:Properly Declared)

## S04-2

### Signaling mechanisms underlying angiogenic effect of COA-Cl

Igarashi Junsuke
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Angiogenesis begins with vessel sprouting initiated by vascular endothelial growth factor (VEGF), followed by vessel maturation promoted by a variety of bio-active molecules including a lipid mediator sphingosine 1-phosphate (S1P). An adenosine-like compound COA-Cl activates ERK1/2 and induces angiogenesis in endothelial cells when co-cultured with fibroblasts. Both angiogenic activity and ERK1/2 activation are sensitive to pharmacological inhibition or gene silencing of the $S1P_1$ receptor subtype for S1P. COA-Cl competes with binding of radio-labeled S1P to the $S1P_1$ receptor in vitro. On the other hand, COA-Cl up-regulates the expression of VEGF gene and increases the secretion of VEGF protein in fibroblasts. Among two major activators of the VEGF gene, COA-Cl induces the expression of a co-transcription factor PGC-1α, but not a transcription factor HIF1α. Gene silencing of PGC-1α attenuates the COA-Cl-induced up-regulation of VEGF. COA-Cl induces VEGF in COS-7 cells over-expressing PGC-1α and its partner transcription factor ERRα. In skeletel muscle and fat tissue, a second messanger cAMP and its down-stream transcription factor CREB activate the expression of the PGC-1α gene. In fibroblasts, COA-Cl increases cAMP content and induces phosphorylation of CREB at Ser133, cAMP-dependent kinase site. In conclusion, COA-Cl activates a signaling cascade comprising cAMP/CREB/PGC-1α/ERRα to induce VEGF secretion from fibroblasts, whereas it directly activates mitogenic signal via the $S1P_1$ receptor pathway in endothelial cells. COA-Cl may therefore promote both steps of angiogenesis, vessel sprouting and maturation. (COI:No)

## S04-3

### Synthesis and evaluation of novel carbocyclic oxetanocin A (COA-Cl) derivatives as potential tube formation agents

Sakakibara Norikazu
*(Faculty of Pharmaceutical Sciences at Kagawa Campus, Tokushima Bunri Univ, Kagawa, Japan)*

We succeeded in the synthesis of eight types of carbocyclic oxetanocin A (COA-Cl) analog: 2-halogenated, aminated or alkoxylated carbocyclic oxetanocin A: COA-F, COA-Br, COA-I, COA-$NH_2$, and COA-OR (R = methyl, ethyl, isopropyl, and *n*-butyl), and six types of various hydroxymethylated or spiro-conjugated cyclobutane rings at the $N^9$-position of the 2-chloropurine moiety of COA-Cl, which were synthesized and evaluated using human umbilical vein endothelial cells. Among these compounds, 100 μM COA-Br, COA-I, and three types of hydroxymethylated cyclobutane rings at the $N^9$-position in the 2-chloropurine moiety of COA-Cl (*cis-trans*-2',3'-bis(hydroxymethyl)cyclobutyl derivative, *trans*-3'-hydroxymethylcyclobutyl analog, and 3',3'-bis(hydroxymethyl)cyclobutyl derivative) had greater angiogenic activity, which were comparable to that of COA-Cl. These data may be important for further development of this class of compounds as potential tube formation agents. (COI:No)

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## S04-4

### Cardioprotection of COA-Cl in myocardial infarction in vivo

Oyama Jun-ichi
*(Dept Advanced Cardiol, Saga University, Saga, Japan)*

Myocardial infarction (MI) is one of the most important reasons for mortality in Japan. Coronary revascularization therapies are most popular treatment and the door-to-balloon time is critical, however, there is a limit to reduce the time and sometimes it did not link to the lower mortality. These unfortunate results underscore the need for improvement of the conventional strategy. Myocardial ischemia causes myocardial necrosis, however, sometimes the development of the collateral vessels which react to the ischemia minimizes the myocardial necrosis. It is important to develop the collateral vessels for the prevention of the excessive damage in MI. However, we do not have the established tool for MI at the present. COA-Cl have the property to induce angiogenesis through VEGF signaling in vitro and in vivo. Therefore, we investigated whether COA-Cl prevent myocardial damage after myocardial infarction through the development of collateral vessels in vivo. The left anterior descending coronary artery was ligated to induce MI in C57/BL6 mice. The mice were divided into the two groups randomly: the group which received COA-Cl and non-treatment group. The mice who were treated with COA-Cl exhibited preserved cardiac function in COA-Cl group evaluated by echocardiography as compared to the control group. Myocardial inflammation including NOS-2 induction was lower in COA-Cl group significantly. We are now evaluating the size of myocardial necrosis and the development of collateral vessels in both groups. In conclusion, COA-Cl can preserve cardiac function after MI and reduce myocardial inflammation in vivo. (COI:No)

## S04-5

### The potential neuroprotective effects of COA-Cl against stroke events

Lu Feng[1]、Okabe Naohiko[1]、Himi Naoyuki[1]、Nakamura-maruyama Emi[1]、Tsukamoto Ikuko[2]、Miyamoto Osamu[1]
*([1]Dept Physiol, Kawasaki Med Sch, Okayama, Japan、[2]Department of Pharmaco-Bio-Informatics, Faculty of Medicine, Kagawa Universi)*

COA-Cl is a novel adenosine analogue which is soluble, stable and easy to synthesize. It has been reported that COA-Cl exerts strong angiogenetic activity in human umbilical endothelial cells, which may be mediated by ERK1/2 activation. As a well-known kinase, ERK1/2 is involved in the over-activated apoptotic process of neurological disorders, including stroke. In the current study, we purpose to investigate the neuroprotective effects of COA-Cl on stroke events. Both rat transient focal cerebral ischemia and intracerebral hemorrhagic models were used for this study. COA-Cl was intracerebroventricularly administrated immediately after model making or 1d delay. On ischemic stroke, COA-Cl reduced the infarct volume, decreased the number of TUNEL positive cells and improved neurological deficits. The level of pERK increased by the administration of COA-Cl in vitro, indicating that the neuroprotective effect of COA-Cl may be mediated by ERK1/2 activation. Delayed continuous administration of COA-Cl also reduced infarct volume and enhanced peri-infarct angiogenesis. On hemorrhagic stroke, the administration of COA-Cl significantly reduced brain edema 1d after ICH and attenuated the sensorimotor deficits. Furthermore, both TUNEL and 8-OHdG positive cells were fewer in COA-Cl treated rats compared with vehicle ones. In conclusion, COA-Cl may exert neuroprotective effects on both ischemic and hemorrhagic stroke, which may be related with its anti-apoptotic and anti-oxidative effects. (COI:No)

# Symposium 5

## DIVERSITY IN PACEMAKING OF SMOOTH MUSCLE ORGANS

March 22 (Tue), 9:00 – 10:30, Room K

## S05-1

### Pacemaker role of pericytes in the microvasculature of visceral organs

Hashitani Hikaru

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In visceral organs that undergo distension upon filling, the microvasculature appear to display intrinsic contractile properties to maintain their flow. Submucosal venules in the bladder generate spontaneous constrictions triggered upon the firing of spontaneous $Ca^{2+}$ transients and transient depolarisations. These events are initiated within venular pericytes upon the spontaneous $Ca^{2+}$ release from sarcoplasmic reticulum (SR) and the opening of $Ca^{2+}$-activated chloride channels that trigger $Ca^{2+}$ influx through L-type voltage-dependent $Ca^{2+}$ channels (VDCC). These L-type VDCC play a critical role in maintaining synchrony within venular pericytes. In the stomach myenteric microvasculature, spontaneous $Ca^{2+}$ transients are evident in both arteriolar smooth muscle cells (SMCs) and capillary pericytes. Spontaneous $Ca^{2+}$ transients initiated in capillary pericytes appear to spread to their neighbouring arteriolar SMCs. Capillary $Ca^{2+}$ transients primarily rely on spontaneous SR $Ca^{2+}$ release, but also require $Ca^{2+}$ influx through T-type VDCC for their synchrony. The opening of T-type VDCC may also contribute to the $Ca^{2+}$ transients that propagate into arteriolar SMCs. Thus, pericytes in the visceral microvasculature may act as the originators of synchronous spontaneous $Ca^{2+}$ transients that regulate contractility of &quotupstream&quot arterioles and &quotdownstream&quot venules so that the microcirculation blood flow meets tissue demand. Pericytes in the microvasculature appear to function as voltage-dependent coupled $Ca^{2+}$ oscillators, their synchrony depending on various VDCC types in a microvasculature bed-dependent manner. (COI:No)

## S05-2

### PACEMAKER MECHANISMS UNDERLYING LYMPHATIC VASOMOTION

Dirk Van_Helden[1], Pierre-Yves Von_der_Weid[1], Mohammad Imtiaz[1]

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Lymphangions, each formed by sequential unidirectional valves in lymphatic vessels pump lymph through rhythmical constrictions of the smooth muscle (SM) in their walls. Such lymphatic vasomotion was originally suggested to occur through a voltage dependent classical heart-like pacemaker mechanism (i.e. membrane clock). However, our detailed studies made on non-perfused lymphatic SM revealed a very different pacemaker mechanism, one driven by the release/refill cycle of intracellular calcium stores (i.e. calcium clock), a pacemaker that has subsequently been shown to underlie activities such as gastrointestinal slow waves, vascular vasomotion and a 2nd heart pacemaker mechanism. Our findings and those recently communicated (1), indicate that under moderate to high perfusion/stretch conditions store inhibition has no effect indicating a classical heart-like pacemaker model is now dominant. In contrast, we find that at low perfusion/stretch conditions inhibition of store uptake or release slows pumping rate. Thus we propose there is a shift in dominance between calcium and membrane clock mechanisms dependent on factors such as pressure/stretch. Activators (e.g. IP3-activating neurotransmitters etc.) will also influence this. We conclude that lymphatic pacemaking occurs through a synergistic interaction between the calcium and membrane clocks, an interaction also functional in the heart.1) Gui et al. (2015) Symposium - Multicellular Inputs in Regulating Muscle Excitability, Reno. (COI:No)

## S05-3

### Regulation of spontaneous contractile activity of the bladder urothelium/lamina propria

Russ Chess-Williams

*(Centre for Urology Research, Bond University)*

Recently the inner epithelial lining of the bladder (urothelium) and its underlying lamina propria have been shown to exhibit spontaneous phasic contractions. On a tissue weight basis, this layer is capable of contractions as great as those of the detrusor smooth muscle. It also has pacemakers which generate spontaneous contractile activity and α1-adrenoceptors and ATP increase activity, whilst β-adrenoceptors inhibit the contractile activity. Stretch of the tissue also stimulates pacemaker activity and this is due to stretch-induced acetylcholine release from the urothelium, which then acts via M3 muscarinic receptors. Electrical field stimulation produces neurogenic increases in spontaneous contractile activity, which is insensitive to cholinergic or adrenergic antagonists and unaffected by P2X receptor desensitisation with αβ-methylene ATP.
Interstitial cells similar to intestinal interstitial cells of Cajal have been identified in the bladder lamina propria and may be responsible for initiating contractions. The physiological function of urothelial/lamina propria contractile activity is uncertain, but it may be responsible for folding of the urothelium in the empty bladder. Also, in the overactive bladder, where gap junctions are increased, the contractile activity may possibly pass to the detrusor muscle causing bladder overactivity.
In conclusion, the urothelium/lamina propria of the bladder possesses pacemakers that generate spontaneous contractile activity. However the transmitter mediating neurogenic responses of this tissue has yet to be identified. (COI:No)

## S05-4

### Role of mucosa in generating spontaneous activity in the guinea pig seminal vesicle

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Seminal vesicles (SVs) expel most of seminal fluid upon sympathetic nerves excitation. However, little is known about its contractile activity during storage phase. We investigated the mechanisms underlying the spontaneous activity in SVs, focusing on the role of the mucosa. Changes in contractility, membrane potential and intracellular $Ca^{2+}$ concentration of smooth muscle cells (SMCs) in the guinea pig SVs were recorded from mucosa-intact and -denuded preparations. Mucosa-intact SVs exhibited periodical spontaneous contractions which were abolished by nifedipine. Removal of the mucosa abolished spontaneous contractions without suppressing neurally-evoked contractions. In mucosa-intact SVs, SMCs generated slow waves (SWs) and spontaneous $Ca^{2+}$ flashes, while mucosa-denuded SMCs remained quiescent. Nifedipine suppressed both SWs and spontaneous $Ca^{2+}$ flashes leaving spontaneous transient depolarizations (STDs) and synchronous spontaneous $Ca^{2+}$ transients. Residual STDs and spontaneous $Ca^{2+}$ transients were abolished by cyclopiazonic acid suggesting that periodical $Ca^{2+}$ release from intracellular stores plays a primary role in generating STDs and the subsequent opening of L-type $Ca^{2+}$ channels to contract SMCs. Unidentified cells in the mucosa of SVs appears to drive spontaneous activity in the SMCs by sending depolarizing signals and/or releasing mucosa-derived humoral factors. (COI:No)

# Symposium 6

Timing regulations in the HPG-axis: interaction among the central-peripheral system.

March 22 (Tue), 9:00 – 10:30, Room D

## S06-1

### Time-lapse analysis of the follicular growth in the cultured ovarian tissue

Komatsu Kouji、Masubuchi Satoru
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The number of oocytes in one ovulation is different among species, however, it is almost same in one species. Ovaries contain numerous follicles of various stages, including primordial, primary, secondary, antral, and Graafian follicles. Therefore, the maintenance of cyclic ovulation necessitates the interaction of follicles and the regulation of follicular growth at the tissue level. To analyze the regulatory mechanism of follicular growth in the ovary, we cultured mouse ovarian tissue slices and attempted a new experimental approach for the simultaneous tracking of the growth and the movement of each follicle in the cultured ovary by time-lapse imaging. Under our culture system, follicular growth, ovulation and follicle atresia were reproduced. In our recent experiments, this culture system and analysis by time-lapse imaging revealed new roles of leukemia inhibitory factor (LIF) and progesterone on the regulation of follicular growth in the ovary. For example, the effects of progesterone on the follicular growth were different depending on the concentration in the culture medium, 100 ng/ml progesterone promoted the growth of primary follicles and the ovulation, on the other hand, 1 μg/ml progesterone suppressed the growth of secondary follicles and the ovulation. This result correlates with the change of the blood concentration of progesterone during the estrus cycle of rodents. In this presentation, we show these results using new ovarian culture system and time-lapse analysis and the possibility of this method in the ovarian research. (COI:No)

## S06-2

### Key Role of Kisspeptin-GPR54 Signaling in Regulation of Mammalian Reproduction

Tsukamura Hiroko、Uenoyama Yoshihisa
*(Grad Sch Bioagricutural Sci, Nagoya Univ, Nagoya, Japan)*

Accumulating evidence suggests that kisspeptin-GPR54 signaling plays a key role in controlling reproductive function via regulating gonadotropin-releasing hormone (GnRH)/gonadotropin release in mammals, including rodents, ruminants, reflex ovulators, and primates. Two modes, surge and pulse, of GnRH/gonadotropin secretion are responsible for ovulation and gametogenesis/steroidogenesis, respectively. Kisspeptin neurons located in the forebrain, such as anteroventral periventricular nucleus (AVPV) and preoptic area (POA), are considered to be responsible for the estrogen-positive feedback action to induce GnRH/gonadotropin surge, and consequently ovulation. On the other hand, kisspeptin neurons in the hypothalamic arcuate nucleus (ARC), in which Kiss1 expression is down-regulated by estrogen, are considered to play a role in regulation of pulsatile release of GnRH/gonadotropin. The present paper focuses on the mechanism mediating the estrogen feedback actions on kisspeptin expression in the brain to understand the mechanism underlying GnRH/gonadotropin surge and pulse generation. This work was supported in part by the Science and Technology Research Promotion Program for Agriculture, Forestry, Fisheries and Food Industry. (COI:No)

## S06-3

### Phase control of the ovarian circadian clock and its function in timed ovulation

Yoshikawa Tomoko[1,2]、Sellix Michael[3]、Jia Shusheng[2,4]、Honma Sato[2]、Honma Ken-ichi[2]、Menaker Michael[5]
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The timing of ovulation is critically important to the success of reproduction. It has been shown that the timing signal from the hypothalamus determines the timing of LH surge in turn induce the timed ovulation. However, discovery of the circadian clock in the mammalian ovaries raised the possibility that ovulation is timed by an interaction between clocks in the suprachiasmatic nucleus (SCN), pituitary and ovary. We first investigated how circadian information is transmitted from the SCN to the ovary. A series of experiments using *Period1-luciferase* transgenic rats revealed that endocrine signals are sufficient to transmit timing cues to the ovary. We next asked if gonadotropins were a candidate for this endocrine signal. LH and FSH phase-shifted the circadian clock of ovarian granulosa cells in vitro, suggesting that gonadotropins function as a timing signal for the ovarian clock. Finally, we asked if the ovarian clock plays a role in determining the timing of ovulation. We found a circadian rhythm of ovarian sensitivity to LH that determines the ovulatory response to gonadotrophins. It is plausible that the circadian clock in the ovary may set the responsiveness of the ovarian follicle to the LH surge. Our results add a new insight to the classic view that gonadotrophins provide the only timing cue for ovulation. (COI:No)

## S06-4

### Suprachiasmatic Nucleus: Effects of Aging

Nakamura Takahiro
*(Lab Animal Physiol, Sch Agri, Meiji Univ, Kawasaki, Japan)*

Circadian timing systems, like most physiological processes, cannot escape the effects of aging. With age, humans experience decreased duration and quality of sleep. While there are undoubtedly many factors contributing to these changes, a body of literature is emerging suggesting that an age-related decline in the central circadian clock in the suprachiasmatic nucleus (SCN) may be a key element responsible. To explore age-related changes in the SCN, we first carried out in vivo multiunit neural activity (MUA) recordings from the SCN of freely-moving young and aged mice. In the result, the amplitude of day-night difference in MUA was significantly reduced in the older mice. However, evidence for age-related disruption of circadian oscillations of clock genes in the SCN has been equivocal. We next performed ex vivo bioluminescent imaging of cultured SCN slices of young and aged PER2::luciferase knock-in (PER2::LUC) mice housed under light-dark (LD) or prolonged constant dark (DD) conditions. Under LD conditions, the amplitude of PER2::LUC rhythms differed only slightly between SCN explants from young and aged animals; under DD conditions, the PER2::LUC rhythms of aged animals showed markedly lower amplitudes and longer circadian periods than those of young animals. Recordings of PER2::LUC rhythms in individual SCN cells using an EM-CCD camera revealed that aged SCN cells showed longer circadian periods and that the rhythms of individual cells rapidly became desynchronized. These data suggest that aging degrades the SCN circadian ensemble, but that recurrent LD cycles mask these effects. (COI:No)

## S06-5

### Recovery from age-related infertility under environmental light-dark cycles adjusted to the intrinsic circadian period

Nakamura Wataru、Takasu Nana
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Female reproductive function changes during aging with the estrous cycle becoming more irregular during the transition to menopause. We found that intermittent shifts of the light-dark cycle disrupted regularity of estrous cycles in middle-aged female mice, whose estrous cycles were regular under unperturbed 24-h light-dark cycles. Although female mice deficient in *Cry1* or *Cry2*, the core components of the molecular circadian clock, exhibited regular estrous cycles during young, they showed accelerated senescence characterized by irregular and unstable estrous cycles and resultant infertility in middle age. Notably, tuning the period length of the environmental light-dark cycles closely to the endogenous one inherent in the Cry-deficient females restored the regularity of the estrous cycles and consequently improved fertility in middle age. These results suggest that reproductive potential can be strongly influenced by age-related changes in the circadian system and normal reproductive functioning can be rescued by manipulation of environmental timing signals. (COI:No)

# Symposium 7

Frontiers in non-invasive, non-labeled methodology for detecting cellular function and environments

March 22 (Tue), 9:00 – 10:30, Room C

## S07-1

### The role of Raman spectroscopy in preventive medicine

Pezzotti Giuseppe[1,2]、Ikegaya Hiroshi[3]、Miyamori Daisuke[3]、Tamamoto Toshiro[4]、Kanamura Narisato[4]、Adachi Tetsuya[4]、Sugano Nobuhiko[5]、Zhu Wenliang[5]、Takahashi Yasuhito[6]、Yamamoto Kengo[6]、Puppulin Leonardo[2]、Yoshinori Marunaka[2]

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Raman spectroscopy screens molecular vibrations and discriminates between healthy and diseased tissues through spectroscopic fingerprints attached to histological tissue characteristics. It possesses potential in locating physiological alterations and facilitat-ing diagnosis before disease manifestation. We have constructed Raman algorithms suiting clinical applications in dentistry, orthopedics, forensic science, and cell physi-ology, and now continue to expand this practice toward new medical applications. Here, some of our recent findings are presented, including assessments of osteoporot-ic bone, tooth enamel with incipient (but yet invisible) decay, cartilage degradation in human joints, protein folding, and lipid crystallization in human skin. Avoiding un-necessary biopsies, Raman spectroscopy improves diagnostic approaches and contrib-utes to reduce the economic burden associated with modern healthcare. (COI:No)

## S07-2

### New method based on Raman spectroscopy to detect in-situ concentrations of HCO3-, Na+ and K+ from physiological relevant solutions.

Puppulin Leonardo[1]、Pezzotti Giuseppe[1,2,3]、Hosogi Shigekuni[1,4]、Nakahari Takashi[1,4]、Marunaka Yoshinori[1,4]

*([1]Dept Cells Physiol, Grad Sch Med Sci, Kyoto Pre Med Univ, Kyoto, Japan 、[2]Dept Ceramic Phys Lab, Kyoto Inst Tech, Kyoto, Japan、[3]Dept Med Eng Treat Bone Joint Dis, Osaka Univ, Osaka, Japan、[4]Dept Bio-Ionomics, Grad Sch Med Sci, Kyoto Pre Med Univ, Kyoto, Japan )*

Accurately and non-destructively measuring the concentration of ions in biological environments can certainly be considered as a pivotal and challenging task in a number of scientific fields, spanning from cell physiology to biomaterials science. In the present research study, we developed a new method based on Raman spectroscopy to measure ionic concentrations in physiologically relevant solutions. The effect of bicarbonate (HCO3-) and cations (Na+, K+) solvated in water were revisited according to high spectrally resolved Raman measurements, which led to unfold phenomenological correlations between the ionic concentrations and the intensity of selected Raman bands. The sensitivity of this spectroscopic technique to detect variations of ions in the order of the tens of mM was proved by measuring concentration gradients developed at the solid/liquid interface of ionic solutions interacting with Si3N4 bioceramic surfaces. (COI:No)

## S07-3

### Noninvasive analysis of cardiac function of inside shells by using MRI and photoplethysmography

Seo Yoshiteru

*(Dept Regul Physiol, Dokkyo Med Univ Schl of Med, Tochigi, Japan)*

I am looking for an animal system that might provide insight how $H_2S$ work as EDRF (endothelium-derived relaxing factor), and picked up *Bathymodiolus* mussels live in deep-sea and depend on symbiotic chemosynthetic bacteria and have a tolerance to $H_2S$ toxicity. However, we have little knowledge of cardiovascular function of mussels. In order to take basic knowledge, cardiac function of the *Mytilus galloprovincialis* was analyzed by 7 T magnetic resonance imaging (MRI). The heart beats were imaged by retrospectively self-gated fast-low-angle-shot sequences and $T_1$-weighted gradient-echo imaging ($T_{1w}$-MRI). Flow of hemolymph was detected by phase-contrast gradient-echo imaging. Based on these results, in addition to the original constant-volume hypothesis (i.e. the total volume of the heart is constant, and ventricle contraction causes a negative pressure in the pericardium), we concluded that i) the negative pressure maintains venous return from the vein to the auricle, and ii) the pressure difference between the auricle and the pericardium drives haemolymph filtration through the auricle walls. Infrared photoplethysmography (IRP) wave was detected by using an infrared reflective sensor fixed on the shell above the heart. IRP wave and $T_{1w}$-MRI were observed simultaneously. As a result, we could confirm relationships between peaks of the IRP wave and the cardiac cycle. Therefore, we can measure not only short-time reaction by MRI, but also can observe long-term behavior by IRP wave. New findings obtained from *Mytilus* and *Bathymodiolus* mussels by using these 2 techniques will present in the meeting. (COI:No)

## S07-4

### Noninvasive measurements of dynamic human brain temperature changes by using magnetic resonance spectroscopy

Yoshioka Yoshichika[1,2]

*([1]Immunol Frontier Res Center (IFReC), Osaka Univ, Suita, Japan、[2]Center for Information & Neural Networks (CiNet), NICT & Osaka Univ, Suita, Japan)*

Temperature and pH are basic physical and chemical quantities for physiological functions. However, the information of these quantities at deep regions of normal human body such as brain, liver and muscles are very scant. We could not say whether our brain temperatures rise or not when we have a fever. fMRI and NIRS show the dynamical fluctuations of regional brain blood flow in association with brain activities. The brain temperature might change by the fluctuations of brain blood flow and of brain tissue heat productions with brain activities. Trials to visualize the body temperatures have been done by magnetic resonance imaging (MRI) and magnetic resonance spectroscopy (MRS). We have succeeded in the visualization of human brain temperature distribution and also in the detection of dynamical brain temperature changes during physiological tasks and with some other maneuvers by MRS. We could show the brain temperature rose when a volunteer caught a cold. We found regional brain temperatures change easily by about 0.5°C during drinking. Regional brain temperatures fell during light tasks such as hand grasp and sedation. On the other hand, the temperature rose by tasks like knee flexion. MRS could provide the noninvasive method to measure and to monitor the temperatures at deep regions of human body. (COI: No)

# Symposium 8

Ion Channels and Cell Function: a Compass for Incurable Diseases

March 22 (Tue), 9:00 – 10:30, Room J

## S08-1

### CFTR and AQP: Two channels deeply involved into incurable diseases

Sohma Yoshiro

*(Dept Pharmacol, Keio Univ Sch Med, Tokyo, Japan)*

Cystic Fibrosis Transmembrane conductance Regulator (CFTR), a unique member of ABC transporter superfamily, functions as an ATP-dependent anion channel after PKA-dependent phosphorylation of a regulatory domain unique to CFTR. CFTR is expressed primarily in epithelial cells and its dysfunction causes Cystic fibrosis (CF), a life-shortening hereditary disease mainly afflicting white Caucasians through insufficient exocrine. On the other hand, we recently reported that CFTR was expressed in pancreatic B-cells and its dysfunction induced diabetes mellitus [1], which suggested that a group of diabetes patients might be caused by acquired CFTR dysfunctions beyond the race.

There are cases where channels are involved in the establishment of diseases beside their dysfunctions. Water channels named aquaporin (AQP) are expressed in various organs and play an important role in water homeostasis. Aquaporin-4 (AQP4) dominantly expressed in astrocytes and contributing blood-brain barrier, has been identified as an autoimmune target in Neuromyelitis optica (NMO) is an inflammatory demyelinating disease, leading to paralysis and blindness. Recently we have succeeded to directly visualize dynamic binding process of single pathogenic autoantibodies to their self-antigen human AQP4 in real time using high speed- atomic force microscopy.

In the symposium, I will introduce the latest findings for the establishment of incurable diseases, CF and NMO, which CFTR and AQP are deeply involved.

[1] Guo JH,,, Sohma Y, Chan HC. Glucose-induced electrical a ctivities and insulin secretion in pancreatic islet B -cells are modulated by CFTR. Nat Commun. 15;5: 4420, 2014. (COI:No)

## S08-2

### Behaviors of the voltage-gated $H^+$ channels under pH disturbances in osteoclasts

Kuno Miyuki

*(Dept Physiol, Osaka City Univ Grad Sch Med, Osaka, Japan)*

Voltage-gated proton ($H^+$) channels are highly proton-selective and the primary determinant for the activation threshold voltage and the current amplitude is the transmembrane pH gradients. Once activated, the high output ($H^+$ secretion) will compensate for the large pH and voltage imbalances across the membranes and, furthermore, it might disrupt the pH homeostasis by extracellular acidification or intracellular alkalization. Both functional/protective and adverse effects of the $H^+$ channels have been reported: the $H^+$ channels are often activated under pathological conditions, like cell acidosis, cell swelling, elevation of temperature, and with innate immunity. The ambient pH is probably a most essential regulator for the $H^+$ channels, but the pH disturbances could be generated in various ways. Here the behaviors of the $H^+$ channels under different pH disturbances were investigated in osteoclasts, bone-resorbing cells. The $H^+$ channel currents were increased by a metabolic acid (butyrate), although the amplitudes were declined gradually during the exposures. On the other hand, an inorganic acid (HCl) decreased the $H^+$ currents, but its removal increased the amplitudes as twice as the control level. Intracellular alkalization by either $NH_4Cl$ or the transmembrane $H^+$ effluxes through V-ATPases and $Na^+$-$H^+$ exchangers not only decreased the driving force for $H^+$ efflux but also facilitated endocytic internalization of the channels available at the plasma membrane. Behaviors of the $H^+$ channels under pH disturbances are likely to be modified differently by the accompanying secondary mechanisms. (COI:No).

## S08-3

### Daikenchuto ameliorates fibrotic stenosis by activating myofibroblast TRPA1 channel

Kurahara Lin[1]、Hiraishi Keizo[1]、Hu Yaopeng[1]、Aoyagi Kunihiko[2]、Inoue Ryuji[1]

*([1]Dept Physiol, Grad Sch Med,Fukuoka Univ, Fukuoka, Japan、[2]Dept Gastroenterol, Grad Sch Med,Fukuoka Univ, Fukuoka, Japan)*

Background---Daikenchuto (DKT) is a traditional oriental herbal medicine, often used to mitigate post-operative ileus and constipation. We explored the anti- fibrotic effects of DKT on signal transduction in intestinal myofibroblast in its association with transient receptor potential ankyrin 1 (TRPA1) channel. Results---In InMyoFib, TRPA1 was expressed at the highest level among TRP family. Active ingredients of DKT, i.e. hydroxy α-sanshool and 6-shogaol can induce Ca2+ influx in InMyoFib, which was antagonized by co-treatment with selective TRPA1 channel blocker HC-030031. DKT ameliorated TGF-β1 induced expression of Type 1 collagen, α-SMA, and N-Cadherin and this was accompanied by the reduction of the phosphorylation of Smad-2, p38-MAPK and of the expression of myocardin, a well-known transcription factor regulating fibrosis signaling at the downstream of TGF-β1 receptor. Importantly, 24-hour incubation with 100μg/ml Japanese Pepper increased the mRNA and protein expressions of TRPA1, which acted as a negative feedback regulator of collagen synthesis in InMyoFibs. In stenotic regions of CD patient's intestine, TRPA1 expression was significantly increased.Conclusions---DKT-induced expression and activation of TRPA1 could be important mechanisms for suppressing intestinal fibrosis. Thus, targeting myofibroblast TRPA1 may serve as a promising therapeutic strategy for fibrotic stenotic changes, which would in part account for the reported beneficial actions of DKT on inflamed intestines. (COI: No)

## S08-4

### CALHM1 and CALHM3 are assembled to form a novel voltage-gated ATP channel

Taruno Akiyuki[1]、Miyazaki Hiroaki[1]、Niisato Naomi[1,3]、Sun Hongxin[1]、Kashio Makiko[1]、Marunaka Yoshinori[1,2]

*([1]Dept Mol Cell Physiol, Kyoto Pref Univ Med, Kyoto, Japan、[2]Dept Bio-Ionomics, Kyoto Pref Univ Med, Kyoto, Japan、[3]Health Sports Sci, Kyoto Gakuen Univ, Kyoto, Japan)*

*CALHM1* encoding a subunit of a voltage-gated ATP-permeable channel was discovered as a susceptibility gene for Alzheimer's disease. In the tongue, CALHM1 plays an essential role in taste perception by mediating the release of ATP, the neurotransmitter conveying taste information, from taste cells lacking synapses to the gustatory nerves. Meanwhile, it was suggested that CALHM1 is an essential but not a sole component for the ATP release channel in taste cells. Heterologous CALHM1 currents show slower activation kinetics compared to CALHM1-dependent currents in taste cells. Here we show that CALHM1 and CALHM3, a CALHM1 homolog expressed in taste buds, are assembled to form a novel voltage-gated ATP channel with functional properties distinct from those of CALHM1 channel. When co-expressed, CALHM1 was co-immunoprecipitated with CALHM3 and vice versa. Non-denaturing BN-PAGE demonstrated that CALHM3 was recruited into a protein complex containing CALHM1. Co-expression of CALHM1 and CALHM3 gave rise to a novel membrane conductance with a more negative $V_{50}$ value and faster activation and deactivation kinetics than CALHM1 channel, while CALHM3 alone does not generate a conductance. Furthermore, ATP release assay demonstrated that the CALHM1/CALHM3 channel was permeable to ATP. Collectively, the CALHM1/CALHM3 channel is possibly a bona fide mediator of physiological extracellular purinergic signaling including neurotransmission of taste. (COI:No)

## S08-5

### Roles of Mast Cells in Renal and Peritoneal Fibrosis in Chronic Kidney Disease and the Therapeutic Usefulness of Mast Cell Stabilizers

Kazama Itsuro

*(Dept Physiol I, Tohoku Univ Grad Sch Med, Sendai, Japan)*

We previously demonstrated that the overexpression of Kv1.3-channels in kidney lymphocytes facilitates the progression of renal fibrosis in chronic kidney disease (CKD) by promoting lymphocyte proliferation and the over-activation of cellular immunity. Recent studies revealed that mast cells, which are also of hematopoietic origin, produce fibroblast growth factors during chronic inflammation and facilitate the progression of organ fibrosis. Using rat models of chronic renal failure (CRF), we have recently demonstrated that mast cells proliferate in situ within the fibrotic peritoneum and increase their activity to produce fibroblast-activating factors. Since treatment with tranilast, a potent mast cell-stabilizer, actually ameliorated the progression of peritoneal fibrosis, mast cells were thought to be responsible for the progression of peritoneal fibrosis. In previous patch-clamp studies, the exocytotic process in mast cells was electrophysiologically detected by the changes in membrane capacitance (Cm). In our recent study using rat peritoneal mast cells, an anti-allergic drug, olopatadine, suppressed the increase in the Cm and directly inhibited the exocytotic process, indicating its high potency as a mast cell stabilizer. Morphologically, this drug induced inward membrane bending and counteracted the cellular surface deformation induced by exocytosis. Together with our in vivo evidence, the studies suggested the usefulness of targeting mast cells in the treatment of organ fibrosis with chronic diseases (COI: No).

## S08-6

### Roles of basolateral TASK2 in the kidney proximal tubule for the acid-base balance: Lessons from TASK2 KO mice

Kawahara Katsumasa、Yasuoka Yukiko
*(Dept Physiol, Kitasato U Sch Med, Tokyo, Japan)*

Potassium channels, ubiquitously expressed in the plant and animal cells, are involved in the maintenance of fundamental cell properties, such as the cell volume and resting cell membrane potential. TASK2 is a member of the two-pore domain K+ channel (K2P channel) family and is expressed in various epithelial tissues, including pancreas, intestine, and kidney (Reyes et al, 1998). Recently, TASK2 knockout animals were established to show plasma electrolyte patterns typical of the human clinical condition of renal tubular acidosis type II (Warth et al, 2004). However, its pathophysiological mechanisms are still unsolved. Our major findings in TASK2 KO and wild-type mice (KO and WT, respectively) are (1) metabolic acidosis without increase in NH3/NH4+ production (pH7.27 in KO (n=6) vs. 7.36 in WT (n=5), (2) no significant difference in urinary NH3/NH4+ excretion between the two: 0.5 (KO) vs. 0.7 mg/mg Cre (WT)), (3) similarly increased urinary excretion of NH3/NH4+ during a 6-days period of HCl loading (21.8 (KO) vs. 16.9 mg/mg Cre (WT)), (4) decreased urine pH in mice with HCl loading, but is not significantly different between the two (pH5.78 (KO) vs. 5.82 (WT)). (5) Immunostaining of carbonic anhydrase type II (CAII) in PCT (proximal convoluted tubule) and IC-A (type-A intercalated cell) of collecting ducts [PCT < IC-A in control] similarly increased when mice were treated with HCl-containing diet (6 d). Thus, we prefer the hypothesis of "pH sensing function of TASK2" to that of "driving force supplier" at the basolateral membrane of the mouse kidney PCT. (COI: No)

## S08-7

### Molecular mechanism for NGF-induced endocytosis of TASK1 channels in rat adrenal medullary cells and PC12 cells

Matsuoka Hidetada、Inoue Masumi
*(Dept Cell and Systems Physiol, Sch Med, UOEH, Fukuoka, Japan)*

TASK channels belong to a family of two-pore domain K+ channels, which produce background K+ currents, and are involved in important physiological functions, such as acidosis detection. We have recently elucidated that TASK1-like channels function as a sensor of acidosis in rat adrenal medullary (AM) cells and thus are indispensable for the endocrine function of AM cells. Here, we studied how the localization of TASK1 channels is regulated in rat AM cells and PC12 cells in response to NGF. Pharmacological and biochemical analyses revealed that TASK1 channels in rat AM and PC12 cells were internalized in a clathrin-dependent manner in response to NGF. This internalization was triggered by binding of AP-2, an adaptor protein for clathrin, to a dileucine motif (LL263/264). The combination of enzyme inhibitors and TrkA mutants revealed the involvement of both phospholipase C and PI3-kinase pathways that converged on PKC with the consequent activation of the non-receptor tyrosine kinase, Src. The mutation analysis of TASK1 channel showed that tyrosine 370 was phosphorylated by Src. Furthermore, proximity ligation assay demonstrated that Src was transiently co-localized with the TASK1 channel but not the TASK1 mutant (Y370F) in response to NGF. Consistent with this result, NGF-induced internalization was markedly diminished in the TASK1 mutant. From these results, we concluded that NGF induces clathrin-dependent endocytosis of TASK1 channels via activating Src with the consequent phosphorylation of tyrosine 370. (COI:No)

# Symposium 9

Signaling pathways regulating vascular development

March 23 (Wed), 15:00 – 16:30, Room J

## S09-1

### The role of accessory protein for heterotrimeric G-protein in vascular formation

Hayashi Hisaki[1]、Mamun Abudullah[1]、Sakima Miho[1]、Takeyama Masayuki[2]、Zako Masahiro[2]、Yagasaki Rina[3]、Nakahara Tsutomu[3]、Sato Motohiko[1]

*([1]Dept Physiol, Aichi Med Univ, Nagakute, Japan、[2]Dept Ophthalmology, Aichi Med Univ, Nagakute, Japan、[3]Dept. Mol. Pharmacol., Kitasato Univ. Sch. Pharmacol., Tokyo, Japan)*

Activator of G-protein signaling 8 (AGS8) is a molecule that directly binds Gβγ subunit of heterotrimeric G-protein. We have recently shown that AGS8 played pivotal roles in VEGF-induced angiogenic events in vitro; tube formation, cell proliferation and migration. Here, we further analyzed the mechanism how AGS8 regulates VEGF-induced angiogenesis. Knockdown of AGS8 by siRNA inhibited VEGF-induced phosphorylation of VEGF receptor type2 (VEGFR-2) in endothelial cells. AGS8 reduced VEGFR-2 on the cell surface, but increased VEFGR-2 in cytoplasmic organelles. The decrease of cell-surface VEGFR-2 was not rescued by endocytosis blockers, suggesting involvement of AGS8 in receptor sorting to the membrane. VEGFR-2 formed protein complex with AGS8-Gβγ in cell. Synthetic peptide, which interfered AGS8-Gβγ interaction, disrupted VEGFR-2-AGS8-Gβγ complex, and inhibited VEGF-induced tube formation of endothelial cells. Role of AGS8 was further analyzed in vitro with laser-induced choroidal neovascularization model of mouse. AGS8 mRNA was upregulated in the choroid after irradiation. Immunofluorescence study indicated that AGS8 protein was expressed in the newly formed vessels, but not in the preexisting ones. Altogether, these results suggest that AGS8-Gβγ regulates VEGF signaling via trafficking of VEGFR-2 from cytoplasm to the cell membrane, and that AGS8-Gβγ plays important roles in angiogenic events. (COI:No)

## S09-2

### PI3K-C2α is required for TGFβ-induced receptor endocytosis and endosomal signaling in endothelial cells

Aki Sho[1]、Yoshioka Kazuaki[1]、Okamoto Yasuo[1]、Takuwa Noriko[2]、Takuwa Yoh[1]

*([1]Dept Physiol, Sch Med, Kanazawa Univ, Kanazawa, Japan、[2]Dept Health and Medical Sciences, Ishikawa Prefectural Nursing Univ, Kahoku, Japan)*

The phosphatidylinositol (PtdIns) 3-kinases (PI3Ks) are a family of enzymes that phosphorylate membrane inositol phospholipids at the 3 position of the inositol ring and comprise three classes. In contrast to the well characterized class I and class III PI3Ks, physiological roles of class II PI3Ks were not well understood. We have recently demonstrated that class II α isoform (PI3K-C2α plays crucial roles in angiogenesis, by analyzing PI3K-C2α KO mice. The major angiogenic factor TGFβ signals via the serine/threonine kinase receptor to induce phosphorylation of Smad2/3 and their nuclear translocation. PI3K-C2α knockdown nearly completely abolished TGFβ-induced phosphorylation and nuclear translocation of Smad2/3 in EC. PI3K-C2α was necessary for TGFβ-induced increase in PtdIns(3,4,)$P_2$ in the plasma membrane and TGFβ receptor internalization into the early endosomes containing SARA, which is required for Smad2/3 phosphorylation, but not for PtdIns(3)P enrichment or localization of SARA in the early endosomes. TGFβ stimulated Smad-dependent VEGF-A expression, VEGF receptor-mediated EC migration and tube formation, which were all abolished by either PI3K-C2α knockdown or a dynamin inhibitor. Finally, TGFβ-induced microvessel formation in Matrigel plugs was attenuated in EC-specific PI3K-C2α-deleted mice. These observations indicate that PI3K-C2α plays the pivotal role in TGFβ receptor endocytosis and thereby Smad2/3 signaling, participating in angiogenic actions of TGFβ. (COI:No)

## S09-3

### Novel adhesion molecule, Ninjurin1 for capillary formation through endothelial-pericytes interactions

Kawabe Jun-ichi

*(Dept CVRI, Internal Med, Asahikawa Med Univ, Asahikawa, Japan)*

Pericytes (PCs), the mural cells of capillary microvessels, play an important role in the formation and maintenance of microvessels. However, little is known about the mechanisms of how PCs regulate angiogenesis. To identify factors that modulate their angiogenesis functions, genes whose levels in PCs were altered during neovessel formation were sorted through a microarray screening. Finally, Ninjurin1 (Ninj1) was selected as a candidate molecule for angiogenesis regulation. Ninj1 was expressed at a higher level in PCs , and also in endothelial cells (ECs). Isolated PCs induced the EC-growth and formation of immature EC-tubes through their production of angiogenic growth factors. This trophic effect was enhanced by siRNA-mediated knock-down (KD) of Ninj1, and conversely reduced by overexpression of Ninj1 in PCs. When PCs and ECs were co-incubated in a 3D-gel culture, PCs was wrapped around EC-tubes to form capillary-like structure. Adhesion between ECs and PCs, and formation of capillary-like vessels were reduced by Ninj1-KD in either PCs or ECs. We examined the role of Ninj1 in pathophysiological angiogenesis using mouse hind limb ischemia model. When expression of Ninj1 was reduced by biodegradable microbeads containing Ninj1-siRNA, the formation of functional microvessels and blood flow recovery in ischemic tissues were decreased. In conclusion, Ninj1 acts as adhesion molecule to association between ECs and PCs, and induce the vascular maturation. Ninj1 plays an important role for formation of functional matured vessels in skeletal muscle to recovery from ischemia. (COI:No)

## S09-4

### The roles of phospholipase C and cyclic AMP in regulation of extracellular matrix in vascular smooth muscle cells

Yokoyama Utako、Yanai Chiharu、Ishikawa Yoshihiro

*(Cardiovascular Research Inst., Yokohama City Univ.)*

Background: Intimal thickening (IT) is required for physiological vascular remodeling in the fetus and pathological remodeling in the adult period. During fetal period, IT is critical for normal neonatal closure of the ductus arteriosus (DA) which is a bypass artery between the aorta and pulmonary arteries. Since we have reported that prostaglandin E2 ($PGE_2$)-EP4 signaling plays a critical role in IT of the DA, we hypothesized that $PGE_2$-EP4 signaling promoted both physiological and pathological vascular remodeling.Methods and Results: We performed microarray analysis using rat DA smooth muscle cells (rDASMCs) stimulated with EP4 agonist, and found that EP4 stimulation increased fibulin-1 (FBLN1), extracellular matrix glycoprotein, which was confirmed by qRT-PCR (270-fold, $p<0.01$). Inhibition of protein kinase A or phospholipase C (PLC) using H-89 or D609 decreased EP4-induced FBLN1 mRNA in rDASMCs (0.4-and 0.4-fold, respectively, $p<0.01$). Immunohistochemistry revealed that FBLN1 was localized at IT of both human and rat DA. Silencing of FBLN1 using siRNAs attenuated EP4-induced DASMC migration (0.6-fold, $p<0.01$). Next, to examine the role of EP4-FBLN1 in the adult vessels, we created vascular injury in EP4-deficient mice. FBLN1 was highly expressed in IT in wild-type mice. Formation of IT was significantly inhibited in EP4-deficient mice (0.3-fold, $p<0.01$). Furthermore, EP4 antagonist inhibited IT and FBLN1 expression in vivo.Conclusion: $PGE_2$-EP4-mediated cAMP and PLC signaling promotes IT via up-regulation of FBLN1 in both fetal and adult vessels. (COI:No)

# Symposium 10

Physiological Properties of Neural Circuits underlying Awake-Sleep States

March 24 (Thu), 15:00 – 16:30, Room E

## S10-1

### Cortical Top-Down inputs during sleep consolidates perceptual memory in mice

Murayama Masanori

*(Lab for Behavioral Neurophysiology, BSI, RIKEN)*

It is hypothesized that the internal (i.e. top-down) inputs have crucial roles for memory consolidation, however this has never been tested before. Here we studied when and how bottom-up and top-down cortical circuits contribute to tactile or visual recognition memory in freely moving mice. We applied optogenetic pathway-specific silencing technique to cortical bidirectional circuits between the primary somatosensory cortex (S1) and the secondary motor cortex (M2), a higher order frontal region. Silencing either the bottom-up (S1 to M2) or top-down (M2 to S1) pathway during the learning or retrieval phase impaired novelty discrimination in a tactile recognition test, while not affecting visual recognition memory. This data suggests that online tactile processing includes cortical top-down and bottom-up processing. Next, we applied closed-loop optogenetic stimulation to silence cortical circuits in a sleep state-specific fashion. During post-learning slow wave sleep (SWS), silencing the top-down but not the bottom-up pathway impaired the consolidation of tactile recognition memory. This top-down pathway silencing during post-learning SWS impaired the slow oscillation flow in top-down direction. Furthermore, the pathway silencing impaired memory reactivation in S1. These data reveal that slow oscillation flow in top-down direction is essential contributors to memory reactivation and consolidation. Take all data together, while the cortical bottom-up and top-down bidirectional pathways cooperate for online processing of sensory information, cortical top-down pathway unidirectionally contributes to memory consolidation during SWS. (COI:No)

## S10-2

### Circadian system regulating sleep-wake rhythms: Analysis using luciferase reporter for clock gene

Honma Sato[1]、Yoshikawa Tomoko[2]、Natsubori Akiyo[3]、Honma Ken-ichi[1]

*([1]Dept.Chronomedicine, Hokkaido Univ. Grad Sch. Med, Sapporo, Japan、[2]Photonic Bioimag, Res Center Coop. Projects, Hokkaido Univ. Grad Sch. Med, Sapporo, Japan、[3]Dept. Neuropsy., Sch.Med, Keio Univ., Tokyo, Japan)*

In mammals, circadian rhythms are regulated by a multi-oscillator system composed of the central clock in the hypothalamic suprachiasmatic nucleus (SCN) and peripheral clocks throughout the body. A molecular feedback loop involving clock genes and their protein products is regarded as an intracellular molecular machinery for circadian rhythm generation. In human subjects under temporal isolation, sleep-wake rhythm often desynchronizes from circadian rhythms of melatonin and core body temperature, suggesting the regulation by another oscillator than the SCN. But the site or molecular mechanism of this extra-SCN oscillator remains to be studied.

We found that chronic methamphetamine (MAP) treatment induced an internal desynchronization of sleep-wake rhythm from the SCN rhythm in rats and mice. Since the MAP-induced rhythms share major characteristics of human sleep-wake rhythm, we examined its brain mechanism by using a luciferase reporter system for clock gene Per2. By chronic MAP treatment, Per2 expression rhythms in several dopaminergic areas shifted in area-specific manner, suggesting that the circadian oscillators of these areas re-organize to form a complex oscillator which desynchronized the sleep-wake rhythms from the SCN. A novel macro-imaging of clock gene expression rhythms further demonstrated the relation among SCN and extra SCN circadian oscillators (COI:No)

## S10-3

### Regulation of sleep/wakefulness and memory by peptidergic neurons in the hypothalamus

Yamanaka Akihiro

*(Dept. Neurotic II, Res Ins Env Med, Nagoya Univ)*

Instinctive behaviors, such as feeding and drinking behaviors, sleep/wakefulness and sexual behavior, are important behaviors to survive or to conserve species. These behaviors are regulated by the neurons in the hypothalamus. Some neurons in the hypothalamus contain neuropeptides and release them as a neurotransmitter. Recent studies revealed that these neuropeptides have a crucial role in the regulation of instinctive behaviors. Orexin and melanin concentrating hormone (MCH) is one of these neuropeptide. Recently we found that MCH neurons are involved in state switching from non-REM sleep to REM sleep. To study the role of MCH neurons in sleep/wakefulness, MCH neurons were specifically ablated by expressing diphtheria toxin A fragment (DTA). These MCH neurons ablated mice showed increased time in wakefulness and decreased time in non-REM sleep. Histochemical study indicates that MCH neurons densely project to the hippocampus and MCH receptor is expressed in the hippocampus. Thus we tested memory of MCH ablated mice using novel object recognition test. Interestingly, these MCH neurons ablated mice showed significantly increased memory compare with control mice. This result suggests that the activity of MCH neurons during non-REM sleep and REM sleep is involved in memory regulation. (COI:No)

## S10-4

### Cortical nNOS/NK1 Neurons Link Homeostatic Sleep Drive to EEG Slow Wave Activity

Thomas Kilduff

*(Center for Neuroscience, Biosciences Division, SRI International, Menlo Park, CA USA)*

Type 1 cortical nNOS neurons are rare GABAergic interneurons that have distinctive properties: they coexpress the Substance P receptor NK1; they receive subcortical inputs from cholinergic and serotonergic neurons; and they send long-range projections. Whereas the majority of cortical neurons express Fos during waking, cortical nNOS neurons express Fos during sleep. When homeostatic sleep drive is manipulated by varying the duration of prior wake, nNOS KO mice showed a greatly diminished homeostatic response to sleep deprivation (SD). However, it was unclear whether cortical nNOS/NK1 neurons are activated throughout NREM sleep or whether their activation is related to sleep pressure accumulating during prior wake. Therefore, we used hypnotic drugs to control the amount of NREM sleep while we varied prior wake duration and the resultant sleep pressure. We found that the proportion of Fos+ cortical nNOS/NK1 neurons was minimal when sleep pressure was low, irrespective of the amount of time spent in NREM. In contrast, a large proportion of cortical nNOS/NK1 neurons was Fos+ when an equivalent amount of sleep was preceded by SD. Thus, although sleep is necessary for cortical nNOS/NK1 neuron activation, the proportion of cells activated is dependent upon the duration of prior wake and the resultant sleep pressure. We conclude that cortical nNOS/NK1 neurons monitor substances that accumulate in the brain during wakefulness. Cortical nNOS neurons appear to be critical integrators in the neuronal network linking state-dependent afferent inputs, homeostatic sleep drive and EEG slow wave activity. (COI:No)

# Symposium 11

Involvements of TRP channels in appetite and energy metabolism

March 22 (Tue), 16:00 – 17:30, Room J

## S11-1

### Gastric hormones regulate insulin release via TRPM2 signaling in islet β-cells

Dezaki Katsuya、Kurashina Tomoyuki、Yada Toshihiko
*(Dept Physiol, Grad Sch Med, Jichi Med Univ, Tochigi, Japan)*

Glucose-stimulated insulin secretion in β-cells is initiated by closure of the ATP-sensitive K+ (KATP) channel, followed by plasma membrane depolarization. In this process, opening of background inward current through non-selective cation channels (NSCCs) might facilitate depolarization after KATP channel closure. It has been reported that the TRPM2 channel, a type of NSCC, in β-cells plays an essential role in insulin secretion. Both glucose metabolism and glucagon-like peptide-1 (GLP-1), an insulin releasing gastric hormone, increase the activity of TRPM2 channels via the cAMP signaling. We further uncovered a novel role of TRPM2 channels in signaling for ghrelin, an insulin inhibiting hormone, in β-cells. Ghrelin markedly counteracted the glucose (8.3 mM)-induced activation of TRPM2 current in islet β-cells. Furthermore, in islets from TRPM2-KO mice, ghrelin failed to attenuate glucose-induced insulin release. These results suggest that ghrelin suppresses glucose-induced insulin secretion by inhibiting TRPM2 channels. Therefore, GLP-1 and ghrelin have reciprocal actions on the cAMP level and TRPM2 channel activity in islet β-cells. GLP-1 and ghrelin are released in a reciprocal pattern: upon meals, plasma level of GLP-1 rises and that of ghrelin falls, which may collaborate to effectively elevate cAMP and activate TRPM2 channels in β-cells, leading to rapid and efficient insulin release for the fine postprandial glucose disposal. (COI:No)

## S11-2

### Thermosensitive TRP channel agonists stimulate brown fat thermogenesis in humans

Yoneshiro Takeshi
*(Dept Biomed Sci, Grad Sch Vet Med, Hokkaido Univ, Sapporo, Japan)*

Obesity is serious health hazards for mortality but has globally increased. Brown adipose tissue (BAT) is a potential target to combat obesity because it contributes to the regulation of energy expenditure (EE) including cold-induced non-shivering thermogenesis. We reported previously that repeated cold exposure elicits a parallel increase in BAT mass and EE and a concomitant decrease in body fatness in humans, but it would seem inapplicable to increase exposure to cold in daily life. It is thus important to explore novel and practical ways for the BAT recruitment. Cold-induced BAT activation through the sympathetic nervous system (SNS) is initiated by the activation of thermosensitive transient receptor potential (TRP) channels in the peripheral tissues. Animal studies have demonstrated that the TRP-SNS-BAT axis can be activated by some food ingredients such as capsaicin, its analogs (capsinoids), and green tea catechins, all of which have agonistic activity at TRPV1 and/or TRPA1 expressing in the sensory nerves of the gastrointestinal tract. Actually, we found in healthy human subjects that a single oral ingestetion of either capsinoids or catechins activated BAT and increased EE even in a thermoneutral room at 27°C. Chronic treatment with capsinoids or catechins resulted in a significant increase in BAT thermogenic capacities. These findings indicate that food ingredients activating thermosensitive TRP channels activate and recruit BAT in humans, thereby increasing whole-body EE. (COI:No)

## S11-3

### Peripheral administration of TRPA1 agonists increase food intake: involvement of the novel subpopulation of vagal afferents with orexigenic function

Iwasaki Yusaku、Yada Toshihiko
*(Dept Physiol, Jichi Med Univ, Shimotsuke, Japan)*

Spices added in the meal are experientially used to stimulate appetite. However, their functional ingredients and action mechanisms are not revealed. Many spices have the agonistic activity for transient receptor potential ankyrin 1 (TRPA1). Vagal afferents innervate almost all peripheral organs, and sense the peripheral energy state and convey them to the brain to regulate feeding. Thus, we hypothesized that TRPA1 agonists exert their orexigenic action via directly interacting with vagal afferents. We examined whether a subpopulation of vagal afferent neurons is activated by TRPA1 agonists, allyl isothiocyanate (AITC) from wasabi and dially trisulfide (DATS) from garlic. AITC and DATS increased cytosolic $Ca^{2+}$ concentration in 40% of single nodose ganglion (NG) neurons isolated from mouse. Cholecystokinin (CCK), a satiety hormone, activates approximately 40% of vagal afferents. We found that the majority (70%) of TRPA1 agonist-responsive NG neurons are unresponsive to CCK, suggesting that TRPA1 agonist- and CCK-responsive neurons are mostly separated subpopulations and TRPA1 agonist-responsive NG neurons may represent a distinct subpopulation with orexigenic function. Moreover, we found that intraperitoneal or oral administration of AITC or DATS increased food intake in mice, and these effects were blunted by desensitization of vagal afferents using capsaicin. In conclusion, we demonstrate that peripheral administration of TRPA1 agonist increases food intake by interacting with the novel subpopulation of vagal afferents with orexigenic role. (COI:No)

## S11-4

### Involvements of thermosensitive TRP channels in the functions of brown adipose tissue

Uchida Kunitoshi[1]、Sun Wuping[1,2]、Tominaga Makoto[1,2]
*([1]Div of Cell Signaling, NIPS (OIIB), NINS, Okazaki, Japan 、[2]Dept of Physiological Science, SOKENDAI, Okazaki, Japan)*

Most of the transient receptor potential (TRP) channels are $Ca^{2+}$-permeable non-selective cation channels. TRP channels are expressed in many tissues and have a wide variety of physiological functions, including detection of various physical and chemical stimuli in vision, taste, olfaction, hearing, touch. In addition, several TRP channels having thermosensing ability have been identified in mammals, as 9 thermosensitive TRP channels (thermo-TRPs) reported in mammals to date (TRPV1, TRPV2, TRPV3, TRPV4, TRPM2, TRPM4, TRPM5, TRPM8 and TRPA1). Especially, TRPV1, TRPV2, TRPV3, TRPV4, TRPM8 and TRPA1 are mainly expressed in sensory neurons and/or skin, and act as sensors for ambient temperature. Interestingly, many of thermo-TRPs are also expressed in tissues which are not exposed to dynamic temperature changes. These thermo-TRPs could have important roles other than temperature sensing. In this presentation, I will talk about the involvement of thermo-TRPs in differentiation and non-shivering thermogenesis in a brown adipose tissue. (COI:No)

# Symposium 12

High Definition Physiology in the Heart, from Molecule to Organ

March 23 (Wed), 9:00 – 10:30, Room K

## S12-1

### Emerging link between genetic variations of sodium channels and susceptibility to lethal arrhythmias

Makita Naomasa、Ishikawa Taisuke

*(Dept Mol Physiol, Nagasaki Univ Grad Sch Biol Sci, Nagasaki, Japan)*

Brugada syndrome is a rare cardiac arrhythmia disorder, characterized by coved-type ST elevation in the right precordial leads and ventricular fibrillation without underling structural heart diseases. It has been described as a monogenic disorder with mutations in SCN5A (cardiac sodium channel Nav1.5) in around 20% of cases. Through a genome-wide association study of 312 individuals with Brugada syndrome and 1,115 controls, with independent replication studies using Caucasian and Japanese cohorts, we confirmed three significant association signals at SCN5A, SCN10A (neuronal sodium channel Nav1.8) and near the transcription factor HEY2 gene. The cumulative effect of the three loci on disease susceptibility was unexpectedly large. Despite the controversy about the expression levels of SCN10A in the heart, and the pathophysiological significance of its rare coding variations in Brugada syndrome, common intronic polymorphisms of SCN10A directly enhance the promoter of SCN5A both in mice and human. These results indicate that common genetic variation can have a strong impact on the predisposition to rare diseases, and open a new era of investigation in the complex genetic architecture in cardiac arrhythmias. (COI:No)

## S12-2

### Numerical model-based investigation of TRPM4 channel in cardiac remodeling-associated arrhythmogenicity

Inoue Ryuji[1]、Hu Yaopeng[1]、Duan Yubin[1]、Takeuchi Ayako[2]、Kurahara-hai Lin[1]、Ichikawa Jun[1]、Matsuoka Satoshi[2]

*([1]Dept Physiol, Fukuoka Univ Sch Med, Fukuoka, Japan、[2]Dept Integ Systems Physiol, Fac Med, Fukui Univ, Fukui, Japan)*

The present study aimed at establishing a theoretical framework for investigating the functionality and pathological impact of stress-sensing/responsive molecules transient receptor potential (TRP) channels in the cardiovascular system (CVS), in light of mathematical modeling and simulations. For this purpose, we focused on a $Ca^{2+}$-activated monovalent cation channel TRPM4, the expression of which greatly increases during cardiac remodeling. To obtain quantitative data usable for numerical simulations, we newly developed ionomycin-permeabilized cell-attached recording technique and utilized voltage-sensing phosphatase and Foerster resonance energy transfer measurement to control and monitor the endogenous $PIP_2$ level, respectively. The obtained data were used to construct appropriate cardiac action potential models to reproduce the observed electrophysiological changes. The results indicated that; (1)upregulation of TRPM4 expression and activity is crucial to increase arrhythmic propensity; (2)decreased $PIP_2$ level likely protects against the heightened arrhythmogenicity by increased TRPM4 channel activity during excessive neurohormonal activities; (3)this appears disrupted by an arrhythmic mutation in the channel. The above approach is the first to integratively elucidate the functionality and pathological impact of TRP family members in the CVS, and expected to advance our understanding about their otherwise too diverse and intricate regulations. (COI: No)

## S12-3

### Regulation of cardiac functio by second messenger

Ishikawa Yoshihiro

*(CVRI, Sch Med, Yokohama City Univ, Yokohama, Japan)*

Cyclic AMP is a major second messenger to regulate cardiac function via the sympathetic nervous system. Norepinephrine released from the synaptic terminal binds to the adrenergic receptor on the cardiac cell membrane, which activates the stimulatory G protein, leading to the activation of adenylyl cyclase and thus production of cAMP. cAMP, as a second messenger, activate protein kinase A, which phosphorylates the L-type calcium channel, increasing the influx of calcium and thus cardiac contractility. Adenylyl cyclase is an enzyme that is made from multiple isoforms, among which the heart expresses the type 5 isoform. This isoform can be inhibited by calcium. Therefore, a negative feedback regulation may exist between cAMP and calcium. cAMP can activate the L-type calcium channel to increase calcium concentration, which inhibits adenylyl cyclase to decrease cAMP production. Because intracellular calcium concentration oscillates in accordance with cardiac beats, this regulatory mechanism may induces additional oscillation of cAMP-calcium within the cardiac myocyte. Disturbance of this regulation may lead to the occurrence of cardiac arrhythmia. Potential use of specific inhibitor or stimulator of cAMP production may be useful to adjust this disturbance. Beneficial effect of beta-blockers may also be related to the suppression of this enzyme activity. We will demonstrate both experimental and simulated results to explain the existence of such regulatory system. (COI:Properly Declared)

## S12-4

### Cardiac excitation wave propagation and arrhythmogenesis

Honjo Haruo

*(Dept Cardiovasc Res, Res Inst Environ Med, Nagoya Univ, Nagoya, Japan)*

Fibrillation is a most important cardiac arrhythmia characterized by electrical desynchronization of cardiac cells. The loss of synchronization results in complex spatiotemporal patterns of excitation wave propagation. While there is general agreement that fibrillation consists of multiple reentrant excitation waves, the mechanisms and dynamics of functional reentry are not fully understood. Conduction disturbance necessary for the initiation of reentry are thought to be caused by abnormalities in membrane properties and/or intercellular electrical coupling. For example, spatial differences in electrical refractory or cell coupling may lead to slow conduction and unidirectional block, initiating reentrant activity. However, slow conduction and block can be caused by the geometry of the excitation waves (i.e., wavefront curvature) without requiring abnormalities or spatial heterogeneities in either membrane properties or cell coupling. In the case of excitation waves with convex wavefront curvature, the local excitatory current supplied by upstream cells is dispersed to a large downstream area, resulting in decreases in conduction velocity and safety margin for propagation. Thus, excitation wavefronts with convex curvature higher than a critical value would not propagate. Such wavefront curvature-dependent functional conduction block plays fundamental roles in the formation of self-sustained electrical vortices and high-frequency excitation. In this talk, we will present our recent experimental data obtained by using high-resolution optical action potential mapping techniques and will discuss the relation to cardiac arrhythmogenesis. (COI:No)

## S12-5

### Multi-scale simulation studies of excitation conduction including cardiac conduction systems and whole ventricles

Inada Shin[1]、Harrell Daniel[2]、Haraguchi Ryo[1]、Ashihara Takashi[3]、Aiba Takeshi[1]、Yamashita Fumiyoshi[4]、Shibata Nitaro[5]、Ikeda Takanori[6]、Mitsui Kazuyuki[7]、Makita Naomasa[2]、Honjo Haruo[8]、Boyett Mark[9]、Nakazawa Kazuo[1]

*([1]National Cerebral and Cardiovascular Center、[2]Nagasaki University, Nagasaki, Japan、[3]Shia University of Medical Science, Otsu, Shiga, Japan、[4]Kyoto University, Kyoto, Japan、[5]Shinjuku Mitsui Building Clinic, Tokyo, Japan、[6]Too University Faculty of Medicine, Tokyo, Japan、[7]Tokyo Denki University, Tokyo, Japan、[8]Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan、[9]University of Manchester, Manchester, United Kingdom)*

We have studied excitation conduction in the cardiac conduction systems and ventricles using computer simulation. In this symposium, we would like to discuss our results according to the followings. (1) Sinoatrial (SA) node. The SA node is a physiological pacemaker. We investigated the importance of heterogeneity in terms of cell size and electrical coupling in relation to the SA node function. (2) Atrioventricular (AV) node. The AV node is the only conduction pathway between the atria and the ventricles. Using our multicellular model, effects of ionic channel blocking on ventricular rate during atrial fibrillation were investigated. (3) Purkinje network. A loss of the expression and function of gap junctions can impair excitation conduction in the His-Purkinje system. We simulated the effect of decreasing gap junction conductance on excitation conduction. (4) Ventricles. We simulated excitation propagation using a supercomputer. The effects of slow conduction in the right ventricle on inducibility of arrhythmia were studied. (COI:No)

# Symposium 13

Neural circuit mechanism in hippocampal activity

March 22 (Tue), 16:00 – 17:30, Room D

## S13-1

### Cooperation and specialization of the bilateral hippocampi in rodents

Shinohara Yoshiaki

*(Laboratory for Neuron-Glia Circuitry, RIKEN Brain Science Institute, Wako)*

While rodent hippocampi are interhemispherically connected, little is known about the anatomical and functional significance. We show that rat CA3-CA1 spine synapses differ in morphology and glutamate receptor subunit composition, depending on the laterality. At the systems level, theta-associated gamma oscillations in CA1 stratum radiatum in anesthetized rats become larger in power and bilateral synchrony after a month of enriched environment housing. This experience-dependent gamma power enhancement is more prominent on the right side and coincided with laterally-biased synaptogenesis in CA1 stratum radiatum. These results suggest that experience influences wiring and operational dynamics of bilateral hippocampi. (COI:No)

## S13-2

### Synaptic plasticity controls hippocampal circuit activity

Kitanishi Takuma

*(Dept Mol Behav Neurosci, Grad Sch Med, Osaka Univ)*

Temporally precise neuronal firing is crucial for information processing in the brain. Neurons often preferentially fire at specific phases of neural oscillations, referred to as phase locking. Phase locking to gamma oscillations is thought to mediate the dynamic interaction of neuronal populations, which is essential for information processing underlying higher-order functions such as learning and memory. However, the cellular mechanisms determining phase locking remain unclear. By devising a virus-mediated approach to perform multi-tetrode recording from genetically manipulated neurons, we demonstrated that synaptic plasticity dependent on the GluR1 subunit of AMPA receptor mediates two dynamic changes in neuronal firing in the hippocampal CA1 area during novel experiences: (i) the establishment of phase-locked firing to slow gamma oscillations, which is thought to originate from the CA3 area, and (ii) the rapid formation of the spatial firing pattern of place cells. The results suggest a series of events potentially underlying the acquisition of new spatial information: slow gamma oscillations induce the two GluR1-dependent changes of CA1 neuronal firing, which in turn determine information flow in the hippocampal-entorhinal system. (COI:No)

## S13-3

### Impaired hippocampal activity at the goal zone on the place preference task in a DISC1 mouse model

Hayashi Yuichiro[1]、Sawa Akira[2]、Hikida Takatoshi[1]

*([1]Medical Innovation Center, Kyoto Univ Grad Sch Med, Kyoto, Japan、[2]Dept Psychiatry and Behav Sci, Johns Hopkins Univ Sch Med, Baltimore MD, USA)*

Learning deficit is a clinical feature of many mental disorders, and is hypothesized to result from an inability to integrate information in neural systems. We showed that transgenic mice expressing a dominant-negative form of DISC1, a risk gene for neuropsychiatric disorders, exhibited impaired performance in a reward-place association task when combined with a mild isolation stress. CA1 cells in the mutant mice showed normal place cell properties, but their activity at the goal zone was diminished. This abnormality in hippocampal activity at the goal zone during the task may underlie the learning deficit observed in the DISC1 mutant mice. (COI:No)

## S13-4

### A prefrontal-thalamo-hippocampal circuit for goal-directed spatial navigation

Ito Hiroshi

*(Max Planck Institute for Brain Research, Frankfurt am Main, Germany)*

Spatial navigation is a fundamental ability for animals to survive in a geometric space. While animals use various strategies for navigation, a prominent feature in rats is their ability to use geometric relationships of landmarks to estimate a distance and direction of a goal location. This led to the idea that rats have an internal model of a local space, or a map, in their brain, which was enforced by the discovery of place cells in the hippocampus. However, while place cells may be ideal for animals to identify their positions, these cells are mostly silent once animals leave the firing fields of the cells. It has thus been a long-standing question how to use place cells to plan a route to a desired location, which is distant from the animal. A role of the hippocampus may be better clarified in a context of wider circuits to implement the computations necessary for route decisions. A growing body of evidence indicates a key role for the medial prefrontal cortex (mPFC) in navigation. We found that the information about next planned movements in mPFC is transferred to area CA1 of the hippocampus using the midline thalamic nucleus reuniens (NR) as a relay. Next movements were represented in CA1 by changing firing rates of place cells, which was largely disrupted by lesioning or optogenetic silencing of NR. Our study suggests that the hippocampus is a part of action planning circuits for navigation and further points to the thalamus as a crucial node for long-range communication between cortical regions. (COI:No)

# Symposium 14

Zinc signaling: its molecular basis on physiopathology

March 22 (Tue), 16:00 – 17:30, Room F

## S14-1

### Zinc signaling: Introduction

Fukada Toshiyuki

*(Mol. Cell. Physiol., Faculty of Pharm., Tokushima Bunri Univ., Tokushima, Japan)*

Zinc, an essential trace element for life, is necessary for physiological conditions, so that involved in variety of cellular functions. Therefore, abnormal zinc homeostasis results in various health problems such as growth retardation, irregular bone formation, neuronal dysfunctions, immunodeficiency, and metabolic diseases like diabetes mellitus, etc. It is now well known that zinc homeostasis is regulated by zinc transporters, ZIPs and ZnTs. Recent investigations have revealed that zinc mediated by zinc transporters acts as a signaling factor (zinc signaling), regulating numbers of physio-pathological events. This symposium aims to share the updated information about the roles of zinc signaling and its molecular mechanisms in mammalian physio-pathogenesis, and to discuss the current probl:ms and future directions. Also, I will introduce the activities of the International Society for Zinc Biology, which brings scientists from a various fields to share a common interest and knowledge in physiology and pathogenesis of zinc biology (http://iszb.org/).

**Ref).** ***Zinc Signals in Cellular Functions and Disorders***, Ed. Fukada and Kambe: Springer, 2014
(COI:No)

## S14-2

### Zinc transporter ZIP13 regulates the adipocyte browning

Fukunaka Ayako[1]、Fujitani Yoshio[1]、Fukada Toshiyuki[2]、Watada Hirotaka[1]

*([1]Grad. Sch. of Med., Juntendo Univ., Tokyo, Japan、[2]Pharma. Sci., Tokushima Bunri Univ.)*

Obesity is caused by a long-time imbalance between energy intake and energy expenditure. Adipose tissues are a major site of the control of energy balance. It comprises two functionally distinct cell types: white adipocyte cells and brown adipocyte cells. White adipocyte cells store excess energy, whereas brown adipocyte cells specialize in energy expenditure. Brown fat-like cells have also been found within white adipose tissue (WAT). These inducible brown fat cells are known as beige fat cells (a process also known as browning). Therefore, the identification of signaling pathways that regulate acquisition of beige fat cell properties by WAT may foster novel therapies for obesity and type 2 diabetes. *Zip13* knockout mice (KO mice) and human Spondylocheirodysplastic Ehlers-Danlos syndrome (SCD-EDS) patients who possess a loss-of-function mutation in ZIP13 showed reduced white-fat mass. We found that KO mice have increased expression levels of brown adipocyte cell markers in their subcutaneous WAT and that this browning phenotype of KO cells occurs in a cell-autonomous manner. Furthermore, KO mice exhibited an increased $O_2$ consumption rate and a remarkable obesity-resistant phenotype under a high-fat diet. These data suggest that zinc transporter ZIP13 negatively regulate adipocyte browning, which might be involved in the pathogenesis of obesity-related diseases. Investigation of the molecular mechanism by which ZIP13 regulates this browning is now underway, and will be addressed in this workshop. (COI:Properly Declared)

## S14-3

### Role of the zinc transporter in intestinal homeostatic self-renewal

Ohashi Wakana[1]、Hase Koji[2]、Fukada Toshiyuki[3]

*([1]Dept. of Mol. Med. Pharmacol., Graduate sch. of Med. and Pharm., Univ. of Toyama, Japan、[2]Div. of Biochem., Faculty of Pharm., Keio Univ., Japan、[3]Mol. and Cellular Physiol., Faculty of Pharm., Tokushima Bunri Univ., Japan)*

Intestinal epithelium forms a physical and biochemical barrier that protects the body from the harmful environment of the lumen, and is continuously replenished by resident adult stem cells. The dysregulation of the self-renewing of intestinal epithelium has been linked to the pathogenesis of the various disease including inflammatory bowel disease and tumorigenesis.
Accumulating evidence indicate the importance of zinc transporters in mediating normal cellular function and tissue homeostasis. It has been indicated that zinc may affect intestinal barrier function, however, the role of zinc transporters in the self-renewing of intestinal epithelium remains to be elucidated.
In the intestine, the expressions of zinc transporters vary along with the stem cell hierarchy. Using the genetic approach, we found that the zinc transporter is important for the self-renewing of intestinal epithelium by maintaining intestinal stem cells. We will discuss the essential role of zinc transporter in the regulation of the homeostasis of self-renewing of the intestinal epithelium. (COI:No)

## S14-4

### A novel splicing variant of zinc transporter ZIP2 controls the pathology of CF and CF-like airway diseases

Shuto Tsuyoshi[1]、Kamei Shunsuke[1,2]、Shuto Keiko[3]、Suico Maryann[1]、Kai Hirofumi[1]

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Zinc ion ($Zn^{2+}$) is an essential dietary metal ion that has pleiotropic effects in many tissues including airway epithelial cells. Patients with cystic fibrosis (CF), a common human hereditary pulmonary disease characterized by mucus hypersecretory and inflammatory phenotypes, have a defect in the regulation of intracellular $Zn^{2+}$ level. However, the cellular and molecular mechanisms of Zn deficiency in the context of CF are largely unknown. Here, we identified in CF and CF-like airway epithelial cells that expression of a novel splicing isoform of zinc transporter ZIP2 (ΔC-ZIP2) is increased, which results in the production of C-terminus-deleted immature ZIP2 protein. Consistently, ZIP2 protein expression and intracellular Zn2+ level were decreased in CF-related cells. Moreover, reduction of intracellular zinc concentration using the zinc chelator TPEN up-regulated the gene expression of mucus and inflammatory cytokines in normal airway epithelial cells. This upregulation is a typical disease-associated characteristic of CF airway epithelial cells, implying that defective intracellular $Zn^{2+}$ regulation, at least in part, contributes to CF pathogenesis. These results provide a novel molecular basis of intracellular $Zn^{2+}$ dysregulation that leads to mucus hypersecretory and inflammatory phenotypes in CF, which is exerted by the splicing alteration of Zip2 gene. (COI:No)

## S14-5

### Zinc transporter-targeting strategy for enhancing zinc absorption

Hashimoto Ayako、Kambe Taiho

*( Grad. Sch. of Biostudies, Kyoto Univ., Kyoto, Japan)*

Zinc deficiency is an important nutritional problem especially in elderly people and women, so we are exploring strategies for enhancing zinc absorption. We focus on the molecular mechanism of zinc absorption process in the small intestine, where the zinc transporter ZIP4 plays an essential role. Mutations in this gene cause acrodermatitis enteropathica (AE), an autosomal-recessive disorder. Several studies have revealed that ZIP4 is dynamically regulated by post-transcriptional mechanisms. ZIP4 accumulates at apical membrane of intestinal epitherial cells by inhibition of ZIP4 endocytosis and degradation, which likely contributes to facilitating zinc absorption during zinc deficiency. Overexpression of ZIP4 protein increases zinc uptake, and thereby cellular zinc levels, suggesting that food components with the ability to increase ZIP4 expression could potentially enhance zinc absorption. In this study, we searched such food components using mouse Hepa cells, which express mZip4 in a manner indistinguishable from that in intestinal enterocytes. We found two soybean extracts, which increased expression of mZip4 protein by inhibition of mZip4 endocytosis, and consequently increased cellular zinc levels. Furthermore we identified soyasaponin Bb as an active component from one extract. Soyasaponin Bb is capable of enhancing apically localized mZip4 and mZip4 AE mutants in transfected polalized Madin-Darby canine kidney cells, and moreover cell surface endogenous hZIP4 in human cells. Our results suggest that ZIP4-targeting may represent a new strategy for improving zinc absorption, and thus zinc nutritional status. (COI:No)

## S14-6

### Zinc trafficking failure: from Acrodermatitis to Alzheimer's disease

Ashley I. Bush, MD PhD
*(Florey Institute, University of Melbourne, Australia)*

Over 20 transmembrane proteins move zinc. Failure of zinc uptake from the gut occurs in Acrodermatitis Enteropathica, caused by mutation of ZIP4[1]. A similar failure of extracellular zinc uptake occurs in brain cortex with aging, which raises extracellular zinc levels and induces the precipitation of the vulnerable peptide, Aβ, into the amyloid pathology of Alzheimer's disease (AD)[2]. Novel proteins that participate in zinc homeostasis include the presenilins[3], where a mutation causes familial AD. Zinc also modulates the stability of apolipoprotein E[4], the major risk allele for AD. Abnormal brain zinc homeostasis is also evident in other neurodegenerative diseases, leading to a redistribution of zinc and impairment of zinc-dependent physiological processes. Zinc supplementation is unable to easily correct these deficiencies since cells are impermeable to passive zinc fluctuations. We have developed novel pharmaceutical agents that chaperone cellular uptake of zinc and are highly active in models of these disorders.

1 Geiser *et al.* Clioquinol Synergistically Augments Rescue by Zinc Supplementation in a Mouse Model of Acrodermatitis Enteropathica. *PLoS ONE* **8**, e72543, (2013)
2 Barnham, K. J. & Bush, A. I. Biological Metals and Metal-Targeting Compounds in Major Neurodegenerative Diseases. *Chem Soc Rev* **43**, 6727-49, (2014)
3 Greenough *et al.* Presenilins Promote the Cellular Uptake of Copper and Zinc and Maintain Cu-Chaperone of SOD1-Dependent Cu/Zn Superoxide Dismutase Activity. *J Biol Chem* **286**, 9776-86, (2011)
4 Xu *et al.* Zinc Affects the Proteolytic Stability of Apolipoprotein E in an Isoform-Dependent Way. *Neurobiol Dis* **81**, 38-48, (2015) (COI:No)

## S14-7

### Zinc Signaling: Conclusion

Kambe Taiho
*(Grad Sch Biostudies, Kyoto Univ)*

As a conclusion of this symposium, I will summarize the crucial role of zinc signaling and its molecular mechanisms in health and diseases, and also discuss the future directions and questions underlying this unique phenomenon. (COI:No)

# Symposium 15

Autonomic Regulation Under Stress

March 23 (Wed), 9:00 – 10:30, Room D

## S15-1

### Contribution of serotonergic neurons in the medullary raphe to physiological responses induced by stress

Ikoma Yoko[1]、Kusumoto Ikue[1]、Yamanaka Akihiro[2]、Ootsuka Youichirou[1,3]、Kuwaki Tomoyuki[1]

*([1]Dept. Physiol., Grad. Sch. Med. Dent. Sci. Kagoshima Univ., Kagoshima, Japan、[2]Dept., Neurosci. II, Res. Inst. Environ. Med., Nagoya Univ., Nagoya, Japan、[3]Centre for Neuroscience, Dept., Human Physiol. Sch. Med., Flinders Univ., SA, Australia)*

Stress causes several physiological responses including tachycardia, hyperthermia and hyperactivity. Activation of neurons in the medullary raphe causes an increase in heart rate and blood pressure. Interestingly, inhibition of the medullary raphe neurons does not affect resting heart rate and blood pressure, but it does attenuate stress-induced tachycardia. Thus it has been suggested that while the medullary raphe may not affect the resting state, it has an important role in the regulation of cardiovascular responses under stressful conditions. The medullary raphe region contains the serotonin synthesizing B1-B3 bulbospinal neurons, suggesting contribution of these serotonergic neurons to the stress-induced cardiovascular responses. In the present study, we tested this possibility by taking an optogenetic approach. We used transgenic mice in which archaerhodopsin-T, a green light-driven neuronal silencer, was expressed selectively in serotonergic neurons in the central nervous system. We performed focal green light stimulation in the medullary raphe to inhibit serotonergic neurons and axons during these tests. We then examined the effect of this selective light-induced inhibition on physiological responses such as heart rate, body temperature, respiration, and movement to stress under free moving. We will report our updated results. (COI:No)

## S15-2

### The effects of social stress on neuronal activation in the hypothalamus and the cardiovascular reaction

Nagaoka Yuya、Ohashi Hiroki、Horiuchi Takatoshi、Sato Fumitaka、Yoshioka Yuumi、Horiuchi Jouji

*( Dept Biomedical Engineering, Toyo Univ, Saitama, Japan)*

It is known that neurons in the hypothalamus play an important role on the autonomic cardiovascular response evoked by psychological stress. Changes regarding social life, such as living atmosphere after move and interpersonal issues at workplace, are also thought to be a kind of psychological stress, but it is unclear how neurons in the hypothalamus participate in the cardiovascular response during the social stress. In addition, orexin-containing neurons are localized within a restricted region of the hypothalamus and may be involved in the cardiovascular response during various types of stress. In this presentation, we reveal the cardiovascular response, distributions of the c-Fos expression and role of the orexin neurons during 2 different types of the psychosocial stressor (home-cage change and social-defeat situation) in conscious Wistar rats. Home cage change stress caused increases in blood pressure (BP), heart rate (HR) and the c-Fos expressed neurons in the hypothalamus, but there were not significant differences compared to non-stress control animals. In contrast, the social defeat stress evoked significant increases in BP, HR and the c-Fos expressed neurons in the hypothalamus. In addition, c-Fos expressions in the orexin neurons of the hypothalamus were increased when compared to control group. These results suggest that neurons in the hypothalamus play a crucial role in the cardiovascular response evoked by the social defeat stress and the orexin neurons participate in the stress-induced response. (COI:No)

## S15-3

### A ventral medial prefrontal cortex-dorsomedial hypothalamus pathway driving psychological stress-induced hyperthermia

Kataoka Naoya[1]、Nakamura Kazuhiro[1,2]

*([1]Dept Integrative Physiol, Nagoya Univ Grad Sch Med, Nagoya, Japan、[2]PRESTO, JST, Japan)*

Psychological stress-induced hyperthermia is a fundamental autonomic stress response in mammals. We have reported that stress induces thermogenesis in brown adipose tissue (BAT), hyperthermia and tachycardia by activating a direct neural pathway from the dorsomedial hypothalamus (DMH) to the rostral medullary raphe. However, the mechanism by which stress signals activate the DMH neurons driving the sympathetic stress responses remains unknown. In this study, we show that the ventral medial prefrontal cortex (vmPFC) is a major source of the stress signals to the DMH by performing *in vivo* physiological experiments and immunohistochemistry. Rats exposed to social defeat stress, a sociopsychological stress model, exhibited increased expression of Fos, a marker for neuronal activation, in DMH-projecting neurons in the vmPFC. Inhibition of vmPFC neurons with bilateral muscimol nanoinjections in free-moving rats significantly reduced BAT thermogenesis and hyperthermia evoked by social defeat stress. Furthermore, selective stimulation of vmPFC-DMH monosynaptic transmission using an in vivo optogenetic technique elicited BAT thermogenesis and an increase in heart rate, which were blocked by antagonizing glutamate receptors in the DMH. These results indicate that the vmPFC-DMH glutamatergic monosynaptic pathway mediates stress signaling to drive the BAT thermogenesis contributing to psychological stress-induced hyperthermia. (COI:No)

## S15-4

### Neuroendocrine and behavioral responses to fear-related or affiliative stimuli: roles of oxytocin

Onaka Tatsushi、Takayanagi Yuki、Yoshida Masahide、Okabe Shota

*(Div Brain Neurophysiol, Dept Physiol, Jichi Medical Univ, Tochigi, Japan)*

Noxious stimuli or stimuli previously paired with noxious stimuli (conditioned fear stimuli) activate oxytocin-synthesizing neurons in the hypothalamus via activation of noradrenaline/prolactin releasing peptide (PrRP)-synthesizing neurons in the medulla oblongata. The upstream of medullary noradrenaline/PrRP neurons to activate oxytocin neurons in response to conditioned fear stimuli has been found to be the medial amygdala by experiments with local lesions. Recently, we found that not only aversive stimuli but also pleasant stroking stimuli activate hypothalamic oxytocin neurons. Roles of activation of oxytocin neurons in response to aversive or affiliative stimuli remain to be clarified. Oxytocin neurons in the hypothalamus expressed the secretin receptor. In order to examine roles of activation of oxytocin neurons, we activated hypothalamic oxytocin neurons by secretin microinjections into the supraoptic nucleus and examined social recognition. Activation of local hypothalamic oxytocin neurons induced oxytocin release from dendrites of oxytocin neurons and facilitated social recognition. This facilitation of social recognition was impaired by local application of an oxytocin receptor antagonist into the medial amygdala. All these data suggest that activation of hypothalamic oxytocin neurons induces oxytocin release in the medial amygdala and facilitates social recognition via activation of the oxytocin receptor within or in the vicinity of the medial amygdala. (COI:No)

# Symposium 16

Crosstalk of human brain research

March 22 (Tue), 9: 00 – 10:30, Room F

## S16-1

### Assessment of human cognitive processing with event-related potentials during passive heat stress and exercise

Nakata Hiroki
*(Fac Human Life Environment, Nara Women's Uni, Nara city, Japan)*

Event-related potentials (ERPs) obtained by time-locked averaging electroencephalography (EEG) have been used to evaluate human cognitive processing, such as sensory, motor, or cognitive events. The ERP waveforms are described according to the latency and amplitude. In ERP studies, the P300 or P3b component is one of the most widely studied components with a parietal distribution on the scalp, and it has been linked to the cognitive processes of context updating, context closure, and event-categorization. The present study demonstrates the effect of passive heat stress and exercise on P300. The peak amplitude of P300 decreased with increases in body temperature during passive heat stress, and the peak latency of P300 gradually decreased. However, these modulations were not observed in the normothermic time control. Repetition of exercise also reduced the peak amplitude of P300, and the peak latency of P300 gradually decreased. In addition, these modulations were different depending on the thermal conditions. The decrease in the peak amplitude of P300 was greater under the 35 degree centigrade thermal condition than the 20 degree condition, even when the intensity of exercise was the same between two thermal conditions. The peak latency of P300 was earlier under the 35 degree condition than the 20 degree condition. ERPs have been used under various psychological, psychiatric, and neurophysiological conditions, but the present studies provide novel approaches to assess human cognitive processing in the fields of exercise and environmental physiology. (COI:No)

## S16-2

### Regulation of regional brain blood flow during exercise

Sato Kohei
*(Japan Womens College of Physical Education, Tokyo, Japan)*

Constant cerebral blood flow (CBF) is vital to human survival. Originally thought to receive steady blood flow, the brain has shown to experience increases in BF during exercise. Because of methodological limitations, almost all previous studies have evaluated the response of mean blood flow velocity in the middle cerebral artery to exercise as a measure of BF response across the whole brain, and there has been limited study of regional differences in CBF regulation during exercise. Yet our recent studies have demonstrated that there appear to be differences in BF responses to exercise between head, anterior, and posterior cerebral arteries. Of particular note, vertebral artery (VA) BF continuously increased during incremental exercise workload despite a decrease in internal carotid artery (ICA) BF at heavy intensity. These different BF responses between ICA and VA are probably mediated by the regional difference in metabolic demand and CO2 reactivity. Moreover, we also observed that BF in the external carotid artery (ECA) was inversely proportional to decrease in ICA BF during heavy dynamic exercise that associated rises in core temperature. The large increase in ECA BF may be an important pathway by which heat is locally dissipated to regulate temperature of the face and head. In this lecture, we highlight BF distribution at head and brain arteries during exercise, and then summarize the integrative mechanisms underlying the regulation of regional BF in head and brain during exercise. (COI:No)

## S16-3

### The relationship between cognitive function, cardiovascular and cerebral blood flow regulations

Ogoh Shigehiko
*(Dept Biomed Eng, Toyo Univ, Saitama, Japan)*

When the response of cardiac output (CO) to exercise is attenuated by β1-blockade, or in patients with heart failure, exercise-induced increase in cerebral blood flow (CBF) is reduced. In our previous study, we examined the influence of changes in CO induced by manipulation of central blood volume on CBF in healthy subjects at rest. We observed that change in CBF was significantly associated with that of CO, and its association was independent of dynamic cerebral autoregulation (CA). This finding suggest that CO is an important physiological factor to determine cerebral hemodynamics. Therefore, the regulation of CO via the arterial baroreflex could expectedly influence CBF regulation. Recently, we identified dynamic CA during acute hypotension with and without the arterial baroreflex-mediated tachycardia (vagal blockade). In this previous study, dynamic CA was attenuated with vagal blockade compared with the conditions with the arterial baroreflex-mediated tachycardia (control and β1-adrenergic blockade conditions). The finding of this previous study suggest that the arterial baroreflex plays an important role in the regulation of CBF indirectly. For example, chronic cardiovascular disease impairs dynamic CA. Moreover, this impairment of CBF regulation may be due to arterial baroreflex dysfunction rather than specific dysfunction of cerebrovascular regulation. In this symposium, I will present the previous studies regarding the interaction between CBF, cognitive function and systemic vascular regulatory system to discuss about the mechanism of change in CBF hemodynamic and cognitive function. (COI:No)

## S16-4

### Cerebral blood flow and cognitive function during heat stress

Shibasaki Manabu
*(Nara Women's University)*

Heat stroke is a life-threatening illness. Heat waves or severe heat stresses increase morbidity and mortality relative to a temperate condition. In the summer of 2010, over 50,000 people were taken to the hospital because of heat-related illness. Approximately 80% of the death toll in that summer occurred among people with age over 65 years. In these aged individuals, impaired thermoregulatory functions including low sweat rate or thermal sensation are serious factors for heat-related illness. However in the viewpoint of preventive medicine, this symposium focuses on more initial symptoms of heat-related illness, which happen to anyone. For example, we feel dizziness or light-headedness when exposed to a hot environment. Subsequently confusion, disorientation or staggering occurs in more severe condition. These symptoms indicate orthostatic intolerance or cognitive dysfunction. A considerable factor of these symptoms is thought to be cerebral blood flow and its regulation. Heat stress reduces cerebral blood flow, which is caused by increased distribution of cardiac output to the cutaneous circulation, profuse sweating, and hypocapnia due to hyperthermia-induced hyperventilation. Thus reduced orthostatic tolerance or impaired cognitive function during heat stress appears to be caused by cerebral hypoperfusion and/or increased brain temperature. I will summarize recent researches regarding to cerebral blood flow focused on orthostatic tolerance and cognitive function during heat stress. (COI:No)

# Symposium 17

Leading methodologies for uncovering hierarchical information processing of neuronal circuits

March 24 (Thu), 15:00 – 16:30, Room D

## S17-1

### Two-photon calcium imaging of neurons and thalamocortical axons in the motor cortex of behaving animals

Tanaka Yasuhiro[1]、Masamizu Yoshito[1]、Tanaka Yasuyo[1]、Matsuzaki Masanori[1,2,3]

*([1]Div. Brain Circuit, NIBB, Okazaki, Japan、[2]SOKENDAI, Okazaki, Japan、[3]Dept. Physiology, Univ. Tokyo, Tokyo, Japan)*

Recent advances in two-photon calcium imaging have enabled us to record the activities of hundreds of neurons as well as those of input axons from various distant information sources. We will show our data obtained with two-photon calcium imaging and hopefully exemplify the usefulness of this technique for studying the neuronal code for animal behavior and the temporal dynamics of the neural activity. We conducted two-photon calcium imaging both of neuronal cell bodies and thalamocortical axons in various layers in mouse primary motor cortex during a self-initiated lever-pull task. Layer 1 and layer 3 thalamocortical axonal boutons show distinct temporal dynamics in their neural activities. As for the activities of neurons in the primary motor cortex, in layer 2/3, the accuracy of neuronal ensemble prediction of lever trajectory remained unchanged as a whole, with a subset of individual neurons retaining high prediction accuracy throughout the training period. However, in layer 5a, the ensemble prediction accuracy steadily improved. The layer 2/3 network may represent coordination of signals from other areas throughout learning, whereas layer 5a may participate in the evolving network representing well-learned movements. The thalamocortical activity evolves to handle diverse temporal dynamics and may be integrated into cortical activity to modify well-learned movements. (COI:No)

## S17-2

### Preconfigured, skewed distribution of firing rates in the hippocampus and entorhinal cortex

Mizuseki Kenji

*(Dept Physiol, Grad Sch Med, Osaka City University, Osaka, Japan)*

The dominant communication across neurons occurs via spikes. Yet, despite the pivotal role of spiking activity in transmitting information, only limited data about the firing rates of unbiased neuronal populations in behaving animals are available. Using large-scale chronic recording methods, we simultaneously monitored the activity of many (~100) neurons in multiple layers of the entorhinal-hippocampal loop in waking and sleeping rats. To reduce sampling bias toward more-active neurons, we recorded from the same neurons for many hours (typically, for more than 4 hours). Importantly, for the subsequent analyses, we included all recorded neurons without omitting low firing rate ones. Using a large database of physiologically characterized neurons, we found that the firing rates of principal neurons in the hippocampal and entorhinal cortex showed a lognormal-like distribution in all brain states. Mean and peak firing rates within place fields of hippocampal neurons were also strongly skewed. Firing rates of the same neurons showed reliable positive correlations in different brain states and testing situations, as well as across familiar and novel environments, suggesting that the firing rate of individual neurons is relatively "fixed". The persistent skewed distribution of firing rates implies that a preconfigured, highly active minority dominates information transmission in cortical networks. (COI:No)

## S17-3

### Inter-areal and inter-laminar circuit for memory retrieval in primate temporal cortex

Takeda Masaki[1,2]

*([1]Grad Sch Med, Juntendo Univ、[2]Grad Sch Med, The Univ of Tokyo)*

The primate temporal cortex locates at the final stage of the ventral visual pathway and implements visual long-term memory. Area TE and area 36 (A36) of the temporal cortex are known to engage in the associative representations of long-term memory of visual objects and to play distinct roles in memory retrieval. While the perceptual activity of visual objects emerges earlier in TE than A36, memory retrieval activity for the sought target emerges earlier in A36 than TE, suggesting that a backward signal flows from A36 to TE during memory retrieval. The inter-area signal, if present, would modify local signal processing implemented by laminar neuronal circuits in the target area TE. To test this hypothesis, we simultaneously recorded from A36 and TE from monkeys performing a pair-association memory task. Two distinct inter-area signal flows were identified during memory retrieval: A36 spiking activity was coherent with low-frequency field activity in either the supragranular or infragranular layer of TE. Of these two flows, only signal flow targeting the infragranular layer of TE was further translaminarly coupled with gamma activity in the supragranular layer of TE. Moreover, this coupling was observed when monkeys succeeded in the retrieval of the sought object but not when they failed. The results suggest that local translaminar processing can be recruited via a layer-specific inter-area network for memory retrieval. (COI:No)

## S17-4

### Studying regional brain functions using functional magnetic resonance imaging (fMRI)

Cheng Kang

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

In this presentation, I will first introduce the principle of Blood Oxygenation Level-Dependent (BOLD) technique, the most widely used functional magnetic resonance imaging (fMRI) approach, as well as newly emerged ultra-high-field MRI and multiband acquisition technologies. I will then use a simple retinotopic-mapping example to demonstrate how a typical fMRI experiment is conducted. Finally, I will introduce the results from our recent fMRI studies exploring functional architectures in human visual cortex as well as intuitive and strategic decision-making processes employed by professional players in the game of shogi. (COI:No)

# Symposium 18

Synaptic basis of behavioral change

March 22 (Tue), 16:00 – 17:30, Room E

## S18-1

### Functional organization of cerebellar modules during skilled behavior

Kitamura Kazuo

*(Dept Physiol, Grad Sch Interdiscipl Res, Univ Yamanashi, Yamanashi, Japan)*

The cerebellar cortex consists of elaborate longitudinal functional modules called zones/microzones. Climbing fibers that arise from the inferior olive project to Purkinje cells in specific cerebellar zones, which are distinguished by their aldolase C/zebrin II expression, and are thought to provide the instructive/error signals for motor learning. To investigate the functional organization of cerebellar zones and its role in learning skilled movements, we have recently developed a transgenic mouse in which cerebellar zones can be visualized *in vivo*. Two-photon calcium imaging in these mice revealed fine scale correspondence between cerebellar zones and complex spike synchrony, indicating precise relationship between cerebellar zones and climbing fiber inputs. Furthermore, chronic calcium imaging experiments in mice performing lick/no-lick auditory discrimination task have uncovered the distinct roles of cerebellar zones and changes in climbing fiber signals during learning. Our results indicate flexible and distinct roles of cerebellar microcircuits and yield important insights into the function of cerebellar modules in motor learning. (COI:No)

## S18-2

### CAPS1 stabilizes docking state of SVs in hippocampal CA3-CA1 synapses

Shinoda Yo[1]、Ishii Chiaki[1]、Fukazawa Yugo[2]、Furuichi Teiichi[1]

*([1]Dept Appl Biol Sci, Fac Sci and Tech, Tokyo Univ of Sci, Chiba, Japan、[2]Div Cell Biol and Neurosci, Fac Med Sci, Univ of Fukui, Fukui, Japan)*

Calcium-dependent activator protein for secretion 1 (CAPS1) is a cytosolic protein, which associates with dense-core vesicle secretion in endocrine cells, however, their neuronal function is still largely unknown because of Caps1 knock-out (KO) results in prenatal death. Here we show that CAPS1 stabilizes the docking state of synaptic vesicle (SV) to presynaptic active zone using forebrain specific Caps1 conditional KO (cKO) mice. The synaptic transmission is strongly reduced and paired-pulse facilitation shows significant alteration in Caps1 cKO. Morphological analysis shows accumulation of SVs in presynapse without any other morphological changing. Interestingly, even though SV accumulation is occurred, the percentage of presynaptic button contained docked vesicle is markedly reduced in Caps1 cKO. These data suggest that CAPS1 stabilizes SV docking state to enhance SV release. (COI:No)

## S18-3

### Brain-behavior phenotyping of genetically engineered mice in research of psychiatric disorders

Takao Keizo[1,2]

*([1]Life Science Research Center, University of Toyama, Toyama, Japan、[2]NIPS, Okazaki, Japan)*

Animal models serve as essential tools for investigating the pathophysiology and treatment of neuropsychiatric disorders. For the past decade, we have sought to identify rodent models of such disorders by analyzing genetically engineered mice with a comprehensive behavioral test battery that covers many distinct behavioral domains. To date, we have screened more than 178 mutant mouse strains and identified several strains with behavioral phenotypes that resemble symptoms of human schizophrenia patients. Among them, we found that mice heterozygous for a null mutation of &alpha-CaMKII exhibited abnormal behaviors related to schizophrenia and other psychiatric disorders. In these mutants, almost all neurons in the dentate gyrus are arrested in a pseudoimmature state at the molecular and electrophysiological levels, a phenomenon referred to as immature dentate gyrus (iDG). The iDG phenotype and shared behavioral abnormalities (including working memory deficit and hyperlocomotor activity) was discovered in Schnurri-2 KO, mutant SNAP-25 knock-in, and forebrain-specific calcineurin KO mice. Furthermore, an iDG-like phenomenon was observed in post-mortem analysis of brains from patients with schizophrenia/bipolar disorder. Based on these observations, we propose that iDG is a potential endophenotype shared by certain types of neuropsychiatric disorders. In this presentation, I summarize recent data describing the iDG phenotype and discuss the potential implications of the data from the mouse models for elucidating the pathophysiology of neuropsychiatric disorders. (COI:No)

## S18-4

### Regulation of synaptic plasticity by a ubiquitin ligase SCRAPPER

Yao Ikuko

*(Dept of Optical Imating, MPRC, Hamamatsu Univ Sch of Med, Shizuoka,Japan)*

Protein synthesis and degradation occur to exert their effects at appropriate times and in appropriate locations. Such spatiotemporal controls serve as mechanisms for balancing environments in cells as well as in individuals. One of the mechanisms that help achieve this balance involves the ubiquitin proteasome system (UPS). The UPS is involved in the mechanism underlying selective protein degradation. We focused on the UPS in the nerve cells, we found SCRAPPER which is a subunit of the SCF-type E3 ubiquitin ligase complex. SCRAPPER localizes synapses and directly binds to Rab3-interacting molecule 1 (RIM1), an essential factor for synaptic vesicle release. Our studies have shown that SCRAPPER is involved in the regulation of neurotransmitter release via the degradation of RIM1, which is essential for the synaptic vesicle release. Increased frequency of miniature excitatory postsynaptic currents (mEPSCs) and reduced paired-pulse facilitation, both of which are caused by increased release probability, showed that Scrapper knockout (SCR-KO) hippocampal neurons had increased levels of spontaneously released neurotransmitter. Upregulation of synaptic vesicle release was induced in SCR-KO neurons. In this time, we also investigated the contributions of the UPS on basic electrophysiological property and presynaptic long term plasticity using SCR-KO. Our data suggest the involvement of Scrapper on basal transmission and long term synaptic plasticity not only in the hippocampus but also in the cortex. Local protein degradation could be one of the regulatory mechanisms underlying neural transmission. (COI:No)

# Symposium 19

Critical events in circadian rhythm-supporting physiological adaptation systems

March 23 (Wed), 15:00 – 16:30, Room F

## S19-1

### Amplitude of circadian oscillations: possible involvement of the p160 coactivator family

Ikeda Masaaki[1,2]、Chiba Yasushi[1,2]、Kumagai Megumi[1,2]、Nakajima Yoshihiro[3]
*([1]Dept Physiol, Saitama Med Univ, Saitama, Japan、[2]Molecular Clock Project, Res Center for Genomic Med, Saitama Med Univ, Saitama, Japan、[3]Health Research Institute, AIST)*

Members of the p160 family of coactivators, which include SRC-1, GRIP1 and pCIP, are involved in the transactivation of nuclear hormone receptors, such as glucocorticoid receptors, by binding to them directly and recruiting CBP/p300 cofactors. It is known that *Bmal1* transcription is positively and negatively regulated by RORα and Rev-Erbα, respectively, via ROR response elements (ROR-REs) located in the *Bmal1* promoter region. In this study, we demonstrate that RORα-stimulated *Bmal1* transcription is significantly enhanced by p160 factors. No enhancement was observed in the *Bmal1* promoter with deleted ROR-REs. Two hybrid studies indicated that RORα directly interacts with p160. Transfection with p160 expression constructs enhanced the amplitude of *Bmal1* promoter oscillation without affecting phases or period lengths. Dominant-negative p160 coactivators reduced the amplitude. These results indicate that p160 has important roles in the regulation of *Bmal1* oscillation. (COI:No)

## S19-2

### Protein modification oscillator shifts gear of circadian clock

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

Circadian clocks in whole body coordinate harmonized circadian rhythms of various physiological functions based on circadian expression of ~10% of genes in genome. Night-oriented and globalized lifestyle cause dysfunction of adaptive protection programs based on circadian clocks, to increase various diseases, such as lifestyle-related diseases and cancer. Circadian clock is worked through repeated shift-up and shift-down of molecular gears with 1 day-period to drive circadian gene expression, which is robustly achieved by coordinated running of the core gear with transcriptional-translational negative feedback loop based on clock genes (Bmal1, Clock, Cry, Per) and regulatory gears based on other molecular events such as clock protein modification. In this study, as a critical regulatory gear, we captured CK2-mediated circadian phosphorylation of BMAL1-Ser90 using live-monitoring analysis, and elucidate oscillatory mechanism of the phosphorylation. A novel role of CRY as a suppressor for BMAL1-Ser90 kinase drives this protein modification oscillator. BMAL1-Ser90 phosphorylation plays a critical role in central and peripheral clocks, and oxidative stress-resistant pathway. Our findings potentially contribute to the medicine from novel approach that targets protein modification oscillator. (COI:No)

## S19-3

### Intracellular $NAD^+$ regulates circadian genes expression pattern

Nakahata Yasukazu、Bessho Yasumasa
*(Grad. Sch. Bio. Sci., NAIST, Ikoma, Japan)*

We and other groups have reported that intracellular $NAD^+$ amount demonstrates circadian oscillation, because the gene expression of rate limiting enzyme of $NAD^+$ salvage pathway "NAMPT" is regulated by circadian clock. Circadian $NAD^+$ oscillation regulates not only output physiological events but also circadian clock itself. $NAD^+$ consuming enzymes SIRT1 deacetylase and poly (ADP-ribose) polymerase 1 (PARP1) control acetylation status of histones, BMAL1 and PER2 and poly ADP ribosylation status of CLOCK to fine-tune the circadian genes oscillation. These findings suggest that controlling intracellular $NAD^+$ amount is crucial for maintaining circadian genes expressions precisely. To assess that, we tried to increase or decrease intracellular $NAD^+$ amount in NIH3T3 cells by genetically or pharmacologically. So far, we found that increasing $NAD^+$ up to 2-fold or decreasing by 50% rarely affect circadian machinery. However, the period of circadian clock was prolonged when $NAD^+$ amount was below 50% compared to control condition. We are now trying to reveal molecular mechanisms how $NAD^+$ amount modulates circadian period. In this talk, I would like to discuss how $NAD^+$ affects circadian machinery and when it happens under physiological or pathological conditions. (COI:No)

## S19-4

### VIP and AVP signaling regulate circadian cellular networks in the suprachiasmatic nucleus during postnatal development

Ono Daisuke[1]、Ono Daisuke[1]、Honma Sato[2]、Honma Ken-ichi[2]
*([1]Photonic Bioimaging Section, Grad. Sch. of Med. Hokkaido Univ. Sapporo, Japan、[2]Dept Chronomedicine, Grad. Sch. of Med. Hokkaido Univ. Sapporo, Japan)*

In mammals, the suprachiasmatic nucleus (SCN) in the hypothalamus plays a critical role in the expression of circadian rhythms. According to the current hypothesis, cellular circadian rhythms are generated by a transcription and translation feedback loop which involves several clock genes and their protein products. Recently we found that mice lacking *Cry1* and *Cry2* (*Cry1,2*$^{-/-}$) exhibited robust circadian rhythms in the cultured neonatal SCN slices. However, the circadian rhythms on the SCN tissue level disappeared during postnatal development, due to desynchronization among cellular rhythms. These results suggest the differential networks for circadian rhythm expression on the SCN tissue level between neonatal period and adulthood. The SCN contains two major neuropeptides arginine vasopressin (AVP) and vasoactive intestinal polypeptide (VIP) in the shell and core regions, respectively. They have been regarded as important factors for cellular signaling in the SCN, but it remains unclear how these molecules work in the network during postnatal development. Here we show that in *Cry1,2*$^{-/-}$ SCN, VIP signaling is critical for the tissue-level circadian rhythms in the neonates, and AVP signaling is substantially attenuated throughout the life. We also found multiple cellular clusters with different circadian periods in the SCN. They were differently integrated by VIP and AVP signaling depending on postnatal development. (COI:No)

## S19-5

### Circadian rhythm and toxicology, Chronotoxicology

Miura Nobuhiko、Ohtani Katsumi
*(Natl. Inst. Occup. Safety Health)*

In the modern society, the globalization developed by technologies such as telecommunication and transport established a 24-hour society. Over 25% exceedance of shift worker ratio to the total employees means that shift work is essential working arrangement. Therefore, shift workers may receive exposure to toxic substances at any hour of day or night, with quite higher concentration than general environment. If shift workers exposed to toxic substances in the time when their biological defense functions reduced, then toxicity may express strongly in their body. It is well known that the administration timing influences the efficacy of various drugs, such as antitumor, antiasthma, antihypertensive and antiangiogenic, as chronotherapy. However, the toxicological viewpoint is still scarce in regard to the toxic severity of toxic substances in a day. We have introduced this viewpoint, chronobiology, into toxicology as Chronotoxicology. In this symposium, we talk about Chronotoxicology with presenting the diurnal variation of heavy metal-induced toxicity in mice, and the newly role of PER2 protein as a biological defense factor. Further, it has been considered that shift work induces circadian disruption and attributes to the severe health disorders such as carcinogenesis, obesity, cardiovascular diseases. We talk about the testicular disorder induced by circadian disruption using shift work model. (COI:No)

# Symposium 20

PIPs-protein interaction in cell physiology

March 24 (Thu), 15:00 – 16:30, Room K

## S20-1

### Spatiotemporal dynamics of the phosphatidylinositol lipids signaling pathway for chemotaxis

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Eukaryotic chemotaxis occurs during a variety of physiological and pathological processes including immunity, neuronal patterning, morphogenesis and nutrient finding. Recent researches of the molecular and cellular biology of chemotaxis have demonstrated that the molecular mechanisms are highly conserved among many eukaryotic cells including human leukocytes and social amoebae, Dictyostelium discoideum. The chemotactic signaling system of Dictyostelium cells consists of G protein-coupled chemoattractant receptors, their coupled trimeric G-proteins and the downstream parallel signaling pathways including the phosphatidylinositol (PtdIns) lipids signaling pathway. PtdIns lipids have been identified as key signaling mediators for random cell migration as well as chemoattractant-induced directional migration, although how the PtdIns lipids are organized spatiotemporally to regulate cellular motility remains to be clarified. Here we report spatiotemporal dynamics of the PtdIns lipids signaling pathway in Dictyostelium cells. We found self-organized traveling waves of PtdIns 3,4,5-trisphosphate, PtdIns 4,5-diphosphate, PI3K and PTEN on the membrane work as signals to control cell migration. Characteristic oscillatory dynamics within the PtdIns lipids signaling system were determined experimentally, and the reaction-diffusion model was developed. Based on the quantitative live imaging analysis of the PtdIns lipids signaling pathway, we will discuss the possible mechanism by which cells can sense and transduce chemotactic signals to control cellular motility for chemotaxis. (COI:No)

## S20-2

### Is $PIP_2$ involved in the effect of insulin on ion channels?

Ishii Kuniaki、Wu Minghua、Takahashi Marie、Oshima Shingo、Nagasawa Yoshinobu、Obara Yutaro

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Many ion channels and transporters require phosphatidylinositol 4,5-bisphosphate ($PIP_2$), a minor component of the plasma membrane, for their function. The $I_{Ks}$ (KCNQ1/KCNE1) channel is one of such $PIP_2$-sensitive ion channels. It is well known that activation of $G_q$PCR inhibits the $I_{Ks}$ via phospholipase C (PLC)-mediated $PIP_2$ reduction. $PIP_2$ is a substrate not only for PLC that breaks $PIP_2$ into $IP_3$ and DAG, but also for phosphoinositide 3 kinase (PI3K) that phosphorylates $PIP_2$ to $PIP_3$. We have reported that insulin suppresses KCNQ1/KCNE1 currents using the *Xenopus* oocyte expression system. The pharmacological experiments indicated that signaling molecule(s) downstream of PI3K and upstream of Akt was involved in the insulin effect. Therefore, we investigated the possible involvement of $PIP_2$ in the insulin-mediated KCNQ1/KCNE1 suppression. We used *Ciona intestinalis* voltage sensing phosphatase (Ci-VSP) to manipulate $PIP_2$ content in the oocytes. The data obtained suggested that $PIP_2$ reduction was responsible for the insulin effect, although a definite conclusion has not been reached yet. When $PIP_2$ was monitored with PLCδPH-GFP, obvious reduction of membrane $PIP_2$ by $G_q$PCR activation was observed. However, insulin did not cause any reduction of membrane $PIP_2$, which does not support our hypothesis of $PIP_2$ involvement in the insulin effect. This might be simply because $PIP_2$ reduction by PI3K activation is too little to be detected by PLCδPH-GFP. Although the result has not been a success, we would like to introduce our attempt to create a new GFP-based probe for $PIP_2$. (COI:No)

## S20-3

### An arrhythmic mutation modifies TRPM4 channel gating via altered PIP2 sensitivity

Hu Yaopeng[1]、Kurahara Lin[1]、Shioi Narumi[2]、Hiraishi Keizo[1]、Ichikawa Jun[1]、Numata Tomohiro[1]、Okamura Yasushi[3]、Inoue Ryuji[1]

*([1]Dept. Physiol., Sch. Med., Fukuoka Univ., Fukuoka, Japan 、[2]Dept. Chem., Fac. Sci., Fukuoka Univ., Fukuoka, Japan 、[3]Dept. Integ. Physiol., Sch. Med., Osaka Univ., Osaka, Japan )*

A $Ca^{2+}$-activated TRPM4 channel is abundantly expressed in the heart, and its activity is greatly upregulated during sustained mechanical and neurohormonal stresses to induce arrhythmic changes. We found that a single arrhythmogenic mutation on its distal N-terminus ($Glu^5$ to $Lys^5$; E7K) greatly affects its gating properties in association with the endogenous $PIP_2$ level. In this study, to more thoroughly understand it, we conducted detailed analyses of this channel with its charge-reversing and -enhancing mutants E7K and $ENE^{5-7}$ respectively.In expression system, intracellular application of a short soluble form of $PIP_2$ $diC_8PIP_2$ restored the channel activity which had faded after membrane excision. The potency of $diC_8PIP_2$ for this action was largest in E7K with altered voltage-dependency. Similar results were also obtained when endogenous $PIP_2$ level (monitored by FRET) was reduced by activation of voltage-sensing phosphatase by depolarizing pulses/ramps of varying durations. Phosphoinositide-binding assay suggested that E7K may have an enhanced affinity to $PI(4,5)P_2$, and co-expression of a TRPM4 N-terminal polypeptide tended to counteract the activation of TRPM4 channel. Finally, numerical simulation based on a modified Luo-Rudy model indicated that depletion of $PIP_2$ may protect against arrhythmic propensity due to increased TRPM4 activity, but this may be severely disrupted by the E7K mutation. (COI:No)

## S20-4

### Function of Voltage-sensing phosphatase in mice sperm

Kawai Takafumi[1]、Miyata Haruhiko[2]、Nakanishi Hiroki[3]、Sakata Souhei[1]、Arima Hiroki[1]、Miyawaki Nana[1]、Okochi Yoshifumi[1]、Watanabe Masahiko[4]、Sakimura Kenji[5]、Sasaki Takehiko[3,6]、Ikawa Masahito[2]、Okamura Yasushi[1]

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Voltage-sensing phosphatase (VSP) consists of the voltage-sensor domain and phosphoinositide phosphatase domain. VSP shows phosphatase activity that is coupled to membrane potential (Murata et al, Nature. 2005). While its expression has been known in secondary spermatocyte in testis of mice, its biological function in sperm remains elusive. In the present study, we examined whether endogenous VSP can regulate PIP2 levels in mice sperm. Furthermore, we tried to examine the biological function of VSP in mice sperms, by focusing on acrosomal reaction, capacitation, sperm motility and so on. To elucidate them, we used knockin mice (VSP-KI mice) in that endogenous VSP gene was replaced by Venus. Our findings suggest that VSP may be important for the function of mice sperm. A possible mechanism underlying it is discussed. (COI:No)

# Symposium 21

The front line of research on the brain of athletes

March 23 (Wed), 9:00 – 10:30, Room E

## S21-1

### Neural substrate for motivational regulation of motor performance

Nishimura Yukio

*(Department of Developmental Physiology, National Institute for physiological Sciences)*

Positive emotion leads to peak performance in various occasions such as examination and competition in sport. It is generally thought that limbic system regulates motivation-driven effort but is not involved in the direct control of movement. The ventral midbrain (VM) including the ventral tegmental area (VTA) plays a critical role in processing motivation. However, the neural mechanism linking the motivational system and motor system is unclear. Here we propose that potential neural substrate for motivational regulation of motor control. Electrophysiological evidences documented that VM directly projects not only to the emotional system such as the orbitofrontal cortex (OFC) but also to the motor system such as the senrorimotor cortex (SMC). In addition, electrical stimulation of VM induced post-stimulus facilitation effect in multiple muscles. The onset latencies of the evoked muscle responses were longer than that of EMG responses evoked by stimulation of the motor cortex. We further found anatomical di-synaptic projections from the VM to cervical spinal cord that innervates upper limb muscles, using retrograde transsynaptic transport of rabies virus. These results suggest that the VM-Spinal multisynaptic projections may be the pathway of motivational driven motor control, and VM might be the key structure which is able to modulate motivation and motor outputs simultaneously. (COI:No)

## S21-2

### Brain activity during motor imagery in athletes

Mizuguchi Nobuaki、Kanosue Kazuyuki

*(Fac Sport Sci, Waseda Univ, Japan)*

Neural mechanism underlying brilliant sport performance would be the general interest. Mechanisms of motor control in sport movements (i.e. complex whole-body movements) are quite different from usually-analyzed hand/arm movements such as grasping and reaching. For example, novice cannot imagine the movement such as a triple salto but can imagine any hand/arm movements. To clarify neural mechanism of motor control in complex whole-body movements, we investigate brain activity during motor imagery of the movements. Previous studies suggest that brain activity during motor imagery with hand/arm is similar to that during motor execution. In addition, activity in the motor related regions during motor imagery of hand movement was related with the capability of motor imagery. Therefore, we consider that brain activity, recorded by functional magnetic resonance imaging (fMRI), during motor imagery of complex whole-body movements would reflect the capability of motor execution of complex whole-body movements. We had an opportunity to record brain activity during motor imagery from a world crass gymnast. The gymnast imagined floor exercises which were performed by another gold medalist during fMRI scan. In the symposium, we will show the difference in brain activity between the world crass gymnast and medium (university) level gymnasts. (COI:No)

## S21-3

### Voxel-based Morphometry in Sports

Aramaki Yu

*(School of Health and Sport Sciences, Chukyo University, Aichi, Japan)*

Voxel-based morphometry (VBM) can be used to estimate the volume of local grey matter from brain structural MRI data. Using VBM, recent studies have revealed that grey matter volume in local brain areas can predict individual personality and skill. Different sports require different personalities and skills. Thus, we can apply VBM to sports. Recently, I have acquired brain structural MRI data from many athletes, including Olympians. To support the hypothesis that a neuroanatomical approach using VBM is useful to understand the neural substrate of sports abilities, here, I present the results of some of our current VBM studies: 1) difference in local grey matter volume between sprinters and long distance runners, 2) increase in grey matter volume by non-dominant hand throwing training, 3) correlation between local grey matter volume and performance under pressure in archery, and 4) difference in local grey matter volume between Olympians and college-level athletes. (COI:No)

## S21-4

### Neural bases of intuitive pass-target selection in soccer experts

Tanaka Keiji[1]、Wan Xiaohong[1,2]、Nagano Tomohisa[3]

*([1]RIKEN Brain Science Institute, Wako, Japan、[2]State Key Laboratory of Cognitive Neuroscience and Learning, Beijing Normal University, Beijing, China、[3]Faculty of Policy Management, Keio University, Fujisawa, Japan)*

Most open-skill sport games demand cognitive skills of quick, or intuitive, decision-making. We examined neural bases of intuitive pass-target selection in expert soccer players using fMRI. 16 professional soccer players who had been trained more than several years in the FC Barcelona youth academy participated the experiments. 14 amateur soccer players also participated the same experiments as a control group. They saw a 1-s motion clip of a soccer game taken from the side. While the first frame of the clip was presented stationary before the motion clip stated, a yellow circle indicated the player that the subject was to play. The player had a ball. The last frame was presented stationary after the motion clip, and if the ball was kept by the player, the subject had to decide, within 2s, to whom he should pass the ball among the three players marked by red circles (the pass task). If the ball was kept by a player of the opponent team, the subject had to report who was keeping the ball (the position task). By double-contrasting brain activities of professional players in the pass task with the position task and amateur players, activities in the head of caudate nucleus appeared. Our previous studies showed that the caudate head was also specifically activated in intuitive generation of the idea of the best next-move in shogi. The circuitry including the caudate head may commonly mediate intuitive problem-solving of experts in various domains. (COI:No)

# Symposium 22

How to evaluate and manipulate physiological characteristics of pulmonary circulation in pulmonary hypertension?

March 22 (Tue), 9:00 – 10:30, Room I

## S22-1

### Pulmonary artery input impedance in a rat model of pulmonary hypertension

Nishikawa Takuya[1]、Saku Keita[2]、Sakamoto Takafumi[3]、Kishi Takuya[2]、Sunagawa Kenji[2]

*([1]Dept Cardiovascular Medicine, Grad Sch Med, Kyushu univ, Fukuoka, Japan、[2]CDIC, Kyushu Univ, Fukuoka, Japan、[3]Dept Cardiovascular Medicine, Kyushu Univ, Fukuoka, Japan)*

Background: Pulmonary hypertension (PH) is a fatal disease which exhibits high pulmonary artery pressure (PAP) and often leads to life threatening right heart failure. The pulmonary arterial resistance has been a standard to characterize the mechanical properties of pulmonary arterial system, whereas arterial input impedance (Z) provides detailed dynamic mechanical characteristics. In this study, we evaluated how PH affects Z of pulmonary artery in Sugen/Hypoxia (SuHx) induced PH model rats.
Method: In Sprague-Dawley rats, we induced PH by an injection of SU5416 (20 mg/kg) and 3 weeks exposure to 10% oxygen. We measured pulmonary artery flow and PAP under irregular cardiac pacing at 0 (n=5), 3 (n=5), and 8 (n=3) weeks after SU5416 injection. We Fourier transformed both signals to frequency domain, and estimated Z.
Result: SuHx significantly and time dependently increased mean PAP (Baseline: 19.3±2.1, 3W: 46.3±5.4, 8W: 53.0±12.0 mmHg, $p<0.01$) and resistance (Baseline: 15.7±3.5, 3W: 49.5±6.7, 8W: 68.7±7.1 mmHg/ml/sec, $p<0.01$), whereas decreased compliance (Baseline: 3.9±1.1, 3W: 2.1±0.7, 8W: 1.7±0.4 $10^{-3}$×ml/mmHg, $p<0.01$).
Conclusion: PH markedly increased resistance and decreased compliance. The fact that changes in resistance are tightly coupled with that in compliance indicates that resistance vessels and compliance vessels are anatomically inseparable in SuHx induced PH model rats. (COI:No)

## S22-2

### A pulmonary artery impedance loading system for evaluating the pathophysiological significance of dynamic right ventricular afterload in pulmonary hypertension

Fukumitsu Masafumi、Kawada Toru、Shimizu Shuji、Uemura Kazunori、Sugimachi Masaru

*(Department of Cardiovascular Dynamics, National Cerebral and Cardiovascular Center, Osaka, Japan)*

**Background**: Pulmonary artery (PA) impedance, describing right ventricular (RV) afterload to pulsatile flow, is different among patients with various etiologies of pulmonary hypertension (PH). The purpose of this study was to examine pathological changes of PA impedance in PH model rats, and to develop a servo-control system to impose pathological PA impedance on the in vivo RV in normal rats. **Method and Results**: PA impedance in normal (n=10) and monocrotaline-induced PH (MCT-PH) rats (n=7) were quantified by a three-element Windkessel model (3-WK), which consists of characteristic impedance ($Z_C$), arterial compliance ($C_P$), and peripheral resistance ($R_P$). In the MCT-PH, $Z_C$ and $R_P$ were increased ($Z_C$: 0.12±0.04 vs 0.05±0.17 mmHg min/ml, P<0.01, $R_P$: 0.57±0.34 vs 0.25±0.11 mmHg min/ml, P<0.05), and $C_P$ was decreased (0.26±0.15 vs 0.70±0.19 ml/mmHg, P<0.001). In other 10 normal rats, a servo-controlled system was connected to the left PA, and altered PA pressure waveform for loading pathological PA impedance specified by the 3-WK. By activating the system, the difference between target and measured PA impedance modulus was decreased from 0.05±0.02 to 0.02±0.01 mmHg min/ml (P<0.001). **Conclusions**: $Z_C$ and $R_P$ were elevated, and $C_P$ was decreased in the MCT-PH. The developed system, that could impose pathological PA impedance, may offer a unique opportunity for quantitative analysis of RV performance in different types of PH. (COI:No).

## S22-3

### Efficacy of early pharmacological intervention for exercise-induced pulmonary arterial hypertension

Matsuda Akimasa、Yamada Norikazu、Ogihara Yoshito、Ito Masaaki

*(Dept of Cardiology, Mie Univ, Tsu, Japan)*

Backgrounds: Pulmonary arterial hypertension (PAH) is a rapidly progressive disorder and early detection of PAH is an important strategic objective to improve outcomes. We will clarify the potentials and issues of right heart catheterization (RHC) during exercise for the early detection of PAH associated with connective tissue disease(CTD). Methods: We evaluated the hemodynamic changes of RHC during exercise in patients with CTD who had more than 25 mmHg of tricuspid regurgitation pressure gradient in cardiac ultrasonography. We defined exercise-induced PAH (EIPAH) as maximum mean pulmonary arterial pressure (MPAP) >30 mmHg during exercise. Patients (n = 23) with EIPAH were stratified into PAH specific drug (D group, n = 5) and no medication (N group, n = 18). We compared D group with N group in hemodynamic response during exercise in follow-up period.Results: One-year follow-up data was obtained in 14 patients (age: 65±9 years, 12 females). D groups were 4 and N groups were 10. One patient of N group progressed to PAH. δMPAP (δ= value during exercise - value at rest) significantly increased in N group (baseline: 22.1±8.0 mmHg, follow-up: 26.6±7.6 mmHg, p<0.005) but not changed in D group (27.8±6.1 mmHg, 27.5±6.8 mmHg). δMPAP/δcardiac output (CO) tended to decrease in D group (8.5±2.1, 4.5±1.5) but significantly increased in N group (3.7±1.5, 5.6±2.6, p<0.05). Conclusion: It is supposed that early pharmacological intervention for EIPAH might inhibit the progression of pulmonary vascular remodeling and improve the hemodynamic abnormal response during exercise. (COI:No)

## S22-4

### Elevation of pulmonary input impedance in low frequency can worsen right ventricle-pulmonary artery coupling and pulmonary vascular reaction

Fuke Soichiro、Tsushima Ryu、Namba Yusuke、Kashihara Yuya、Moriya Tomoka、Tanaka Masamichi、Yumoto Akihisa、Saito Hironori、Sato Tetsuya

*(Dept Cardiology, Jpn Red Cross Okayama Hosp, Okayama, Japan)*

[Background] The relationship between pulmonary input impedance(Zn) and pulmonary vascular reaction(VR) to increased flow is not well known. [Methods] Nineteen patients suspected of having pulmonary hypertension(PH) were enrolled. Pressure and flow velocity in the main pulmonary trunk was simultaneously recorded. The characteristic impedance(Zc) was calculated in a time-domain manner, and Zn was calculated by harmonic decomposed manner. Right ventricular(RV) pressure and flow velocity were also recorded. The coupling efficiency(Ees/Ea) was calculated by using single beat-estimation of the peak hydromotive pressure. The exercise stress was given in stepwise manner by a supine ergometer. The pulmonary VR was defined as the slope of the plot of mean pulmonary pressure(mPAP) vs. cardiac output(CO).[Results] The mPAP was 17±8mmHg, CO; 4.2±1.4L/min. Ees/Ea was worsened along with elevation of mPAP (R=-0.606, p=0.006). Z0, |Z1| and Zc were significantly correlated with Ees/Ea (R=-0.552, p=0.014; R=-0.551, p=0.015; R=-0.497, p=0.030, respectively). The mPAP at peak exercise was 34±13mmHg, and pulmonary VR;5.2 ±2.9mmHg/L/min. |Z1|, |Z2|, |Z4|, and Zc were significantly correlated with pulmonary VR (R=0.669, p=0.001; R=0.713, p=0.001; R=0.459, p=0.048; R=0.483, p=0.036, respectively).[Conclusion] Elevation of Zc and pulmonary input impedance in low frequency resulting in decoupling of Ees/Ea and impaired pulmonary VR are observed in PH patients. (COI:No)

# Symposium 23

Women Scientists Symposium: The analysis of principles underlying the neuronal network formation in the developmental stage.

March 22 (Tue), 16:00 – 17:30, Room C

## S23-1

### The effects of D/L-Valine on tongue movement as the sensory system of taste during development

Yoshida Chiaki、Nakayama Kurita、Arata Akiko

*(Dept. of Physiol, Hyogo Coll. of Med, Nishinomiya, Japan)*

Sense of taste on the surface of the tongue and sent to the brain can feel sweetness, sourness, saltiness, bitterness and umami. At first the gustatory nerve connects to the solitary tract nucleus, and toward reticular formation; secondly, connects to parabrachial nucleus and reaches taste area in cortex. We produced isolated brainstem-spinal cord intact tongue preparation including solitary tract nucleus, parabrachial nucleus, and facial nucleus to analyze taste circuit keeping sensory-motor connection. We examined the effects of sweet amino acid D-valine and bitter amino acid L-valine on tongue movement focused on perinatal period (the embryonic day 16 (E16)-the postnatal day 3 (P3)). The tongue movement was recorded by bipolar-tungsten electrode inserted to tongue muscle in vitro preparation, and we also examined the behavior concerning taste in vivo P0-P3 rats; that was counted mouth movement as mastication. The tongue movement was irregular, and D/L valine effects were invisible in E16, but the tongue movement detected clearly and the effects of those were seen in E18. Application of D-valine to tongue increased tongue movement, but L-valine inhibited tongue movement or showed long delayed effect in P0-3 rat. In behavioral study, rat pups tend to drink D-valine but they avoided to drink L-valine. The differences of D/L valine were detected clearly. This avoidance behavior in P3 rat was stronger than that in P2. These results suggested that D/L-valine might have different certain taste receptors and the hate memory of bitter taste would be established in P2-P3 rats. (COI:No)

## S23-2

### Development of inhibitory system in the mouse spinal cord.

Shimizu Chigusa

*(Department of Molecular Anatomy, School of Medicine, University of the Ryukyus, Japan)*

In the mammalian spinal cord, glycine is one of the predominant inhibitory neurotransmitters as well as GABA. It is not clear about the formation of inhibitory neural circuits in the spinal cord because development of glycinergic network is not known. To reveal this, I examined developmental localization of glycine transporter 2 (GlyT2), by which glycine is removed from the synaptic cleft, in the developing mouse cervical spinal cord. Furthermore, the developmental localization of GlyT2 was compared with that of glutamic acid decarboxylase (GAD), a marker of GABAergic terminal, or vesicular GABA transporter (VGAT), a marker of inhibitory terminal by immunohistochemistry.(1)GAD was first detected in the ventral part on embryonic day 12 (E12). GlyT2 expression was started on E14. (2)On postnatal day 0 (P0), GAD was expressed in both dorsal (DH) and ventral horn (VH). However, GlyT2 was localized in the VH. (3)On P7, GAD and GlyT2 were co-localized in the VH. On P14, the level of GAD expression was decreased and that of GlyT2 was increased. Present results suggested as follows: there was ventral-to- dorsal gradient in development of both glycinergic and GABAergic system. Development of glycinergic system was delayed in 2days compared to GABAergic neural circuit. After P14, GABAergic synapses might be formed and sift to glycinergic synapses in the developing spinal cord, in particular ventral horn. This work was supported by Grants-in Aid the Ministry of Education, Culture, Sports, Science, and Technology of Japan Kiban C (Nos. 23500413 and 25430066)(COI No)

## S23-3

### Type 1 metabotropic glutamate receptor maintains mature retinogeniculate synaptic connectivity in the visual thalamus

Narushima Madoka[1]、Uchigashima Motokazu[2]、Yagasaki Yuki[1]、Harada Takeshi[3]、Nagumo Yasuyuki[1]、Uesaka Naofumi[4]、Watanabe Takaki[4]、Hashimoto Kouichi[5]、Aiba Atsu[3]、Watanabe Masahiko[2]、Miyata Mariko[1,6]、Kano Masanobu[4]

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In the dorsal lateral geniculate nucleus (dLGN), retinal afferents make eye-specific innervation pattern that is established around birth. During postnatal development, redundant retinogeniculate (RG) synapses are eliminated and matured synapses are maintained in a visual experience-dependent manner. The maintenance phase opens around postnatal day 20 (P20) since one week of visual deprivation from P20 causes abnormal remodeling of RG synapses. We found age-dependent increase of type 1 metabotropic glutamate receptor (mGluR1) expression that reached a plateau around P20 in the dLGN. In mGluR1 knock-out (KO) mice, formation and elimination of RG synapses was normal until P20 but the synapses were remodeled in mGluR1-KO mice older than P28 even in normal rearing condition. Importantly, pharmacological blockade or knock down of mGluR1 in the dLGN of wild-type mice triggered remodeling of RG synapses. Furthermore, activation of mGluR1 in the dLGN rescued remodeling induced by visual deprivation. These results indicate that mGluR1 is crucial for the experience-dependent maintenance of mature RG synaptic connectivity. (COI:No)

## S23-4

### Early auditory experiences shape neuronal circuit to form auditory memory in zebra finch song learnig.

Yazaki-Sugiyama Yoko

*(Neuronal Mechanism of Critical Period Unit, Okinawa Institute of Science and Technology (OIST) Graduate University)*

Sensory experiences during early life intensively shape neuronal circuits during well-timed windows of brain development, which would contribute to higher cognitive function, such as learning. Experience-dependent inhibitory circuit maturation and it's controlling of plasticity in mammalian visual and auditory cortex has been well studied. Similar to human speech acquisition, vocal learning in songbirds depends on early auditory experiences. During early development, juvenile songbirds listen to and form auditory memories of the adult tutor song, then they vocally match them to shape their own song in later sensorimotor learning. We have investigated whether early auditory experiences with tutor songs in the sensory learning phase can shape auditory cortical circuits in the juvenile zebra finch brain, presumably to form a memory of the song. We then identified the neuronal substrate for tutor song memory by recording single-neuron activity in the higher-level auditory cortex, called the NCM. After tutor song experience, a small subset of NCM neurons exhibit highly selective auditory responses to the tutor song. Moreover, blockade of GABAergic inhibition decreased the selectivity. Taken togather, it suggests that similar to mammalian cortex, recruitment of GABA-mediated inhibition shapes auditory cortical circuits to form a tutor song memory in zebra finch song learning during early development. (COI:No)

# Symposium 24

Health enhancement strategy derived from the investigation of mechanism for the regulation of oral functions.

March 22 (Tue), 16:00 – 17:30, Room I

## S24-1

### Mechanism-based novel analgesic treatment for oral ulcerative mucositis

Ono Kentaro[1]、Hitomi Suzuro[1]、Yamaguchi Kiichiro[1,2]、Ito Misa[1,3]、Nodai Tomotaka[1,4]、Inenaga Kiyotoshi[1]

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Oral ulcer induces severe and painful hypersensitivity to pungency and physical contact during meals. Particularly, in cancer patients, chemotherapy-induced oral ulcerative mucositis causes intractable pain, leading to delay and interruption in therapy. However, there are few effective treatments for oral ulcer-induced pain. To examine the pain mechanism in oral ulcer, we investigated pathophysiological characteristics of oral mucositis and evaluated spontaneous pain and mechanical allodynia in pre-clinical models by using our proprietary pain assay system. Oral ulceration by 50% acetic acid treatment causes spontaneous pain and mechanical allodynia. After administration of the chemotherapy drug 5-fluorouracil, the spontaneous pain and mechanical allodynia were exaggerated and mediated by prostanoid-dependent continuous TRPV1 activation and bacterial/mitochondrial toxins-dependent mechanical sensitization of TRPA1, respectively. After administration of the other chemotherapy drug cisplatin, the TRPA1-mediated mechanical allodynia was similarly exaggerated while the spontaneous pain was not caused probably due to activation of phagocytosis. Based on these pain mechanisms, we revealed that topical treatments with antibacterial drugs, TRPV1 and/or TRPA1 antagonists and the TRPV1/A1 channel pore-passing anesthetic QX-314 were more effective procedures than general anti-inflammatory treatments. (COI:No)

## S24-2

### Developmental changes of glutamatergic synaptic properties in rat jaw-closing motoneurons

Nakamura Shiro[1]、Nagata Shoko[2]、Nakayama Kiyomi[1]、Mochizuki Ayako[1]、Kiyomoto Masaaki[1]、Yamamoto Matsuo[2]、Inoue Tomio[1]

*([1]Dept Oral Physiol, Showa Univ Sch Dent, Tokyo Japan、[2]Dept Periodontol, Showa Univ Sch Dent, Tokyo, Japan)*

Feeding behavior of mammals drastically changes from suckling to chewing during early postnatal period. In this study, we examined the developmental changes of miniature excitatory postsynaptic currents (mEPSCs) and voltage responses evoked by focal dendritic stimulation in the retrogradely-labeled masseter motoneurons (MMNs) from P2-5, 9-12 and 14-16 Wistar rats. AMPA receptor- and NMDA receptor-mediated mEPSCs were observed at a holding potential of -60 and +40 mV mV, respectively. There were no significant differences in the amplitude and frequency of the AMPA components between three age groups, whereas those of NMDA components were significantly larger at P2-5 than at P14-17 MMN ($P < 0.05$). We next photostimulated the dendrites of MMNs using laser photolysis of caged glutamate in P2-5 and 9-12 MMNs. In P2-5 MMNs, we found that, with increasing laser intensity, the photostimulation-evoked responses grew in amplitude up to a certain threshold, where a step-like increase in the somatic voltage amplitude, similar to the NMDA spikes. The step-like depolarization was completely abolished by application of NMDA receptor antagonist. However, fewer P9-12 MMNs exhibited NMDA spikes compared to P2-5 MMNs. These results suggest that the glutamatergic synaptic inputs and the dendritic properties of the MMNs change during postnatal development and such changes may contribute to the developmental transition of feeding behavior from suckling to mastication. (COI: No)

## S24-3

### The role of GLP-1 releasing neurons in appetite regulation : application possibility in obesity drug.

Hisadome Kazunari

*(Dept. Oral Physiol, Grad. School. Dent. Med, Hokkaido Univ, Sapporo, Japan)*

Obesity is a serious problem in modern society, particularly because obesity is associated with other diseases, e.g. diabetes, cardiovascular disorder. Obesity has been tackled more or less successful with diet, life style changes or surgery. Although pharmacological agents have been used to treat obesity, therapeutic efficiency is only limited or inconclusive because molecular targets for appetite and body weight control is not well understood.Glucagon-like peptide-1 (GLP-1) is one of gut hormones act as a satiety signal in the brain. However, circulating GLP-1 is unlikely to reach receptors in the brain because of its rapid metabolization in the blood. On the other hand, there is a small population of GLP-1 releasing neurons in the brainstem, and we hypothesized that the GLP-1 releasing neurons may play an important role in generation of satiety signals.To investigate the physiological properties of GLP-1 releasing neurons, we used transgenic mice that express Yellow fluorescent protein (YFP) in these neurons, and performed a patch-clamp recording from the GLP-1 releasing neurons identified by YFP expression.We found that GLP-1 releasing neurons were directly activated by Leptin. Cholecysytokinin (CCK-8) also activated the neurons presynaptically. In contrast, orexin and ghrelin showed no effects. These data suggested that GLP-1 releasing neurons may play a role in the modulation of feeding relaying satiety signals from the periphery to the central nervous system.We discus the functional role of GLP-1 releasing neurons the regulation of food intake. (COI:No)

## S24-4

### The mechanism for sweet suppressive effect of leptin in mouse taste receptor cells.

Yoshida Ryusuke[1]、Shigemura Noriatsu[1]、Ninomiya Yuzo[1,2]

*([1]Sect. of Oral Neurosci., Grad. Sch. of Dental Sci., Kyushu Univ. 、[2]Div. of Sensory Physiol., Research and Development Center for Taste and Odor Sensing, Kyushu Univ.)*

Leptin regulates food intake, energy expenditure and body weight by activating leptin receptors (Ob-R) of the hypothalamus and other peripheral tissues. Leptin is also known to selectively suppress gustatory responses to sweet compounds. However, the molecular mechanism by which leptin suppresses sweet responses has not been elucidated. In this study, we demonstrate that leptin's effect on sweet responses is mediated by functional leptin receptor (Ob-Rb) and ATP gated $K^+$ ($K_{ATP}$) channel. Ob-Rb was abundantly expressed in taste cells expressing a sweet receptor component T1R3 but not in those expressing a Type I cell marker GLAST or a Type III cell marker GAD67. Leptin suppressed sweet responses but not bitter and sour responses of identified taste cells. This effect was suppressed by leptin antagonist and was not observed in leptin receptor deficient db/db mice. $K_{ATP}$ channel subunit SUR1 was well coexpressed with Ob-Rb in T1R3 expressing taste cells. $K_{ATP}$ channel blocker glibenclamide inhibited sweet suppressive effect of leptin in dose dependent manner. In addition, $K_{ATP}$ channel activator diazoxide mimicked the sweet suppressive effect of leptin. These results indicate that leptin suppresses taste responses of sweet sensitive taste cells via activation of Ob-Rb and $K_{ATP}$ channel. Sweet suppressive effect of leptin was almost abolished in diet induced obese (DIO) mice, suggesting that sweet-sensitive taste cells become leptin resistant by diet induced obesity. (COI:No)

## S24-5

### Regulation of hemodynamics in major salivary gland by autonomic nerves system.

Sato Toshiya

*(Div. of Physiol., Dept. of Oral Biol., Sch. of Dent., Health Sci. Univ. Hokkaido)*

Salivary gland hemodynamics play an important role in saliva production because salivary fluid is created from blood plasma. Previously, we demonstrated parasympathetic cholinergic and non-cholinergic (VIP-ergic) vasodilation in submandibular glands evoked by electrical stimulation of orofacial sensory nerves. The activation of parasympathetic nerves innervating glandular blood vessels is thought to be important in the reflex condition because of the rapidity with which glandular blood flow increases. It seems likely that the control of parasympathetic vasodilation differs among major salivary glands because the distribution of serous and mucous acini varies among the three glands. In the present study, we examined hemodynamics in the submandibular, parotid and sublingual glands during electrical stimulation of the central cut end of the lingual nerve in urethane-anesthetized rats using a laser speckle imaging flow meter. Stimulation of the left lingual nerve induced intensity- and frequency- dependent parasympathetic vasodilation in glands predominantly on the ipsilateral side. The magnitude of the changes in vascular conductance was greater in the submandibular gland than the other two glands. Vasodilation in the parotid and submandibular glands is mainly evoked by cholinergic fibers, while vasodilation in the sublingual gland is evoked by cholinergic and non-cholinergic (VIP-ergic) fibers. Thus, our results indicate that vasodilation occurring via the somato-autonomic reflex in major salivary glands is regulated by different neural mechanisms. (COI:No)

# Symposium 25

Unknown biological effects of important metabolites

March 24 (Thu), 9:00 – 10:30, Room H

## S25-1

### 5-Aminolevulinic acid: foe or friend to the cellular metabolism?

Morimoto Yuji

*(Dept. Integrative Physiology and Bio-Nano Medicine)*

5-Aminolevulinic acid (ALA) is widely distributed in both plant and animal cells, and it is the common precursor of heme. In animal cells, ALA is formed from glycine and succinyl CoA by ALA synthase in mitochondria. Administration of ALA enhances the cancer-specific accumulation of porphyrins. ALA-induced porphyrins can be fluoresced and visualized in neoplastic regions. This technique exploits ALA-induced differences in fluorescent signatures between normal and cancer tissues. Therefore, the technique is generally termed fluorescence diagnosis or photodynamic diagnosis (BMC Res Notes 2011, 4:66).

However, whether ALA application affects cellular kinetics is not clear. Most of previous reports showed that ALA may be a prooxidant (J Appl Physiol 1992, 72:226), corresponding to the damage of proteins related to porphyrin biosynthesis, while some other papers indicated the promotive effect of ALA on cellular metabolism(Int J Dermatol 2008, 47:1298). In this paper, our latest data regarding ALA role in cellular proliferation will be presented. (COI:No)

## S25-2

### Visualizing glucose transport of a variety of cells using fluorescently labeled glucose derivatives

Yamada Katsuya

*(Depr Physiol, Hirosaki Univ Grad Sch Med, Hirosaki, Japan)*

Mammalian cells utilize glucose as a carbon source and an essential fuel, taking it up in a stereoselective manner through glucose transporters like GLUTs, whereby only D- and not L-, glucose is recognized. Cellular uptake of D-glucose through GLUTs has been monitored by 2-NBDG, a green fluorescence emitting D-glucose analogue, for a wide variety of cells including brain, pancreas, and tumor cells. To precisely evaluate stereoselectivity of the uptake, we developed 2-NBDLG, the first commercially available fluorescent L-glucose analogue (fLG), as a negative control substrate. Unexpectedly, 2-NBDLG was taken up into a specific population of tumor cells. A combined use of 2-NBDLG with 2-TRLG, a membrane-impermeable L-glucose analogue emitting red fluorescence, demonstrated cellular heterogeneity in 2-NBDLG uptake as well as membrane states in graded, two-color fluorescence. Clinical studies using living ascites cells from cancer patients are currently underway, where two fLGs, 2-NBDLG and 2-TRLG, are applied simultaneously. Such methods might help characterize functional properties of living tumor cells in clinical settings. (COI:Properly Declared)

## S25-3

### Polyunsaturated fatty acids to keep and/or enhance cellular function in culture

Nakamura Takao、Sato Daisuke

*(Dept Biomed Inform Eng, Grad Sch Med Sci, Yamagata Univ, Yamagata, Japan)*

Polyunsaturated fatty acids (PUFAs) are known to play some important roles in cell function: for example, some of them become ligands of at least a part of PPARs, and control genes and disease states. They may further have other roles unknown.

Yet in cases of cell culture, little fatty acids are contained in conventional medium partly because biological cells are considered to synthesize saturated and monounsaturated fatty acids by themselves. In addition, in cases of cell lines in particular, PUFAs sometimes impair their function probably because of their characteristics similar to those of tumor cells. However, in culture using primary or stem cells, PUFAs may be essential to keep the cellular function normal.

Since the contractile performance of cardiomyocytes was known to be extremely low in primary cell culture, we compared fatty acid composition of cultured cardiomyocytes, harvested from rat embryos and cultured for 2 weeks, with that of age-comparable neonatal rats, and found that several PUFAs were considerably lower in cultured cells than in neonatal ventricles. And recently, we confirmed that the performance dramatically improved even with a simple supplementation of docosahexaenoic acid in medium: cardiomyocyte shortening caused by spontaneous contraction (no load) became around 6 folds.

The results suggest that, at least in primary cell culture, PUFA is one of the essential key factors to improve cardiomyocyte performance, and that PUFAs might have very important roles in maintaining or enhancing cellular function in culture of any kinds of cells except for cell lines.

(COI: No)

## S25-4

### Chiral amino acid metabolomics : A new frontier in physiological research

Mita Masashi[1]、Hamase Kenji[2]

*([1]Frontier Science Div, Shiseido, Tokyo, Japan、[2]Grad Sch Pharmaceutical Science, Kyushu Univ, Fukuoka, Japan)*

Almost all amino acids in the living world are in the L-forms, and the mirror images, D-amino acids, have been considered to be absent especially in higher animals. However, this homochirality hypothesis has been overturned, and recent progress in analytical technologies certified the presence and functions of D-amino acids in mammals including human. We have developed Chiral amino acid metabolomics technology based on two-dimensional high performance liquid chromatography to identify D- and L-amino acids as metabolites. Chiral amino acid metabolomics will provide the useful information for basic sciences, medicine and food research. According to the research in central nervous system, the amount of D-serine reaches a peak during the early postnatal period, and controls synaptic receptor activity to acquire kinesthetic memory in the cerebellum. Also we elucidated for the first time that one of the intractable diseases Amyotrophic Lateral Sclerosis (ALS), which is a neurodegenerative disease of selective motoneuronal death, has mechanism that causes imbalance of D-serine in the spinal cord by chiral amino acid metabolomic profiling. Chiral amino acid metabolomics platform is now available to create valuable insights for physiological functions of D-amino acids in mammals. To further clarify the potential of Chiral Amino Acid Metabolomics, we are searching for medical solutions, such as early diagnosis methods for not only neurological disease but also cardiovascular disease and metabolic disease. (COI:Properly Declared)

# Symposium 26

Molecular mechanisms underlying synapse formation and remodeling during development and age-related synaptopathy

March 22 (Tue), 9:00 – 10:30, Room E

## S26-1

### Role of LRRTM1 and LRRTM2 in synapse development, synaptic plasticity and memory

Tabrez Siddiqui[1], Steven Connor[2], Fergil Mills[2], Parisa Tari[2], Sarah Au-Yeung[2], Hiroshi Kawabe[3], Shernaz Bamji[2], Nils Brose[3], YuTian Wang[2], AnnMarie Craig[2]

*([1]Dept. of Physiology and Pathopysiology, University of Manitoba, Canada, [2]University of British Columbia, Vancouver, British Columbia, [3]Max Planck Institute of Experimental Medicine, Goettingen, Germany)*

The development of accurate synaptic connections between neurons is a central principle of brain organization. LRRTMs are synaptic cell adhesion proteins that may regulate synaptic connectivity in the brain. They trigger local presynaptic differentiation when presented to axons, and are characterized by region-restricted expression patterns in the brain, indicating possible roles in cell-selective synapse development and function. Postsynaptic LRRTM1 and LRRTM2 bind across the synaptic cleft to presynaptic neurexins, and mutations in LRRTM and NRXN genes increase the risk of developing psychiatric disorders. To assess these functions in vivo, we studied synapse development and plasticity in mice lacking both LRRTM1 and LRRTM2. We found that LRRTM1 and LRRTM2 regulate the density and functional and morphological integrity of excitatory synapses on CA1 pyramidal neurons in the hippocampus. We further discovered that LRRTM1 and LRRTM2 are essential for the maintenance of long-term potentiation in the CA3-CA1 Schaffer collateral pathway and for normal contextual fear conditioning, which is a hippocampus-dependent memory task. Taken together, these data implicate LRRTMs as mediators of synapse formation, synaptic plasticity and memory and suggest mechanisms by which mutations in LRRTMs may contribute to neuropsychiatric disorders. (COI:No)

## S26-2

### The frontotemporal dementia related gene, granulin regulates developmental synapse elimination in the cerebellum

Uesaka Naofumi、Kano Masanobu

*(Dept. Neurophysiol., Grad Sch Med, Univ of Tokyo, Tokyo, Japan)*

In postnatal development of vertebrates, mature neural circuits are established through refinement of early-formed immature circuits with numerous redundant connections. An important process of refinement is synapse elimination, in which some synapses are selectively strengthened/maintained and the others are weakened/eliminated. Although several molecules required for synapse elimination have been identified, many aspects of the mechanisms of synapse elimination remain to be elucidated. We have screened candidate molecules whose deletion causes significant changes in climbing fiber to Purkinje cell synapse elimination in the developing cerebellum. We found that knockout or knockdown of granulin, a causative gene of frontotemporal dementia, in postsynaptic Purkinje cells accelerated elimination of redundant climbing fibers and reduced the amplitude of synaptic inputs from strong climbing fibers that are presumed to survive developmental synapse elimination. As for putative receptors for granulin protein, we found that knockdown of sortilin, which is known to bind to granulin protein, in climbing fibers resulted in the similar phenotypes to those caused by the progranulin deletion in Purkinje cells. Moreover, the effects of sortilin knockdown in climbing fibers were occluded by simultaneous deletion of granulin in Purkinje cells. We conclude that granulin protein functions as a strengthening/maintenance factor for climbing fiber to Purkinje cell synapses through sortilin in climbing fibers. (COI:No)

## S26-3

### Maturation of actin cytoskeleton in dendritic spines and the age-dependent role in synaptic plasticity

Hanamura Kenji[1]、Kojima Nobuhiko[2]、Hiroki Yasuda[3]、Yamazaki Hiroyuki[1]、Shirao Tomoaki[1]

*([1]Dept Neurobiol & Behav, Gunma Univ Grad Sch of Med, Maebashi, Japan、[2]Faculty of Life Sci., Toyo Univ、[3]ERSC, Gunma Univ Grad Sch of Med)*

Dendritic spines have two major structural elements, PSDs and actin cytoskeleton. An actin-binding proteins in dendritic spines, drebrin has two major isoforms, drebrin E (DE) and drebrin A (DA). The isoform conversion from DE to DA occurs during synapse formation. To characterize the nature of these two isoforms, we introduced GFP-tagged proteins to cultured hippocampal neurons. Although both GFP-DE and GFP-DA accumulated in dendritic spines, they showed distinct turnover in dendritic spines. Fluorescence recovery after photobleaching (FRAP) demonstrated that stable fraction of GFP-DA is significantly higher than GFP-DE in dendritic spines. To examine the physiological role of this isoform conversion, we have generated knockout mice in which a DA-specific exon was deleted from the drebrin gene (named DAKO mice). In adult DAKO mouse brain DE continued to be expressed instead of DA. Electrophysiological study using hippocampal slices revealed that LTP induced by high frequency stimulation of CA1 synapses was impaired in adult DAKO mice, but not in adolescents. In parallel with the electrophysiological phenotype these mice exhibit impaired hippocampus-dependent fear memory in an age-dependent manner. The impairment was evident in adult mice, but not in adolescents. Thus, our data indicate that the isoform conversion of drebrin is critical and DA is indispensable for normal synaptic plasticity and hippocampus-dependent types of fear memory in adult brain. (COI:No)

## S26-4

### Molecular mechanism of active zone organization at neuromuscular synapse during development, maturation and aging

Nishimune Hiroshi

*(Dept. Anatomy and Cell Biol., Sch Med, Univ. Kansas, Kansas City, USA )*

Neural circuits convey information through synapses by releasing synaptic vesicles at presynaptic active zones. Thus, active zones play essential roles in the function, maturation, aging and pathology of the nervous system. The molecular mechanism of active zone organization at mammalian neuromuscular junctions (NMJs) involves synapse organizer laminin β2, laminin receptor voltage-dependent calcium channels (VDCCs), and active zone-specific proteins, such as Bassoon. Muscle-derived laminin β2 accumulates in synaptic cleft and binds extracellularly and specifically to P/Q-type VDCCs to anchor the channels at presynaptic terminals. VDCCs function as scaffolding proteins and interact intracellularly with active zone-specific protein Bassoon. Accumulation of active zone proteins at the presynaptic VDCCs forms the synaptic vesicle release site. During postnatal maturation, NMJs maintain the density of active zones while NMJs triple their size, which is likely to contribute to the efficiency of synaptic transmission. However during aging, active zones become impaired and active zone specific proteins are lost from presynaptic terminals. Super resolution microscopy analyses start to reveal localization of active zone proteins during maturation and their loss during aging. Propitiously, muscle exercise ameliorates the active zone impairment in aged NMJs. These findings suggest that NMJ active zones are plastic structure that needs to be maintained for the function of this synapse. (COI:No).

## S26-5

### Glial activity and age-related memory impairment in *Drosophila*

Junjiro Horiuchi

*(Tokyo Metropolitan Institute of Medical Science)*

Age-related memory impairment is a debilitating consequence of aging. In *Drosophila*, age-related impairments occur in two types of memory, short lasting 1 hr memory, and long lasting long-term memory (LTM). We demonstrate that both of these impairments occur through alterations in glial activity. Age-related impairments in 1 hr memory are regulated by neuronal protein kinase A (PKA) activity. We find that PKA activity in neurons regulates amounts of a glial metabolic enzyme, pyruvate carboxylase. Pyruvate carboxylase inhibits glial production of D-serine, a neuromodulator important for neuronal NMDA receptor activity. We show that increasing amounts of D-serine ameliorate age-related impairments in 1 hr memory. Consolidation of long-term memory (LTM) requires increases in activity of a glial transcription factor, Repo. We find that Repo regulates expression of a glial glutamate transporter, dEAAT1. dEAAT1 is required for LTM, and functions to reduce glutamate activity at synapses. Both Repo and dEAAT1 activity decrease upon aging, and increasing activity of both ameliorates age-related defects in LTM. Thus, age-related memory impairments are caused by an inability of glia to produce sufficient D-serine during memory formation, and an inability of glia to inhibit glutamate activity during memory consolidation. (COI:No)

# Symposium 27

Frontiers of chrono-nutritional research on physiology

March 23 (Wed), 15:00 – 16:30, Room K

## S27-1

### Chrono-nutritional study on the peripheral circadian clocks

Tahara Yu[1,2]、Shibata Shigenobu[2]

*([1]WIAS, Waseda Univ, Tokyo, Japan、[2]Lab Physiol, Pharmacol, Waseda Univ, Tokyo, Japan)*

Many physiological events show day-night fluctuations in mammals are regulated by the circadian clock system, located in the every tissues and cells. Environmental information such as light, food, or stress is an important cue for keeping their clock phase to an appropriate time and for maintaining their homeostasis, thus the manipulating light or food timings could regulate our circadian clocks. Recently we proposed a new research strategy chrono-nutrition, which aims to understand the timing of nutrient intake for the circadian clock, nutrient efficacy, and homeostasis. Our laboratory investigated that food-induced entrainment of peripheral circadian clocks includes insulin-dependent clock gene transcriptions, and nutrient such as high-digestible starch, DHA/EPA have power of clock entrainment through insulin secretion. In addition, we screened several nutrients and found that caffeine and amino acids produced potent entrainment of peripheral clocks. Additionally, we are investigating that short-chain fatty acid produced by micro biota shows phase-entrainment power of peripheral clocks. In this talk, I will overview our recent researches about chrono-nutrition, and discuss the importance of animal studies for the translational research in this field. This work was partially supported by the Council for Science, Technology and Innovation, SIP, Technologies for creating next-generation agriculture, forestry and fisheries. (COI:No)

## S27-2

### Short-term feeding at the wrong time is sufficient to induce obesity with hyperphagia, physical inactivity, hyperleptinemia, hyperinsulinemia, hypercholesterolemia and fatty liver in mice

Oishi Katsutaka[1,2,3]

*([1]Biol Clock Res Gr, AIST, Tsukuba, Japan、[2]Dept Comput Biol Med Sci, Grad Sch Front Sci, Univ Tokyo, Kashiwa, Japan、[3]Dept Appl Biol Sci, Grad Sch Sci Tech, Tokyo Univ Sci, Noda, Japan)*

Feeding at unusual times of the day (inactive phase) is thought to be associated with obesity and metabolic disorders in experimental animals and in humans. The present study aimed to understand the underlying mechanisms through which time-of-day-dependent feeding influences metabolic homeostasis. We compared metabolic functions between mice fed only during the sleep phase (DF, daytime feeding) and those fed only during the active phase (NF, nighttime feeding). After one week of time-imposed restricted feeding (RF), DF mice gained more weight, and developed hyperphagia, hyperinsulinemia, higher feed efficiency and adiposity than NF mice. The daily amount of running on the wheel was obviously reduced by DF. The amount of daily food consumption and hypothalamic mRNA expression of orexigenic neuropeptides were significantly higher in DF than in NF mice, although plasma leptin levels were significantly higher in DF mice. Significantly more lipids accumulated in the livers of DF than NF mice, which resulted from the increased expression of lipogenic genes. Temporal expression of circadian clock genes became synchronized to RF in the liver but not in skeletal muscle. Feeding at an unusual time of day (inactive phase) causes obesity and metabolic disorders by inducing leptin resistance, hyperphagia, physical inactivity, hepatic fat accumulation and adiposity. (COI:No)

## S27-3

### Association between human chorotype as assessed by midpoint of sleep, and dietary intake and health-related quality of life

Mito Natsuko

*(Yokohama National Univ, Kanagawa, Japan)*

It is not clear whether human chronotype is associated with dietary intake. Then, we analyzed the association between chronotype as assessed by the midpoint of sleep, and dietary intake in young and elderly Japanese women. Furthermore, we assessed the effect of chronotype on health-related quality of life in elderly Japanese women. The subjects were classified into quintiles with respect to the midpoint of sleep, from the earliest to the latest quintile. In the study of young women, the late midpoint of sleep was significantly associated with poor dietary intake of certain nutrients and foods. In the study of elderly women, the subjects were women over 65 years of age in the three-generation study of women on diets and health study. Health-related QOL and depression was assessed by the Japanese version short-form 36-item health survey (SF36) score and Center for epidemiologic studies depression scale (CES-D) score, respectively. In contrast to the results of young subjects, almost dietary intakes were not different between the groups by quintile of midpoint of sleep in the elderly subjects. On the other hands, the subjects with late midpoint of sleep adjusted sleep duration showed low physical functioning, low general health perception, and high CES-D score. These results suggested that the association between chronotype and dietary intakes may be affected by age. It was also suggested that chronotype as assessed by midpoint of sleep is associated with certain health-related QOL and mental health in elderly Japanese women. (COI:No)

## S27-4

### Chronotype or genotype? Determinants of diurnal variation of gastric motility and appetite sensations

Nagai Narumi

*(Dept Food Sci & Nutr, Sch Human Sci & Env, Univ of Hyogo, Hyogo, Japan)*

The endogenous circadian clock regulates daily oscillations in various behavioral and physiological processes of the human body, including those associated with digestive activity. With respect to the stomach, gastric motility shows circadian rhythm as well as mealtime variations in healthy subjects. Similarly, it has been reported that ghrelin, an appetite regulating hormone produced by the stomach, is secreted in anticipation of regularly scheduled mealtime. Indeed, the expression of ghrelin and *PER gene* is rhythmic in light-dark cycles and is synchronized to prior feeding, suggesting that the anticipatory activity, such as gastric motility before breakfast, depends on an endogenous circadian timing system. These findings raise the possibility that attenuated gastric locomotor activity before breakfast may reflect the modulation of peripheral and/or central circadian timing system. This presentation will describe a series of studies focusing on: 1) gastric motility before breakfast, and 2) diurnal variation of gastric motility, appetite sensations, and autonomic nervous system activities in young healthy women with different chronotype or genotypes.

This work was partially supported by the Japanese Council for Science, Technology and Innovation, SIP (Project ID 14533567), "Technologies for creating next-generation agriculture, forestry and fisheries" (funding agency: Bio-oriented Technology Research Advancement Institution, NARO).

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(COI:No)

# Symposium 28

Molecular pathogenesis of psychiatric and neurological disorders

March 22 (Tue), 9:00 – 10:30, Room G

## S28-1

### Appoptosin-Mediated Caspase Cleavage of Tau Contributes to Pathogenesis of Progressive Supranuclear Palsy and other taupathies

Xu Huaxi

*(Neurodegenerative Disease Research Program, Sanford Burnham Prebys Medical Discovery Institute, La Jolla, USA)*

Progressive supranuclear palsy (PSP) is a movement disorder characterized by tau neuropathology where the underlying mechanism is unknown. An SNP (rs1768208 C/T) has been identified as a strong risk factor for PSP. Recently, we identified a much higher T-allele occurrence and increased levels of the proapoptotic protein appoptosin in PSP patients. Elevations in appoptosin correlate with activated caspase-3 and caspase-cleaved tau levels. Appoptosin overexpression increased caspase-mediated tau cleavage, tau aggregation, and synaptic dysfunction, whereas appoptosin deficiency reduced tau cleavage and aggregation. Appoptosin transduction impaired multiple motor functions and exacerbated neuropathology in tau-transgenic mice in a manner dependent on caspase-3 and tau. Increased appoptosin and caspase-3-cleaved tau were also observed in brain samples of patients with Alzheimer's disease and frontotemporal dementia with tau inclusions. Additionally, appoptosin hetrozygous knockout mice exhibit significantly reduced long-term depression (LTD) and slowed memory decay, suggesting its key role in synaptic function. Our findings reveal a novel role for appoptosin in neurological disorders with tau neuropathology, linking caspase-3-mediated tau cleavage to synaptic dysfunction and behavioral/motor defects. (COI:No)

## S28-2

### Increased L1 copy number in neuronal genome of schizophrenia and implications for its pathophysiology

Iwamoto Kazuya

*(Dept Mol Psychiatry, Grad Sch Med, Univ of Tokyo, Japan)*

Schizophrenia is a sever psychiatric disease with unknown etiology. Accumulating evidence suggests that retrotransposition capability of long interspersed nuclear element-1 (L1) in neural progenitor cells provide somatic mosaicism among brain cells. We examined copy number of L1 in the brain genome of patients with schizophrenia, and found significant increase of L1 content in patients compared to controls. Increased L1 was also identified in iPS cell-derived neurons from schizophrenia patients with 22q11 deletions, and animal models of schizophrenia. In addition, whole-genome sequencing analysis revealed brain-specific L1 insertion in patients localized preferentially to synapse- and schizophrenia-related genes. Increased retrotransposition activity of L1 triggered by environmental and/or genetic risk factors may contribute to the susceptibility and pathophysiology of schizophrenia. (COI:No)

## S28-3

### Antidepressant action mediated through upregulation of dopamine D1 receptor system in the hippocampal dentate gyrus

Nishi Akinori

*(Dept of Pharmacol, Kurume Univ Sch of Med, Kurume, Japan)*

Understanding of the neurobiology underlying depression allows development of better therapeutic approach. The monoamine and monoamine receptor hypotheses of depression have been derived from analyses of antidepressant action. Antidepressants that inhibit reuptake of serotonin and/or noradrenaline are widely used, but dopamine system is not a major target of antidepressants. Here, we show that activation of dopamine D1 receptor signaling in the hippocampal dentate gyrus elicits antidepressant effects. Chronic treatment of mice with a selective serotonin reuptake inhibitor, fluoxetine, induced the expression of D1 receptors, but not other dopamine receptors, in mature granule cells of the dentate gyrus. The high expression of D1 receptors resulted in activation of cAMP/PKA signaling. In vivo microdialysis analysis revealed that the serotonin responses to a novel environment in the dentate gyrus were suppressed after chronic fluoxetine treatment due to high activity of D1 receptors. In behavioral studies, D1 receptor agonism was shown to enhance antidepressant action of fluoxetine in mice chronically subjected to restraint stress. These findings suggest that upregulation of dopamine D1 receptors after chronic antidepressant treatment contributes to antidepressant actions. The dopamine D1 receptor system can be a therapeutic target especially in antidepressant-resistant depression. (COI:No)

## S28-4

### Deficiency of tRNA modification causes the development of X-linked mental retardation

Wei Fan-Yan、Nagayoshi Yu、Tomizawa Kazuhito

*(Dept. Mol. Physiol. Faculty of Life Sci. Kumamoto. Univ. Kumamoto, Japan)*

Genetic mutations in X chromosome-linked genes have been associated with mental retardation (XLMR). Recently, linkage analyses performed in Belgian, Chinese and Japanese families have identified Ftsj1 gene as a novel candidate gene. Ftsj1 shares homology with a bacterial 23S rRNA methyltransferase FTSJ. However, the molecular function of Ftsj1 and its pathological relevance in mental retardation have remained unknown. We generated Ftsj1 knockout (KO) mice and performed a comprehensive analysis to reveal the physiological functions. Ftsj1 is responsible for the 2-O-methylation of cytosolic transfer RNAs (tRNAs) at position 32 and 34. The deficiency of Ftsj1-mediated methylation caused a significant decrease of protein translation in KO mice. Accordingly, there was a marked decreased of synaptic proteins including glutamate receptors and signaling molecules. The KO mice exhibited abnormal spine morphology and decreased LTP/LTD levels, which ultimately contributed to the impaired spatial learning. There results suggest that the hypomodified tRNAs impairs neuronal protein synthesis, which contributes to the development of mental retardation in Ftsj1-deficient mice and human. (COI:No)

# Symposium 29

Translational research on circadian rhythm

March 23 (Wed), 9:00 – 10:30, Room F

## S29-1

### Circadian clock development : From basic to translational

Yagita Kazuhiro
*(Dept. Physiol. Systems Biosci. Kyoto Pref. Univ. Med.)*

Circadian clock develops cell-autonomously and closely correlates with the cellular differentiation which is regulated by epigenetic mechanisms such as DNA methylation. Methyl-CpG-binding protein 2 (Mecp2) is an X-linked gene encoding a methylated DNA binding nuclear protein which regulates transcriptional activity. The mutation of MECP2 in humans is associated with Rett syndrome (RTT), a neurodevelopmental disorder. RTT patients frequently exhibit abnormal sleep patterns and sleep-associated problems, in addition to autistic symptoms, raising the possibility of circadian clock dysfunction in RTT. We successfully generated Mecp2-deficient mice on the wild-type C57BL/6 background and PER2Luciferase (PER2Luc) knock-in background by utilizing the clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 system. Generated Mecp2-deficient mice recapitulated reduced activity in mouse models of RTT, and their activity rhythms were diminished in constant dark conditions. Bioluminescence rhythms were analyzed using photomultiplier tubes (PMT) and high-sensitivity EMCCD camera-based microscopy in order to evaluate the molecular clockwork in the master pacemaker suprachiasmatic nucleus (SCN) with or without lacking Mecp2, PER2Luc. Real-time bioluminescence imaging revealed that the amplitude of PER2Luc driven circadian oscillation was significantly attenuated in Mecp2 deficient SCN neurons. Together, these results demonstrate that Mecp2 deficiency abrogates the circadian pacemaking ability of the SCN, which may be a therapeutic target to treat the sleep problems of RTT patients. (COI:No)

## S29-2

### Circadian effect of hypoxia to mice locomotor activities

Masubuchi Satoru
*(Dept Physiol, Aichi Med Univ, Aichi, Japan)*

Altitude sickness occurs when we climb to high altitudes quickly. Low oxygen causes symptoms such as a headache, loss of appetite, and sleep trouble. Recently, many people try overnight rush climbing to see sunrise from the top of high altitude mountain such as Mt. Fuji (Bullet climbing). This hard climbing is warmed to increase the risk of altitude sickness. We examined the circadian effects of acute and short time hypoxia to animal behavior. Locomotor activities of ICR mice were continuously monitored by pyroelectric infrared sensors under 12h-12h light-dark condition (light on time was defined as ZT12) for more than 2wks. Next, mice were moved to 8% oxygen at various time points in a day (Day-time, ZT0-9; Dusk-time, ZT6-15; Night-time, ZT12-21; Dawn-time, ZT18-ZT3). After exposure, animals were backed to room-air and behavior monitoring. The effects of hypoxia to activities were time dependent. Day-time hypoxia reduced activities of 3-15h after exposure. In contrast, Night-time hypoxia increased activities of 0-3h after exposure. Dawn-time hypoxia had bidirectional effects. Activities of 0-3h after exposure were increased and activities of 9-18h after exposure were reduced. Dusk-time hypoxia did not changed activities. Because of strong and long-lasing behavior changes, we focused on Day-time hypoxia. *mPer1* increment and *mBMAL1* decays in the forebrain suggest the contribution of these clock genes expression changes to activity suppression by Day-time hypoxia. Our results provide a possibility the hypoxia at nighttime in human (resting phase: corresponds to Day-time in mice) has stronger effects which trigger altitude sickness. (COI:No)

## S29-3

### The clock, mitochondria, and heart function

Kohsaka Akira、Maeda Masanobu
*(Dept Physiol, Wakayama Med Univ, Wakayama, Japan)*

Heart function varies across the sleep/wake cycle to meet the metabolic requirements of the body. The time-dependent heart function relies on the circadian clock system in which a core set of clock genes play a major role. The molecular clocks reside in nearly all cells throughout the body, and the heart also possesses its own clock which is thought to generate local rhythms. However, recent studies have shown that the molecular clock in the heart also participates in such fundamental biological processes as cardiac energy metabolism. These include glucose and lipid transports, fatty acid oxidation, and mitochondrial biogenesis in the heart. We have recently reported that mice with heart-specific ablation of the circadian clock gene *Bmal1* develop heart failure with age. The heart tissue without *Bmal1* function shows sever defects in mitochondrial structure and function such as mitochondrial quality and the electron transport chain/oxidative phosphorylation. These findings indicate that the molecular clock in the heart is closely coupled with the regulation of mitochondrial dynamics and the production of bioenergy, and subsequently maintains heart function. Here, we will discuss the importance of maintaining mitochondrial bioenergy by the molecular clock and its relevance to the development of heart failure. (COI:No)

## S29-4

### Epidemiological evidence about health risk of Shift work.

Kubo Tatsuhiko
*(Univ of Occup and Environ Health, Japan.)*

Most potent exposure on circadian rhythm disruption on human among work place is shift work. Shift work is a method of organization of working time in which workers succeed one another at the workplace so that the establishment can operate longer than the hours of work of individual workers. Roughly 15-20% of workers are estimated to work on shift schedules worldwide. In Japan, the number of night shift workers has been still increasing. The prevalence of night work among Japanese employees was 13.3% in the year 1997, 17.8% in 2002, 17.9% in 2007, and 21.8% in 2012. It was estimated that in Japan twelve million workers currently engaged in night work.

This presentation will try to introduce not only current evidence of shift work, but current limitation of epidemiological studies about shift work. Numbers of epidemiological studies about health impact of shift work have published yet; there are some common limitations for those studies; which are difficulty in work schedule follow-up, potential selection bias due to the healthy worker effect, and confounding by socioeconomic factors. Among those, the first limitation is about exposure assessment. Personal status of work schedule varies over time and precise long term follow up is rarely accomplished. Further there are various types of work schedule for shift work. It is assumed that various types of shift schedule have different impacts on health though, it is not easy to collect precise work schedule data for each worker. To make breakthrough, advance in physiology or chronobiology on circadian rhythm disruption assessment is a key. Translational collaboration among academic fields is much needed. (COI:No)

# Symposium 30

The Sympathetic Nervous System in Cardiovascular and Cardiometabolic Disease

March 23 (Wed), 15:00 – 16:30, Room G

## S30-1

### Effects of neuronal noradrenaline uptake blockade on sympathetic nerve activity in chronic heart failure rats

Kawada Toru[1]、Akiyama Tsuyoshi[2]、Shimizu Shuji[1]、Sugimachi Masaru[1]

*([1]Dept Cardiovasc Dynamics, Natl Cereb and Cardiovasc Ctr, Suita, Japan 、[2]Dept Cardiac Physiology, Natl Cereb and Cardiovasc Ctr, Suita, Japan )*

**Background:** Desipramine is a tricyclic antidepressant which inhibits neuronal noradrenaline uptake and to a minor extent serotonin uptake. An increase in brain noradrenaline levels can inhibit sympathetic outflow from the central nervous system via activation of presynaptic $\alpha_2$-adrenergic receptors. Chronic heart failure (CHF) is characterized by autonomic abnormality with excess sympathetic activation. The present study tested whether intravenous desipramine would antagonize the excess sympathetic activation in CHF. **Methods:** A rat model of CHF was created by coronary artery ligation (n=6). After 9.5±0.7 weeks, carotid sinus baroreceptor regions were isolated and changes in sympathetic nerve activity (SNA) in response to pressure input were measured under anesthetized conditions. Effects of intravenous desipramine (1 mg/kg) were examined, and were compared with those in normal control (NC) rats (n=8). **Results:** The response range of SNA was 55.0±5.9% in NC and 43.0±10.7% in CHF. Desipramine significantly reduced the response range of SNA to 38.6±4.6% in NC and 23.8±10.1% in CHF ($P<0.01$ by two-way analysis of variance). The minimum SNA was 47.6±5.6% in NC and 57.2±9.7% in CHF. Desipramine significantly decreased the minimum SNA to 21.9±5.4% in NC and 36.7±11.4% in CHF ($P<0.01$). **Conclusion:** Neuronal noradrenaline uptake blockade suppresses SNA in both NC and CHF rats, suggesting that the mechanism of presynaptic $\alpha_2$ adrenergic inhibition is maintained in CHF. (COI:No)

## S30-2

### Metabolic disease and the sympathetic nervous system

Lambert Gavin

*(Baker IDI Heart & Diabetes Institute, Melbourne, Australia)*

While the sympathetic nervous system (SNS) is recognised as being implicit in cardiovascular control, it is important to appreciate that activation of the SNS also exerts substantial metabolic effects. Sympathetic nervous activation is evident in overweight and obese subjects. Stimulation of the SNS in this context, even in young adults, exerts detrimental end organ effects on the heart, kidney and vasculature. The metabolic syndrome is associated with adverse health outcomes and represents a growing problem in both developed and developing countries. While the mechanisms underpinning the metabolic alterations defining the syndrome are not completely understood, a growing body of evidence derived from both experimental and clinical studies indicates an important role of SNS activation in this scenario. Indeed, lifestyle interventions commonly recommended as first line treatments for patients with the metabolic syndrome such as diet and exercise have been shown to be associated with SNS inhibition and improvement in metabolic control. Pharmacological and device-based approaches to directly target SNS activation are available and have provided evidence to support the important role played by the SNS, particularly in the area of blood pressure and glucose control. While early evidence is encouraging, whether therapeutically targeting SNS overactivity exerts beneficial effects in regards to the prevention of the development of the metabolic syndrome and the reduction of its adverse consequences remains to be determined. (COI:No)

## S30-3

### The relationship between Depression and Coronary Heart Disease

David Barton

*(Monash Alfred Psychiatry Research Centre and Central Clinical School Monash University)*

Psychiatric and Coronary Heart Disease (CHD) are both highly prevalent conditions which both confer adverse outcomes on one another. Depression is the third leading cause of disease burden worldwide and is predicted to be the leading cause by 2030. Depression and CHD are conditions that both significantly decrease quality of life for the patient and impose a significant economic burden on society. There is now convincing evidence linking depression and CHD showing that Major Depression is an independent risk factor for developing heart disease and in an otherwise healthy person doubles the risk of developing CHD. Various candidate mechanisms might explain how depression increases the risk for CHD and subsequent cardiac mortality and morbidity. Some of these include behavioural and lifestyle factors, the sympathetic nervous system, platelet function, autoimmune and inflammatory mechanisms. Sympathetic hyperactivity has been implicated in increased cardiovascular morbidity and mortality in people suffering major depression, and it has long been known that 'stress' and heart disease is linked. Mental stress is one of the many cognitive symptoms that people with major depression suffer from, and laboratory mental stress has been shown to activate sympathetic nervous outflow. Depression is present in 1 of 5 outpatients with coronary heart disease and in 1 in 3 outpatients with congestive heart failure, yet the majority of cases are not recognised or appropriately treated. The present findings underscore the need to consider depression as a common and modifiable risk factor for CHD events. (COI:No)

## S30-4

### Management of Mental Illness and comorbid Cardiometabolic Disease

Arup Dhar

*(Human Neurotransmitters Laboratory, Baker IDI Heart & Diabetes Institute; Alfred Psychiatry, Alfred Health; Faculty of Medicine, Nursing & Health Sciences, Monash University)*

Those who suffer mental health illnesses, such as major depressive disorder, schizophrenia and, cognitive impairment commonly suffer comorbid cardiometabolic conditions. Mental health illnesses and cardiometabolic conditions have significant socioeconomic importance with the World Health Organisation states that they will be the leading two causes of global burden of disease by 2030. When they occur together the outcomes for both the mental health and cardiometabolic conditions significantly worsen evidenced with poorer quality of life, morbidity and mortality. The activation of autonomic stress pathways have been implicated as a potential neurochemical mechanism that links mental health illnesses and cardiometabolic disturbance. Psychological stress experienced by those suffering mental health illnesses has been shown to cause dysregulation of the sympathetic nervous system. There are a number of complexities in the management of psychiatric illness with some treatments conferring a lower cardiometabolic risk whilst others confer quite the opposite. Better understanding of the common pathophysiological pathways of both mental health and cardiometabolic disturbance will help us target more appropriate treatments, thus alleviating the morbidity, mortality and cost burdens of such conditions. There is a growing body of evidence that targeting the sympathetic nervous system may be therapeutically beneficial in reducing the aforementioned burdens of disease. (COI:No)

## S30-5

### Sympathetic modulation therapy in cardiovascular disease

Markus Schlaich

*(Baker IDI Heart and Diabetes Institute, Melbourne, and Royal Perth Hospital / University of Western Australia, Perth, Australia)*

The renal nerves play an important role in kidney function and blood pressure regulation. The major effects elicited by stimulation of efferent renal sympathetic nerves include sodium and water retention, vasoconstriction and release of renin. Afferent sensory nerve fibers are important mediators of renal signaling to integrative centres in the hypothalamus capable of regulating central sympathetic outflow. Anatomically, renal efferent and afferent nerves lie within the adventitia of the renal arteries and form a network that can be targeted by interventional approaches. While the introduction of catheter-based renal denervation demonstrated substantial blood pressure lowering effects in several clinical trials, data from the recent sham-controlled Symplicity HTN-3 trial questioned the efficacy of renal denervation. Efforts to unravel the reasons for these discrepant results have focused on a range of potential confounders including anatomical and procedural aspects. Indeed, data form post-hoc analyses indicate that sufficient renal denervation may not have been achieved in the majority of patients in Symplicity HTN-3. Furthermore, recent evidence from human post-mortem and functional animal studies revealed new insights into the anatomical distribution of renal nerves and their accessibility by intravascular therapeutic approaches. Integrating these findings into newly designed clinical trials will be key to determine the true potential of renal denervation in the treatment of hypertension and other clinical conditions characterized by increased sympathetic drive. (COI:No)

# Symposium 31

Central presentation of embodiments and its role in the functional organization and re-organization of cognitive-behavioral linkage.

March 23 (Wed), 15:00 – 16:30, Room E

## S31-1

### Self and other's body shared and differentiated in the parietal mirror neuron system

Murata Akira
*(Dept Physiol, Faculty Med, Kinki Univ, Osakasayama, Japan)*

The body is represented in the brain according to spatiotemporal dynamic manner. The body perception is based on visual, somatosensory, and intrinsic motor signals that update the internal representation of one's own body state, and therefor is key for sensory motor control. This system is also concerned to perception of action contribution to whether own self or other, namely "who" system proposed by Jeannerod. The network between the inferior parietal cortex and the ventral premotor cortex (F5), namely the ventro-dorsal stream, plays a pivotal role in visually-guided hand actions. Mirror neurons were found in this ventro-dorsal stream. The fundamental principle of mirror neuron is that the one's own action representation is shared with representation of other's action. However, we postulate that the mirror neurons in the parietal cortex contribute to not only shared representation of self and other, but also to the distinction between self and other. It is suspected that the ventro-dorsal stream is in common neural substrates with the "who" system. We discuss the functional role of the ventro-dorsal stream to the internal representation of one's own and other's body. (COI:No)

## S31-2

### Embodied-brain systems science and modelling

Ota Jun
*(Research into Artifacts, Center for Engineering (RACE), The University of Tokyo)*

The purpose of Embodied-brain systems program, which is one of those in MEXT Grant-in-Aid for Scientific Research on Innovative Areas, is to elucidate the neural mechanisms of the body representation in the brain and the mechanism of the long-term changes in this representation and to apply these findings to rehabilitation interventions.
As the Japanese society ages rapidly, we are experiencing a sharp increase in the number of cases of motor paralysis and other dysfunctions resulting from motor dysfunction, stroke, and neurodegenerative diseases. Thus, establishing effective rehabilitation techniques to overcome these motor dysfunctions is of paramount importance. We assume that we create and maintain a model of the body in the brain (body representation in the brain - internal representation of the body. Indicators of posture and body structure that are updated moment-to-moment by a wide range of sensory inputs that are related to motor performance.
We will attempt to combine brain science and rehabilitation medicine by using systems engineering. We thereby intend to gain an integrated understanding of motor control and somatognosia in order to create a new academic discipline that is known as embodied-brain systems science.
From the viewpoint of system modelling, we aim to construct mathematical models of the “slow dynamics” alteration of the body representation within the brain, influenced by fast dynamics. Understanding slow dynamics will be the key to rehabilitation therapy, which have been central to research. (COI:No)

## S31-3

### Pathophysiology of musician’s dystonia as revealed by multimodal neuroimaging

Hanakawa Takashi
*(Integrative Brain Imaging Center, National Center of Neurology and Psychiatry)*

Musician’s dystonia (MD) is a neurological disorder characterized by loss of voluntary motor control and muscular coordination during playing of a musical instrument. Pathophysiological mechanisms of MD remain unknown while aberrant neuroplastic changes are presumed to play a role (Furuya and Hanakawa 2015). We have been conducting a series of multi-modal imaging studies to advance the understanding of pathophysiology of MD. A functional magnetic resonance imaging study during a finger-tapping task revealed abnormally enhanced cerebeller activity and functional connectivity between the cerebellum and premotor/motor cortex in pianist’s dystonia (Kita and colleagues SfN2014). In consistent, we also found preliminary evidence for enhanced cerebeller activity in another type of MD. Meanwhile, an anatomical connectivity study with diffusion magnetic resonance imaging primarily showed abnormality of transcallosal fibers, including ones connecting between bilateral motor cortices, in pianist’s dystonia (Kita and colleagues SfN2015). Thus far, we have obtained fragmented pieces of evidence suggesting the involvement of aberrant reorganization of motor networks in MD. A remaining challenge is to organize those pieces into a full picture, which is sufficiently clear enough to allow us to develop efficient functional treatment for musician’s dystonia. (COI:No)

# Symposium 32

Effects of rehabilitation on brain stroke and the animal experiments

March 24 (Thu), 9:00 – 10:30, Room F

## S32-1

### Clinical issues in animal experiments of stroke and rehabilitation

Imai Itsuki

*(Dept Physical therapy, Nasu Neurosurgical Center, Tochigi, Japan)*

Over the past few decades, a considerable number of studies have been conducted on the effects of rehabilitation after stroke. Recently, animal studies provide insights into the brain events underlying behavioral recovery after stroke. These findings have significant influence on the rehabilitation after stroke in patients. In this symposium, I focus on the motor recovery after stroke from three perspectives; 1) rehabilitation timing (early or delayed), 2) critical periods (within several months after stroke), 3) rehabilitation dose (amount). In general, there is a consensus between animal experiments and human studies that earlier rehabilitation is more effective than delayed intervention. There is also a broad agreement in the time window of the critical period shown in the basic (animal) and clinical research. On the other hand, the doses of movement practice currently provided during stroke rehabilitation are substantially smaller than those used in the animal experiments. Therefore, it is possible that the doses of current rehabilitation are not adequate to drive the neural reorganization needed to promote poststroke motor function optimally. In conclusion, physiological studies with use of animals are very important to systematize the physical therapy scientifically. Vice versa, animal studies will be more valuable when they are translated into the human patients by physical therapists. Closer interaction between members in physiology and physical therapy is required. (COI:No)

## S32-2

### Rehabilitation promoted rapid motor recovery but delayed motor map reorganization in a rat cortical ischemic infarct model

Nishibe Mariko、Yamashita Toshihide

*(Department of Molecular Neuroscience, Graduate School of Medicine, Osaka University)*

Report 1: Consensus in the field of Neurorehabilitation is that neural reorganization helps support behavioral recovery after a brain injury. The temporal relationship of cortical map plasticity and functional improvement is, however, complex. We used a rat model of ischemic injury confined within the cortical map area of the caudal forelimb area. We then examined using the intracortical microstimulation (ICMS) how spared, adjacent cortical map of rostral forelimb area may be modulated over time, specifically upon 1) completion of receiving a rehabilitative therapy and 2) 3 weeks after completing a therapy. We found that map plasticity and behavioral recovery did not temporarily correlate, either with or without the rehabilitation therapy. Training-induced improvements were not reflected in spared motor maps until substantially later, suggesting early motor training after injury can help shape the evolving neural network. Report 2: Reelin, a glycoprotein, is essential in the construct of layer organization during development as well as in the adult neural plasticity. We first examined the consequence of such a highly disturbed cortical lamination on the cortical motor representations using ICMS. The general topographical layout of motor representations was pertained in homozygous reeler mice. Thus, reelin may not be necessary in the establishment of the ordered topographical motor representations. We will further investigate the role of reelin in cortical plasticity. (COI:No)

## S32-3

### The mechanism of motor map reorganization induced by rehabilitative therapy after stroke

Okabe Naohiko[1]、Shiromoto Takashi[1,2]、Lu Feng[1]、Maruyama Emi[1]、Himi Naoyuki[1]、Narita Kazuhiko[1]、Miyamoto Osamu[1]

*([1]Dept Physiol 2, Kawasaki Med Sch.、[2]Dept Stroke Med. Kawasaki Med Sch.)*

After brain injury, preserved brain areas compensate lost functions which were executed by damaged brain area. In these compensatory changes, survived neurons undergo morphological changes such as dendritic branching, axon sprouting and synapse formation to remodel neural network. Motor map reorganization is a compensatory change in motor system. Previous study demonstrated that motor map reorganization contributes to functional recovery and can be promoted by rehabilitative therapy. However, the mechanism how rehabilitative therapy promotes motor map reorganization is unknown. We have investigated how rehabilitative therapy modifies motor map reorganization using intracortical microstimulation (ICMS) and neural tracers. After stroke on caudal forelimb area (CFA) of the primary motor area, rehabilitative therapy enlarged preserved rostral forelimb area (RFA) of the secondary motor area in a time dependent manner. In anatomical neural connectivity analysis, rehabilitative therapy did not exert significant effect on the neural projection to RFA from other brain area. However, rehabilitative therapy significantly increased corticospinal axon fibers from RFA to spinal cord. Axon fiber was increased specifically in the spinal segments which innervate rehabilitative training related muscles. Our results suggest rehabilitative therapy may contribute the functional recovery by neural network remodeling in spinal cord. (COI:No)

## S32-4

### Motor skills training is more beneficial on motor functional recovery compared with treadmill exercise after intracerebral hemorrhage in rats

Ishida Kazuto[1]、Tamakoshi Keigo[1,2]、Takamatsu Yasuyuki[1,3]、Shibata Atsushi[1]

*([1]Dept Phys Ther, Grad Sch Med, Nagoya Univ, Nagoya, Japan、[2]Dept Phys Ther, Niigata Univ Health and Welfare, Niigata, Japan、[3]Dept Rehab, National Hosp Organ, Higashi Nagoya National Hosp, Nagoya, Japan)*

Therapeutic exercise (Ex) is beneficial on the motor functional recovery after stroke. The effects probably vary with the different kinds of Ex. We investigated the effects of two kinds of Ex, treadmill Ex and motor skills training (MST) on the motor functional recovery and synaptic plasticity following intracerebral hemorrhage (ICH) in rats. Male Wistar rats were injected with collagenase into the left striatum to induce ICH. Animals were assigned to ICH or Sham, and each was divided into Ex and no Ex group after ICH surgery. Motor deficit score (MDS) was assessed daily after surgery. Volume of tissue lost, dendritic morphology and PSD-95 protein level in the striatum or motor cortex were analyzed at 14 or 28 days after surgery. ICH surgery caused significantly gross motor impairment and recovered over time. In both Ex methods, MDS were significantly improved. In treadmill study, the dendritic length and arborization was more complexed than that of ICH only in the contralateral striatum and ipsilateral motor cortex, but PSD-95 level was not changed. In MST, the PSD-95 protein expression in the motor cortex was significantly increased in the late phase and that in the striatum was significantly increased in the early phase by MST after ICH. These data suggest that MST could enhance the plastic changes and bring more efficient effects of motor recovery than treadmill Ex. (COI:No)

# Symposium 33

Structure based approaches to the functioning mechanisms of ion channels and the search for their novel inhibitors

March 24 (Thu), 15:00 – 16:30, Room J

## S33-1

### Structural insights into divalent cation modulations and ligand specificity of ATP-gated P2X receptor channels

Fujiwara Yuichiro[1]、Kasuya Go[2]、Hattori Motoyuki[2,3]、Nureki Osamu[2]

*([1]Dept Physiol, Grad Sch Med, Osaka Univ, Suita, Japan、[2]Dept Biol Sci, Grad Sch Sci, Univ Tokyo, Tokyo, Japan、[3]Dept Physiol Biophys, Sch of Life Sci, Fudan Univ, Shanghai, China)*

P2X receptors are extracellular ATP-gated cation channels involved in diverse physiological processes from muscle contraction to neurotransmission to pain. Despite the structure determinations of P2X4 in the apo, closed, and in the ATP-bound, open states, the molecular mechanisms of the gating modulation and the ligand recognition remain elusive. Such elements of receptor activation are reportedly associated with pathophysiological implication, and elucidation of the mechanisms will be applicable for drug development. Here we present the two crystal structures of P2X: in complex with a weak affinity agonist, CTP; in complex with extracellular $Zn^{2+}$ and ATP. Structure-based electrophysiological analysis identified key hydrogen bonds between the nucleotide base of ligands and the side chain of the basic residues in the binding pocket. Electrophysiological analysis also revealed the binding site specific modulation by divalent cations. Our work, thus, provides the novel structural basis for the ligand specificity and the gating modulation by extracellular divalent cations. (COI:No)

## S33-2

### Strategy to isolate G Protein-Gated Inward Rectifier $K^+$ (Kir) Channel Blockers

Inanobe Atsushi、Kurachi Yoshihisa

*(Dept Pharmacol, Grad Sch Med, Osaka Univ, Osaka, Japan)*

G protein-gated Kir channels control the cell excitability by hyperpolarizing membrane potentials. Their activity is strictly regulated by downstream of GPCR activation. However, it becomes apparent that excessive activity of the channels contributes to the symptoms of several diseases: an augmentation of constitutive activity of acetylcholine-induced Kir channels (Kir3.1/Kir3.4) in chronic atrial fibrillation, and an increase in the strength of GABA signaling (including Kir channels consisting of Kir3.2 and Kir3.1/Kir3.2) in Down's syndrome caused by trisomy of human chromosome 21. Therefore, the development of novel agents specifically blocking these Kir channels has been advanced to aim toward drug therapy.Several techniques such as automated patch clamp systems and compound libraries derived from preceding Kir1.1 blockers facilitate the isolation of compounds which block Kir channels. It is also demonstrated that $K^+$ transporter-deficient yeast strains are useful as a host strain of a cell growth-based drug screening system to isolate potential $K^+$ channel modulators. These diverse approaches enable to work as comprehensive screening of the blockers. In this talk, we would like to exemplify the isolation of Kir3.2 blocker and introduce its mode of action to Kir channels. (COI:No)

## S33-3

### Structure-based analysis of the function of Kir2.1 channel using a novel $I_{K1}$ blocker PA-6: Interplay between PA-6 and intracellular spermine

Takanari Hiroki[1]、Marcel Van-der-heyden[2]、Anna Stary-weinzinger[3]

*([1]Dept. Pathophysiol, Oita Univ Sch Med, Oita, Japan、[2]Dept Med Physiol, Univ Med Ctr Utrecht, Utrecht, The Netherlands、[3]Dept. Pharmacol Toxicol, Univ Vienna, Vienna, Austria)*

**[Background]** Intracellular spermine (SPM) blocks outward current through Kir2.1 channel by hydrophilic binding with the channel, which provide inward rectification of $I_{K1}$. We investigated structure-based mechanisms of the inward rectification of $I_{K1}$ by analyzing the interplay between a specific $I_{K1}$ blocker PA-6 and intracellular SPM.
**[Methods]** HEK293T cells were transfected with human Kir2.1, and used for inside-out patch clamp experiment with a ramp protocol from -100 mV to 100 mV in 5 seconds. Both wild type and mutant (E224A, D172N) channels were tested.
**[Results]** PA-6 (200 nM) alone completely blocked $I_{K1}$. SPM (0.1 μM) decreased the outward component of $I_{K1}$ by 70%, and the inward component by 5%. The blocking effect of PA-6 was reduced by 0.1 μM SPM; PA-6 in the presence of SPM provided block on the outward component of $I_{K1}$ by approximately 40%. Both PA-6 and SPM had less effect on E224A-KIR2.1 current, and no reciprocal interference was observed. SPM showed inward rectification on D172N-Kir2.1 current, which was canceled by PA-6. Summing up, concomitant existence of D172 and E224 provides potent binding of SPM on Kir2.1 channel, while lacking of D172 may weaken SPM-binding on the channel which can be easily overtaken by PA-6.
**[Conclusion]** PA-6 may have an overlapping binding site on Kir2.1 channels with SPM. PA-6 was useful for understanding the structure-based blocking effect of SPM on Kir2.1 channel. (COI:No)

## S33-4

### Revisiting $K^+$ dependence of conductance and gating of strong inward rectifier $K^+$ channels

Ishihara Keiko、Itoh Masayuki、Igata Sachiyo、Takano Makoto

*(Dept Physiol, Kurume Univ Sch Med, Kurume, Japan)*

Kir2 strong inward rectifier $K^+$ channels determine the resting potentials of cardiac myocytes and neuronal cells. Importantly, their open-channel conductance depends roughly on square root of external $K^+$ concentration ($[K^+]_o$) and their voltage dependence of rectification shifts with changes in $K^+$ equilibrium potential ($E_K$) when $[K^+]_o$ is altered. Strong inward rectifier $K^+$ currents are completely inhibited under the external $K^+$-free condition, but the mechanism has been enigmatic. Our recent study revealed that Kir2.1 channels showing significant outward rectifying $K^+$ conductance under the external $K^+$-free condition are completely blocked by physiological concentrations of cytoplasmic polyamines; a shift of $E_K$ toward very negative voltages increases the block and inhibits the outward currents in the entire voltage range. Consistently, the external $K^+$ free inhibition was not found with mutant Kir2.1 in which low-affinity sites for the polyamine block in the cytoplasmic pore are mutated. Our study also indicated that both high- and low-affinity blocks of Kir2.1 depend on the difference between membrane potential and $E_K$ when external or internal $K^+$ concentrations are varied. The shift of affinities for Kir2 block with external $K^+$ has been ascribed to a competition between external permeant $K^+$ ions and internal blocking cations. Considering that the low-and high-affinity sites are located in the wide inner pore, enhancement of the block by lowering internal $K^+$ concentration may also reflect the physiological condition. (COI:No)

# Symposium 34

Restoration and Regeneration of the Gastrointestinal Tract

March 24 (Thu), 15:00 – 16:30, Room I

## S34-1

### Gastrointestinal smooth muscle regeneration using absorbable extracellular matrix containing growth factors

Ueno Tomio、Nagano Hiroaki

*(Dept Surg & Surg Oncol, Grad Sch Med, Yamaguchi Univ, Ube, Japan)*

Small Intestinal Submucosa (SIS) is a biodegradable, collagen-rich matrix containing functional growth factors similar to those in native submucosa. We have reported encouraging outcomes on the regeneration of the smooth muscle created within an artificial defect in the rodent stomach, or cecum, and in the small intestines both in dogs and in micro-mini pigs using SIS grafts. In the rodent stomach, we also investigated the feasibility of SIS with mesenchymal stem cells (MSCs). *In vitro* contractility and immunohistochemical examinations were performed. In the canine model, *in vivo* motor activity was also evaluated. We observed the regrowth of smooth muscle cells in the grafted tissue accompanied by the regeneration of neural cells. We verified peristalsis through the segment, including the reorganized area, as an isoperistaltic migrating motor activity. The SIS grafts seeded with MSCs prior to transplantation appeared to support improved regeneration compared with grafts not seeded with MSCs. The actual length of the smooth muscle layer was observed to be significantly greater in seeded SIS groups than in unseeded SIS groups. Tracing the transplanted MSCs by heterologous GFP expression indicated the presence of GFP in the regenerated interstitial tissue with fibroblast-like cells in the seeded SIS groups, suggesting that MSCs facilitate the repair of injured tissue by neighboring native cells including fibroblasts. Overall, our results indicate that the SIS graft may possibly be used as a bio-absorbable scaffold for the regeneration of the smooth muscle layers in animals. (COI:No)

## S34-2

### Adult Neurogenesis of Enteric Nervous System after Benzalchonium Chloride Treatment in *c-kit* Mutant Mice

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The adult neurogenesis in enteric nervous system (ENS) rarely occurs *in vivo*. In this study we demonstrate that significant adult neurogenesis occurs in *c-kit* mutant mice ($W/W^v$) after intestinal myenteric plexus (MP) ablation by treating benzalchonium chloride (BAC). BAC treatment induces selective myenteric plexus (MP) ablation, and almost all neurons in MP disappeared in a few days after the treatment. One week after the ablation, however, the substantial penetration of nerve fibers from non-damaged area was observed in MP layer and the longitudinal muscle layer both in wild type and $W/W^v$. Two weeks after BAC treatment, in addition to the penetrating fibers from surrounding non-injured area, a few ectopic neurons, which were distributed in the subserosal layer or the longitudinal muscle layer, appeared in wild type mice, whereas substantial number of ectopic neurons appeared in $W/W^v$ mice. These ectopic neurons expressed either excitatory or inhibitory intrinsic motor neuron markers. Under electron microscopic observation, those ectopic neurons formed a ganglion-like structure including glial cells, synaptic vesicles, and basal lamina. Intriguingly, wild type mice orally administrated Imatinib, which is an inhibitor of c-Kit and used as an anticancer agent for GIST (gastrointestinal stromal tumor), markedly induced the appearance of ectopic neurons after BAC treatment. These results would suggest that in ENS adult neurogenesis after neuron injury is negatively regulated by c-Kit signaling *in vivo*. (COI:No)

## S34-3

### Challenge to the stem cell transplantation therapy against intestinal aganglionosis

Fujimura Takumi[1,2]、Shibata Shinsuke[2]、Shimojima Naoki[1]、Morikawa Yasuhide[3]、Okano Hideyuki[2]、Kuroda Tatsuo[1]

*([1]Dept PedSurg, Sch of Med, Keio Univ、[2]Dept Physiol, Sch of Med, Keio Univ、[3]Dept Ped Surg, International University of Health and Welfare)*

Background/Purpose: Intestinal aganglionosis has been remained as a field of therapeutic challenge. Some patients with intestinal aganglionosis are not fully recovered because of their long aganglionic segment. For the future innovative therapeutic options against this intractable disease, neural crest derived cell transplantation is one of the best candidates which can create the enteric neuron and can improve motility disorder.Method: The genetically labelled mouse neural crest derived cells were cultured and transplanted in the aganglionic colon in the chemicallyg-induced aganglionosis mice. The histological recovery was evaluated by immunohistochemistry that include some markers for neural lineage cells. The functional recovery by cell transplantation therapy was evaluated with the rate of body weight and with the stool weight change.Results: The transplanted cells were detected in the aganglonic segmenst of the colon for several months after operation and they showed the neural lineage differentiation. Only the model mice from transplanted group showed an improvement of the bowel function.Conclusion: Transplanted neural crest derived cells survived and facilitated the improvement of the intestinal motility in the transplanted group. These results suggest that usefulness of the cell transplantation therapy for future innovative therapeutic approach against intestinal aganglionosis. (COI:No)

## S34-4

### What is the general action of ghrelin in vertebrates?: Study focused on gastrointestinal motor stimulating action.

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Ghrelin (GRLN) has been identified in vertebrates from fish to mammals, and is known to regulate growth hormone release, food intake and glucose and lipid metabolisms. Structural similarity of GRLN and its receptor with motilin and the motilin receptor proceeded functional studies of GRLN in gastrointestinal (GI) tract. In mammalian GI tract, GRLN stimulates gastric acid release, motility and proliferation of epithelial cells. We used some non-mammalian vertebrates, and examined the effect of GRLN on contractility of GI tract as well as the expression of GRLN receptor mRNA to determine whether motor stimulating action is common through vertebrates. Expression level of GRLN receptor mRNA differed depending on the animal species and on GI regions. Region-dependent expression of GRLN receptor mRNA is remarkable in avian, and the expression level of stomach in chicken changes with growth. When GI contraction was examined using non-stimulated or stimulated strips, GRLN did not cause any mechanical responses in GI strips from goldfish, rainbow trout, bullfrog and Japanese fire belly newts even applied the homologous GRLN. On the other hand, GRLN caused contraction or potentiation of electrical stimulation-induced contraction in chicken and mouse. The results of our study show that motor stimulating action of GRLN demonstrating in rodents is not conserved across vertebrates, and suggest that attentions should be paid to evaluate physiological function of GRLN using model animals. (COI:No)

## S34-5

### Alteration of neuromuscular transmissions in the colon following the onset and resolution of inflammation.

Shiina Takahiko[1]、Gurung Yam[1,2]、Naitou Kiyotada[1]、Nakamori Hiroyuki[1]、Sano Yuuki[1]、Horii Kazuhiro[1]、Shimizu Yasutake[1]

*([1]Dept Basic Vet Sci, Lab Physiol, Unit Grad Sch Vet Sci, Gifu Univ, Gifu, Japan、[2]Inst Agri Animal Sci, Tribhuvan Univ, Kathamandu, Nepal)*

The enteric nervous system (ENS) plays an essential role in the regulation of gastrointestinal function. During inflammatory processes, the regulation of gastrointestinal functions by ENS is disrupted. Alteration of neuronal transmissions following a bout of inflammation may persist at least partially. However, it is unclear how prior inflammation affects the subpopulation of ENS. Therefore, the aim of the present study was to determine whether trinitrobenzene sulfonic acid (TNBS)-induced colitis leads to alterations in enteric neuronal transmission that persist beyond the resolution of inflammation. We assessed the mechanical responses induced by application of electrical field stimulation (EFS) in isolated segments of the distal colon. EFS evoked nitrergic relaxation followed by cholinergic and tachykininergic contractions, which were attenuated in the inflamed colon. Both the nitrergic relaxation and the cholinergic and tachykininergic contractile responses were recovered at post-inflammatory stage. In addition, non-tachykininergic and non-cholinergic excitatory neural components were expressed following the resolution of inflammation. These results suggest that colonic inflammation causes indiscriminate damage to ENS but that neuronal components are restored and new excitatory neural components, compensating for the contractile responses in smooth muscle after colitis, are expressed. (COI:Properly Declared)

# Symposium 35

From glucosensing to neural and endocrine regulation

March 22 (Tue), 16:00 – 17:30, Room B

## S35-1

### Glucose-sensing Receptor in Pancreatic Beta-cells

Kojima Itaru、Nakagawa Yuko
*(Dept Cell Biol, IMCR, Gunma Univ, Maebashi, Japan)*

Glucose is a primary stimulator of insulin secretion in pancreatic beta-cells. It has been long thought that glucose stimulates insulin secretion solely by a mechanism dependent on glucose metabolism. We have shown recently that cell-surface glucose-sensing receptor is expressed in pancreatic beta-cells. This receptor is comprised of the T1R3 subunit of the sweet taste receptor expressed in the taste buds, and is activated by physiological concentrations of glucose. This receptor is coupled to both the calcium and cAMP messenger systems and induces rapid changes in cytoplasmic free calcium and cAMP concentrations. When beta-cells are stimulated by a high concentration of glucose, glucose first acts on the glucose-sensing receptor, evokes rapid elevations of cytoplasmic free calcium and cAMP, and activates the metabolic pathway of glucose. Glucose then enters beta-cells, is metabolized though already activated metabolic pathway, and ATP production is augmented. Collectively, both receptor-mediated and metabolism-dependent pathways act coordinately to stimulate insulin secretion. In fact, when the glucose-sensing receptor is blocked by either deletion of the T1R3 subunit or administration of an inhibitor of T1R3, glucose-induced ATP production and insulin secretion are markedly reduced. The glucose-sensing receptor plays an important role in the action of glucose in pancreatic beta-cells. (COI:No)

## S35-2

### $Na^+,K^+$-ATPase in the hypothalamic arcuate nucleus serves as glucose sensor to regulate feeding behavior

Yada Toshihiko、Kurita Hideharu、Nakata Masanori
*(Dept Physiol, Jichi Med Univ, Sch Med, Tochigi, Japan)*

Reduction in the blood glucose has been considered one of the major peripheral factors that induce appetite under fasting conditions. The neurons that are activated by lowering glucose (LG), the glucose-inhibited (GI) neurons, in the hypothalamic arcuate nucleus (ARC) were suggested to be implicated in feeding. However, their glucose-sensing mechanism and physiological role remain to be determined. We here report that $Na^+,K^+$-ATPase (NKA) in ARC senses LG to stimulate feeding. LG reduced NKA's substrate ATP level in isolated ARC slices. LG decreased intracellular concentration of nicotinamide adenine dinucleotide phosphate ($[NAD(P)H]_i$), indicating reduced ATP level, and increased $[Na^+]_i$, reflecting reduced NKA activity, in ARC GI neurons that responded to LG with $[Ca^{2+}]_i$ increases. Intracerebroventricular injection of the NKA inhibitor ouabain activated agouti-related protein (AgRP) and neuropeptide Y (NPY) neurons in ARC, and evoked NPY-dependent food intake. Ouabain increased $[Ca^{2+}]_i$ in single NPY/AgRP neurons in ARC. By contrast, SSA412, a specific activator of NKA, counteracted fasting-induced food intake and LG-induced $[Ca^{2+}]_i$ increases in ARC GI neurons. These results reveal that LG suppresses NKA activity in ARC, thereby inducing GI neuron activation and NPY/AgRP-dependent appetite. This study identifies ARC NKA as a hypothalamic sensor and converter of LG to key neuronal activity and feeding behaviour. (COI:No)

## S35-3

### Regulation of hepatic glucose metabolism by the shuttling of glucokinase between the nucleus and the cytoplasm

Toyoda Yukiyasu
*(Dept Pathobiochem, Fac Pharm Meijo Univ, Nagoya, Japan)*

Glucokinase (GK) (EC 2.7.1.1) catalyzes the phosphorylation of D-glucose to D-glucose 6-phosphate (P), and functions as a glucose sensor for glucose homeostasis in the pancreas, liver, and brain. Lowered functioning of GK contributes to the pathogenesis of hyperglycemia in type 2 diabetes (2DM). GK is considered a candidate target for anti-diabetic drugs for 2DM. Hepatic glucose metabolism is regulated via the shuttling of GK between the nucleus and the cytoplasm in response to external stimuli. .In the postabsorptive state, GK exists predominantly in the nucleus in association with GK regulatory protein (GKRP) with majority of GK in an inactive state. In the postprandial state, D-glucose and D-fructose enter the hepatocytes, and the latter hexose is converted to D-fructose 1-P by ketohexokinase. Both fructose 1-P and D-glucose act to release GK from GKRP. GK then moves into the cytoplasm and rapid glucose utilization takes place under these conditions. The GK-GKRP system is essential for proper glucose sensing in mammals. In addition, hepatic GK translocation is impaired in diabetic subjects. Hyperglycemia in patient with 2DM would be improved by the stimulation of the translocation of GK out of the nucleus. (COI:No)

## S35-4

### Molecular mechanisms of glucose-induced memory enhancement: an involvement of epigenetic modulation of BDNF and FGF-1 genes expression

Oomura Yutaka、Hossain Shamim、Katafuchi Toshihiko
*(Dept Integr Physiol, Grad Sch Med Sci, Kyushu Univ, Japan)*

We have previously reported that intrahippocampal injection of 7 mM glucose, which is similar to the glucose concentration of the cerebrospinal fluid during food intake, facilitates spatial learning and memory in rats and that the high glucose enhanced basal synaptic response and tetanus-induced long term potentiation. In the present study, we found that, when glucose was increased from 3.5 mM to 7 mM, neuronal cell lines (Neuro2A) showed an increase in expression of brain-derived neurotrophic factor (BDNF) and fibroblast growth factor-1 (FGF-1), which were both known as memory related molecules, along with the enhanced phosphorylation of AKT (PKB) and CREB. In addition, the restricted glucose water drinking increased the number of dendritic spines in the mouse hippocampus. Furthermore, the glucose-induced upregulation of BDNF was blocked by the knock down of CREB using lentiviruses encoding short hairpin-RNA against CREB, while high glucose increased CREB recruitment onto the BDNF and FGF-1 promoter regions. Interestingly, glucose stimulation reduced histone deacetylase 2 (HDAC2) recruitment and increased the acetylated histones (H3K9 and H3K27) near the BDNF and FGF-1 promoter regions in the neuronal cell line and hippocampal tissues. These findings, taken together, suggest that glucose enhances spatial learning and memory by upregulation of BDNF and FGF-1 genes expression through the increases in phosphorylated CREB and epigenetic modulation of the genes. (COI:No)

## S35-5

### Role of sweet taste receptor in the control of energy homeostasis

Kohno Daisuke[1,2]、Koike Miho[1]、Kojima Itaru[3]、Kitamura Tadahiro[2]、Yada Toshihiko[4]
*([1]ASRLD Unit, Gunma University、[2]Institute for Molecular and Cellular Regulation, Gunma University、[3]Department of Cell Biology, Institute for Molecular and Cellular Regulation, Gunma University、[4]Department of Physiology, Division of Integrative Physiology, Jichi Medical University)*

The hypothalamic feeding center plays an important role in energy homeostasis. The mechanisms underlying nutrient sensing, including glucose sensing, in the feeding center are not fully understood. The sweet taste receptor is a heterodimer of T1R2 and T1R3, and senses sweet taste. T1R2 and T1R3 are widely distributed in the body, e.g., in the tongue, pancreas, hypothalamus, and adipose tissue. To explore the role of sweet taste receptors in the hypothalamic feeding center, the distribution of sweet taste receptors and the effect of an artificial sweetener were studied in the hypothalamic arcuate nucleus (ARC) of mice. T1R2 and T1R3 were widely distributed in the ARC neurons, including the POMC neurons of the satiety center. An artificial sweetener, sucralose, at $10^{-5}$-$10^{-2}$ M, dose-dependently increased cytosolic $Ca^{2+}$ concentration ($[Ca^{2+}]i$) in isolated single ARC neurons. Sucralose-responsive neurons also responded to glucose at 10 mM and leptin. Sucralose-induced $[Ca^{2+}]i$ increases were suppressed by an inhibitor of sweet taste receptor and a L-type $Ca^{2+}$ channel blocker. These data suggest that sweet taste receptors are distributed in ARC neurons, and that sweet signal plays a role in the activation of satiety neurons through an sweet taste receptor and L-type $Ca^{2+}$ channel mediated pathway. (COI:No)

# Symposium 36

Neuropsychiatric disorders as neural network malfunctions
sponsored by: Grant-in-Aid for Scientific Research on Innovative Areas (Non-linear Neuro-oscillology)

March 23 (Wed), 9:00 – 10:30, Room G

## S36-1

### Aberrant oscillation property of Perkinje cells is caused by decreased tonic inhibition in granule cells in mice model of Angelman syndrome.

Egawa Kiyoshi[1]、Fukuda Atsuo[2]
*([1]Hokkaido Univ. Hospital Pediatric dept.、[2]Hamamatsu Uni. Sch. of Med. Neurophysiology dept.)*

Angelman syndrome(AS) is a neurodevelopmental disorder caused by a functional deficit of UBE3A. Abnormal movement is one of the characteristic features in AS, which are attributed to cerebellar ataxia. To clarify its mechanisms, we evaluated the spontaneous firing properties of Purkinje cells (PCs) in acute slices of Ube3a K.O. mice using single-unit recordings. The most of recorded PCs from WT and Ube3a K.O. mice showed either tonic firing pattern (stable firing rate) or trimodal patterns (recurrence of tonic and burst-like firing intermitted by silent periods). While only half of the WT PCs presented a tonic pattern, the proportion reached 80% in PCs of Ube3a K.O. mice. It has been shown that synaptic inputs to PCs can toggle the firing pattern from trimodal to tonic. Thus, the predominance of tonic firing pattern can be caused by hyperexcitability of presynaptic neurons. Indeed, we have shown that extrasynaptic $GABA_A$ receptor-mediated tonic inhibition is decreased in granule cells of Ube3a K.O. mice and that the required current injection to reach action potential is significantly smaller in these cells. Administering low doses of THIP, a selective extrasynaptic $GABA_A$-receptor agonist, altered firing pattern from tonic to trimodal in Ube3a K.O. mice. These results suggest that decrement of tonic inhibition in granule cells could modify oscillation property of PCs and cause cerebellar ataxia in AS. Similar mechanism can be applicable to other brain dysfunctions in AS such as memory impairment or epilepsy. (COI:No)

## S36-2

### *De novo* Kv2.1 mutants causing infantile generalized seizures and psychomotor developmental delay inhibit repetitive neuronal firing

Akita Tenpei[1]、Saitsu Hirotomo[2]、Matsumoto Naomichi[2]、Fukuda Atsuo[1]
*([1]Dept Neurophysiol, Hamamatsu Univ Sch Med, Hamamatsu, Japan、[2]Dept Hum Genet, Yokohama City Univ Grad Sch Med, Yokohama, Japan)*

Infantile epileptic seizures and/or psychomotor developmental delay can be caused by mutations in the genes encoding voltage-gated $K^+$ channels (Kv). The pathophysiological mechanism of the diseases, however, is not fully understood. Recently, we found two novel *de novo* heterozygous missense mutations in *KCNB1*, encoding the major delayed rectifier Kv2.1 in cortical and hippocampal pyramidal neurons, in two patients in our whole exome sequencing data of a total of 437 patients with infantile epilepsy. Both patients exhibited a delay in motor development after birth, and then developed severe generalized seizures 1-1.5 years after birth with diffuse high-amplitude spike-and-wave electroencephalogram discharges. They now have intellectual disabilities. One mutation occurred at one of positively charged residues (p.R306C) in the S4 voltage sensor domain of Kv2.1 and strongly disrupted sensitivity and cooperativity of the sensor. Another mutation occurred at the "gating hinge" (p.G401R) in the S6 pore domain and nullified the channel function with a dominant-negative effect on endogenous Kv2. Both Kv2.1 mutants expressed in primary cortical pyramidal neurons greatly inhibited repetitive firing of action potential spikes through reducing the depth of interspike voltages. Thus our results suggest that insufficient firing of pyramidal neurons disturbs both development and stability of neuronal circuits, resulting in the disease phenotypes. (COI:No)

## S36-3

### Parkinson's disease as a network disorder

Chiken Satomi[1,2]、Nambu Atsushi[1,2]
*([1]Div Syst Neurophysiol, Natl Inst Physiol Sci, Okazaki, Japan、[2]Dept Physiol Sci, SOKENDAI, Okazaki, Japan)*

The basal ganglia (BG) play a crucial role in controlling voluntary movements, and their dysfunction causes severe movement disorders. The BG receive inputs from a wide area of the cerebral cortices, and project back to the original cortices via the thalamus. The output nuclei of the BG, which convey processed information to the thalamus, are the internal segment of the globus pallidus (GPi) and substantia nigra pars reticulata (SNr). Motor signals originating from the cortex are processed through cortico-BG networks, i.e., the *hyperdirect*, *direct* and *indirect* pathways, and induce dynamic motor-related activity changes in the GPi/SNr. The GPi and SNr are consisted of GABAergic inhibitory neurons and continuously fire at high frequency. Movement-related GPi/SNr inhibition through the *direct* pathway acts to release movements via disinhibition of the thalamus and cortex, whereas movement-related GPi/SNr excitation through the *hyperdirect* and *indirect* pathways acts to inhibit unnecessary motor programs. The signal transmission through these pathways at appropriate timing and balance is essential to properly release voluntary movements. Actually, neuronal activities in animal models of movement disorders, such as Parkinson's disease and dystonia, indicate abnormal information processing through the cortico-BG networks. We will discuss how the cortico-BG network disorder causes abnormal neuronal activities and motor symptoms in movement disorders. (COI:No)

## S36-4

### Beta and gamma oscillations in the cerebral cortex

Mushiake Hajime
*(Dept Physiol, Grad Sch Med, Tohoku Univ, Sendai, Japan)*

Beta oscillations and gamma oscillations are distinct high-frequency oscillations that are observed in the monkey and rodents. However physiological significance remains unclear. I will present two recent studies performed in our laboratory. The first topic covers the beta and gamma oscillation in the local field potential from the medial motor areas. We trained animals to perform bimanual sequence tasks that require updating and maintaining the motor program depending on behavioral context. We found that beta power was attenuated during early delay periods of updating trials but was increased during maintenance trials, while there was a reciprocal increase in high-gamma power during updating trials. Moreover, transient attenuation of beta power during maintenance trials resulted in the erroneous selection of an action sequence. Therefore, these findings suggested that increases and decreases in beta power in conjunction with the appropriate high-gamma signal contribute to updating and maintain the action plan. The second topic covers subtypes of the neuronal inhibitory and oscillations. Cortical interneurons are classified into several subtypes. We focused on two subtypes: Parvalbumin (PV)-expressing cells and somatostatin (SOM) cells. We used mice lacking glutamate decarboxylase67, primarily in PV cells (PV-GAD67mice) or in SOM cells (SOM-GAD67mice).We then compared LFPs between PV-GAD 67 and SOM-GAD 67mice. We found that PV cells play important roles in persistence of the up state and in laminar distribution of gamma and beta oscillations, whereas SOM and PV cells may make an asymmetric contribution to regulate up-state and delta oscillations. (COI:No)

# Symposium 37

Multiple crosstalk between chemical/interoceptive sensory and behavioral control systems in feeding behavior

March 22 (Tue), 9:00 – 10:30, Room H

## S37-1

### The behavioral functions of the basolateral amygdala neurons and its efferents on the retrieval of conditioned taste aversion in rats

Inui Tadashi、Shimura Tsuyoshi
*(Div. Behav. Physiol., Dept. Behav. Sci., Grad. Sch. Human Sci., Osaka Univ.)*

Humans and animals acquire an aversion to a taste associated with malaise, referred to as conditioned taste aversion (CTA). We have used the CTA paradigm for investigating the neural mechanisms of ingestive behavior based on taste memory. We here explored the role of the basolateral amygdala (BLA) on the retrieval of CTA using pharmacological inactivation technique. Rats implanted with guide cannulae into the BLA received a pairing of 5-mM saccharin solution as a conditioned stimulus (CS) with malaise-inducing lithium chloride. On the following days, we analyzed the rat's approach and ingestive behaviors after the microinjections of $GABA_A$ receptors agonist muscimol into the BLA. It was found that the rats inactivated with the BLA failed to slow down approach speed to, withdraw from, and terminate licking to the CS solution. These results indicate that the BLA mediates these specific behavioral functions on the retrieval of CTA. We further examined which efferent projections from the BLA are involved in the CTA retrieval. We tried to detect the activated efferents by the CS presentation, using a manganese-enhanced MRI (MEMRI) technique. The imaging analyses demonstrated that the efferents from the BLA to the nucleus accumbens, bed nucleus of the stria terminalis, and central amygdala are significantly activated. These efferents are suggested to mediate the CTA retrieval. We discuss the correlations between the behavioral deficits in the muscimol-injection experiment and the activated efferents in the MEMRI experiment. (COI:No)

## S37-2

### Motivation-related olfactory neural circuit involved in feeding behavior

Yamaguchi Masahiro
*(Dept Physiol, Grad Sch Med, Univ of Tokyo, Tokyo, Japan)*

Among many functional systems regulating feeding behavior, olfaction plays crucial role in detecting and judging edible object. Since olfaction is a fundamental sensation that elicits emotional and motivated behavior, olfaction likely regulates feeding behavior via emotional and motivated responses. However, little is known about the underlying circuit mechanisms. Olfactory sensory signal is transferred from the olfactory epithelium to the olfactory bulb and then to the olfactory cortex. Among various regions of the olfactory cortex, olfactory tubercle (OT) is characteristic in that it has medium spiny neurons as principal neurons and constitutes a part of the ventral striatum. The OT is therefore a candidate structure that links olfactory input to motivated behaviors. In fact, we found that odors associated with food reward activate anteromedial domain of the OT while odors associated with electrical foot shock punishment activate lateral domain of the OT, indicating the existence of functional domain in the OT representing different motivated behaviors. We further examined how the anteromedial domain of the OT, which is activated during odor-induced food seeking behavior, develops in neonatal mice. While the domain is not well developed at birth, its structural maturation and food-driven activation became evident around day 15 after birth. Interestingly, this time period corresponded to the time period when feeding habit shifts from mother milk to food pellet. The result suggests a crucial role of the OT in learning novel, edible food in naonates, which might control the feeding habit of animals throughout life. (COI:No)

## S37-3

### The role of the vagal afferent nerve on energy homeostasis

Toshinai Koji
*(Faculty Wellness, Shigakkan Univ, Obu, Japan)*

The vagal afferent nerve is a system forming the basis of the energy homeostasis regulation. Ghrelin and glucagon-like peptide-1 (GLP-1) secreted from the gastrointestinal endocrine cells function as an orexigenic and anorectic peptide, respectively. We here indicate that peripheral ghrelin and GLP-1 interact with each other at the vagal afferent for the control of energy balance. The administration of ghrelin to rats and mice after GLP-1 administration did not stimulate food intake and the injection of GLP-1 after ghrelin administration did not suppress it. We identified the neurons receiving information from both the stomach and ileum using double retrograde tracers. The neurons expressed both GHS-R and GLP-1R mRNAs. The blockade effect of firstly administered peptide for the firing response of vagal afferent and the inward of calcium or the outward potassium in nodose ganglion (NG) neuron by secondly administered peptide. These suggest that gut-derived peptides regulated short-term feeding via the vagal afferent system. The system was disrupted by the high fat diet (HFD) treatment in mice. Subcutaneous administration of ghrelin to mice fed HFD for 4 weeks failed to induce feeding. The ghrelin resistance was caused by dysregulation of ghrelin signaling via the vagal afferent. The dysregulation may be initiated by only one-day HFD intake. One-day HFD treatment induced inflammatory responses in NG and the colon. Celiac vagotomy reduced HFD-induced inflammation in NG. In conclusion, vagal afferents play an important role in energy homeostasis. The injury of vagal afferent system may triggers for obesity and dysregulation of energy homeostasis. (COI:No)

## S37-4

### Food Digestion, Visceral Sensation, and Behaviors in Normal and Pathological Conditions

Fukudo Shin
*(Tohoku University Graduate School of Medicine)*

Food digestion, visceral sensation, and human behaviors have mutual relationship. Many diseases have abnormal features of this trias. They are eating disorders, simple obesity, functional dyspepsia, and irritable bowel syndrome (IBS). Neuroimaging clarified that neural pathways of visceral sensation play crucial roles in these diseases. Structural and functional alterations in the amygdala, anterior cingulate cortex, insula, and prefrontal cortex (PFC) were found in these diseases. Corticotropin-releasing hormone (CRH) is a major mediator of stress response in the brain-gut axis. CRH forms positive feedback system with noradrenergic neurons which play a fundamental role in the formation of anxiety and/or depression. Administration of CRH aggravated visceral sensorimotor response in IBS patients. Conversely, administration of CRH antagonists likely alleviates IBS pathophysiology. Serotonin (5-HT) is another candidate in association to brain-gut function in IBS. Link between visceral sensation and emotion can be modified by psychotherapeutic interventions as well as neuromodification like repetitive transcranial magnetic stimulation (rTMS). Analgesic suggestion induces activation of the dorsolateral prefrontal cortex (DLPFC) and rTMS to the DLPFC causes suppression of the composite feelings of visceral sensation and negative emotion. Furthermore, we found dopamine release from the putamen and caudate nucleus when suggestion induces visceral analgesia in humans. Further studies on food digestion, visceral sensation and behaviors in normal subjects and patients are warranted.
(COI: Properly Declared)

# Symposium 38

New development of functional analyses of protein (Ser/Thr) phosphatases

March 22 (Tue), 16:00 – 17:30, Room K

## S38-1

### Recent topics in natural product-derived protein phosphatase inhibitors

Wakimoto Toshiyuki

*(Grad Sch Pharm, Hokkaido Univ, Sapporo, Japan)*

Over the past three decades, several natural products, that specifically inhibit protein phosphatase 1 and 2A, had been discovered from actinomycetes, cyanobacteria and marine sponges. Recent developments in genome sequencing technology have been applied to the free-living microorganisms, leading to the discovery of biosynthetic gene clusters of actinomycete-derived tautomycin and fostriecin, as well as microcystin from cyanobacteria. All these molecules are biosynthesized by modular-type enzymes, such as polyketide synthase (PKS) and non-ribosomal peptide synthetase (NRPS).On the other hand, marine sponges form a highly complex consortium with diverse symbiotic microorganisms. The biosynthetic gene of okadaic acid has been unknown so far, whereas the okadaic acid-binding protein presumably involved in biosynthesis or sequestration has been isolated from the sponge *Halichondria okadai*. We recently obtained the biosynthetic gene cluster of calyculin A isolated from the Japanese marine sponge *Discodermia calyx* by taking advantage of the metagenomic mining approach. With the desired PKS-NRPS hybrid gene clusters in hand, a symbiotic bacterium encoding the gene cluster was identified by fluorescence *in situ* hybridization and laser microdissection for PCR analysis. Moreover, a new pyrophosphate analog, phosphocalyculin A was found to be the less toxic end product of the biosynthesis. These findings highlight that the activity of the phosphatase inhibitor is regulated by enzymatic phosphorylation of calyculin A itself in the sponge-microbe association. (COI:No)

## S38-2

### Vascular intrinsic circadian rhythm of myosin phosphatase activity

Hirano Katsuya

*(Dept Cardiovasc Physiol, Fac Med, Kagawa Univ, Miki-cho, Kita-gun, Kagawa, Japan)*

The onset of cardiovascular events, such as coronary vasospasm, exhibits circadian variation. The vascular intrinsic oscillation in vascular contractility play a fundamental role for diurnal variation of occurrence of cardiovascular events. The vascular contractility is regulated by the reversible phosphorylation of 20-kDa myosin light chain (MLC). The balance between the activity of MLC kinase and phosphatase determines the level of MLC phosphorylation for a given degree of $Ca^{2+}$ signal. The activity of MLC phosphatase is negatively regulated by the phosphorylation of MYPT1, a regulatory subunit of MLC phosphatase, which is catalyzed by Rho-associated coiled-coil protein kinase, ROCK. We found that agonist-stimulated, but not resting level of MLC phosphorylation circadianly oscillated in phase with the oscillation of MYT1 phosphorylation in cultured vascular smooth muscle cells. The oscillation of MYPT1 phosphorylation was attributable to the circadian oscillation of the expression of ROCK, which was generated by a clock gene RORα. In the α-toxin-permeabilized preparations of aorta of wild-type mice, thromboxane $A_2$ analog produced a greater degree of contraction and MLC phosphorylation under the fixed level of cytosolic $Ca^{2+}$ at Zeitgeber time 0 (ZT0) than that seen at ZT12. This diurnal variation was abolished in Staggere mice, which are deficient in functional RORα. These findings suggest that RORα constructs a vascular intrinsic clock system that generates the circadian rhythm of the myosin phosphatase activity, which is functionally translated to the circadian oscillation of vascular contractility. (COI:No)

## S38-3

### Effects of PP2A inhibitors on smooth muscle contraction

Takeya Kosuke、Ishida Minori、Miyazu Motoi、Takai Akira

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Okadaic acid has been used as a powerful tool to study physiological functions of type 1 and 2A protein phosphatases. In smooth muscles, okadaic acid showed concentration-dependent dual effects. At high concentration (>10 μM), okadaic acid induces $Ca^{2+}$-independent smooth muscle contractions. At low concentration (<3 μM), it induces relaxation in pre-contracted smooth muscles. Although it has been assumed that the force-inhibiting effect of okadaic acid could be due to the inhibition of PP2A, the bona fide target(s) of okadaic acid has not been determined yet.

In this study, we examined the in vivo target of okadaic acid by using other potent PP2A inhibitors, fostriecin and rubratoxin A. We also addressed the question whether okadaic acid affects myosin light chain phosphorylation.

All three PP2A inhibitors (okadaic acid, fostriecin and rubratoxin A) induced relaxation in guinea pig taenia cecum as well as in bovine ciliary muscle strips pre-contracted by ionomycin, a $Ca^{2+}$ ionophore. They also induced relaxation in taenia cecum strips pre-contracted by carbachol, while only rubratoxin A did in ciliary muscle pre-contracted by carbachol. The level of myosin light chain phosphorylation did not change in okadaic acid-relaxed ciliary muscle pre-contracted by ionomycin.Based on these results we concluded that: 1) the force-inhibiting effect of okadaic acid is attributable to PP2A inhibition; 2) in bovine ciliary muscle, carbachol would activate unknown pathways that provide resistance to okadaic acid and fostriecin; 3) PP2A would not be in the upstream of myosin phosphorylation pathway.
(COI:No)

## S38-4

### Manipulating temporal regulation of myosin phosphatase signaling

Eto Masumi[1]、Kitazawa Toshio[2]、Murayama Takashi[3]

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Understanding mechanisms regulating the motility of smooth muscle (SM), a major constituent of hollow organ walls, is vital to develop more precise and effective means of diagnosing and treating various diseases, such as hypertension, gastroparesis, asthma, urinary incontinence, and erectile dysfunctions. Accumulating evidence strongly suggest that the myosin phosphatase (MLCP), which is classified into type-1 Ser/Thr phosphatase, regulates phosphorylation of myosin and governs motility of SM and other cell types in response to agonist stimulation and nitric oxide release. Cell-permeable antagonists for Ser/Thr phosphatases, such as okadaic acid and calyculin A, are powerful tools for studying their cellular roles. Nonetheless, there is the major limitation in the pharmacological approach that these compounds are unable to distinguish over 300 cellular Ser/Thr phosphatases, each of which regulates a specific subset of protein phosphorylation. To more precisely define pathophysiological functions, new research probes specific to each cellular phosphatase are needed. We have discovered and characterized an endogenous inhibitor protein specific to MLCP, named CPI-17. In this session, we will discuss the development of an unconventional dominant active mutant of CPI-17 that can selectively manipulate MLCP among cellular protein phosphatases. (COI:No)

# Symposium 39

Adaptation to Gravitational Changes

March 22 (Tue), 16:00 – 17:30, Room G

## S39-1

### Mouse Habitat Cage Unit -A new space device for a comprehensive mouse analysis-

Shimbo Miki[1,2]、Kudo Takashi[1]、Shiba Dai[3]、Shirakawa Masaki[3]、Takahashi Satoru[1]

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The question whether mammals can produce offspring in outer space is among the biggest issues in space exploration. Conditions differ between ground and space environment, such as space radiation and gravity, which cause tremendous biological consequences to the body. Whether the epigenetic changes caused by space environment are inheritable or not also remains unknown. The "Mouse Epigenetics" project is therefore operated to solve these questions. This project will launch male mice to the Japanese Experimental Module, "Kibo", onboard the International Space Station, house them for 30 days, and return alive to ground. Returned mice will be examined to comprehensively analyze the long-term effects of space environment, especially epigenetic changes. Furthermore, pups will be generated from sperms of these mice to identify the impact to next generations. To enable these experiments, we developed the mouse Habitat Cage Unit (HCU) for installation in the CBEF in Kibo, equipped with sections providing microgravity and artificial 1 *g*. By comparing the gravitational differences generated by CBEF in space, and ground-based 1 *g* will define the impacts of microgravity and the space environment. Another striking innovation that our HCU performs is the introduction of individually housed male mice within the space environment for reproduction studies. Thus the HCU would hold the key for mankind to prosper through outer space, in the future. (COI:No)

## S39-2

### Possible role of a metabolic sensor in muscle adaptations under microgravity environment

Egawa Tatsuro[1,2]、Hayashi Tatsuya[1]、Goto Katsumasa[2]

*([1]Lab Sports and Exercise Medicine, Grad Sch Human and Environment, Kyoto Univ, Kyoto, Japan、[2]Lab Physiol, Grad Sch Health Sciences, Toyohashi SOZO Univ, Toyohashi, Japan)*

Exposure to microgravity environment induces skeletal muscle atrophy by modulating multiple molecular responses. Recent studies have suggested an important role of a "metabolic sensor" 5'AMP-activated protein kinase (AMPK) in muscle mass adaptations. AMPK is activated by metabolic stresses including fasting, hypoxia, ischemia, hormones, exercise, or intracellular $Ca^{2+}$ release, and maintains protein, glucose, and lipid metabolic homeostasis. However, it remains unclear that a role of AMPK in microgravity-associated skeletal muscle adaptation. Recently, we have been showing several evidences regarding a role of AMPK in the gravitational unloading (hindlimb suspension)-induced muscle adaptations (e.g. muscle mass, protein synthesis systems, and protein degradation systems). The antigravitational slow-twitch soleus muscle exhibited 30% decrease in mass following 2-week hindlimb suspension in wild-type mice, but it was 15% in muscle-specific AMPK-defective mice. The protein synthesis systems were down-regulated identically in both type of mice. In contrast, the protein degradation systems, especially ubiquitin-proteasome systems were not fully activated in soleus muscle of AMPK-defective mice, compared with wild-type mice. These observations suggest that AMPK may play a role in a proper adaptation of muscle mass under microgravity environment.The authors declare that there are no conflicts of interest. (COI:No)

## S39-3

### Effects of heat stimulus on skeletal muscle properties

Ohira Takashi、Sudoh Masamichi、Kusakari Yoichiro、Minamisawa Susumu

*(Depart. Cell Physiol, The Jikei University School of Medicine, Tokyo, Japan)*

Activity-dependent stimuli, such as neural and mechanical stimuli, are important for the maintenance of skeletal muscle properties. Recently, heat stimulus has been also focused on as another significant factor. Thus, effects of application of heat stimulus on the morphological properties of denervated or intact soleus muscles were studied in rats. Denervation was performed by cutting the sciatic nerves unilaterally at the gluteal region in all rats (n=16). They were randomly separated into heat stimulus and control groups (n=8 each). Heat stimulus was applied to the rats under anesthesia by immersing their hindlimbs in a warm-water bath (42 °C, 30 min/day, every other day from 1 day after surgery). Control group was also anesthetized in the same way. After 2 weeks, soleus muscles were sampled bilaterally and wet weight, water content, and fiber cross-sectional area were determined. Hypertrophy of muscle fibers in the limb with normal innervation was caused by application of heat stimulus. Furthermore, denervation-related atrophy was partly suppressed in response to heat stimulus. The beneficial effects of heat stimulus are induced regardless of innervation. These results suggested that external warming-up may stimulate protein synthesis and suppress protein degradation in skeletal muscle. And it is indicated that heat treatment can be a possible measure for maintenance and enhancement of skeletal muscle properties. (COI:No)

## S39-4

### Effect of the remodeled short radius centrifuge with ergometric exercise device as a countermeasure for space flight deconditioning caused by -6° head-down bed rest

Nishimura Naoki[1]、Iwase Satoshi[1]、Tanaka Kunihiko[2]、Mano Tadaaki[2]

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We have previously reported that daily artificial gravity with step-up ergometric exercise protocol is effective as a countermeasure for almost spaceflight deconditioning caused by 20 days of simulated microgravity by -6° head-down bed rest. Since the size of device diameter is limited to 3 m due to the planned location on the International space station, a new device was designed and manufactured. Orthostatic intolerance caused by 10 days -6° head-down bed rest was improved by step-up protocol using the device. However, several deconditioned variables including bone metabolism could not be completely prevented, probably due to the attenuated strength of artificial gravity and ergometric exercise. We conclude that a new protocol should be designed to fulfill the role of artificial gravity with exercise. In this symposium, we will talk about usefulness and future prospects of remodeled short radius centrifuge with ergometric exercise device as a countermeasure for space flight deconditioning caused by -6° head-down bed rest. (COI:No)

# Symposium 40

Frontier Studies in Behavioral Neuroendocrinology on Physiological Events

March 23 (Wed), 15:00 – 16:30, Room D

## S40-1

### Molecular mechanisms underlying low glucose-induced ghrelin secretion from gut X/A-like cells

Harada Kazuki[1]、Kitaguchi Tetsuya[2,3]、Oya Manami[1]、Numano Rika[4]、Sato Takahiro[5]、Kojima Masayasu[5]、Tsuboi Takashi[1]

*([1]Dept. Life Sci., Grad. Sch. Art. Sci., Univ. Tokyo, Tokyo, Japan、[2]Cell Signaling Group, WABIOS, Singapore、[3]Org. Univ. Res. Init., Waseda Univ., Tokyo, Japan、[4]Dept. Environ. Life Sci., EIIRIS, Toyohashi Univ. Technol., Toyohashi, Japan、[5]Mol. Genet., Inst. Life Sci., Kurume Univ., Kurume, Japan)*

Ghrelin is a stomach-derived peptide which plays a fundamental role in the regulation of food intake. Although ghrelin secretion from gut X/A-like cells is known to be regulated by neurotransmitters, hormones and nutrients, the precise molecular mechanism underlying ghrelin secretion has not been elucidated. To address this question, we used ghrelin-secreting mouse ghrelinoma 3-1 (MGN3-1) cells. We found that lowering the extracellular glucose concentration from 25 mM to 10 mM induced an increase in intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]_i$), whereas that from 25 mM to 5 mM had no effect. Overexpression of a dominant-negative form of an ATP-sensitive $K^+$ ($K_{ATP}$) channel suppressed the 10 mM glucose-induced $[Ca^{2+}]_i$ increase and ghrelin secretion. Application of a low dose of $K_{ATP}$ channel opener, diazoxide, under 25 mM glucose induced an increase in $[Ca^{2+}]_i$ and ghrelin secretion. In contrast, application of a low dose of $K_{ATP}$ channel closer, tolbutamide, under 5 mM glucose induced an increase in $[Ca^{2+}]_i$ and ghrelin secretion. Furthermore, inhibition of voltage-dependent $Ca^{2+}$ channels (VDCCs) suppressed the 10 mM glucose-induced $[Ca^{2+}]_i$ increase and ghrelin secretion. These results suggest that low glucose-dependent ghrelin secretion is mediated by $K_{ATP}$ channels and VDCCs. (COI:No)

## S40-2

### Investigation of physiological roles of vasopressin in central nervous system using receptor knock out mice

Nakamura Kazuaki、Kyaw Aung、Tanoue Akito

*(Dept. Pharmacol, NCCHD, Tokyo, Japan)*

The neurohypophysial hormone arginine vasopressin (AVP) is not only known to be involved in various physiological regulatory processes as a peripheral hormone, but also known to work as a neuropeptide that is capable of influencing a wide variety of brain functions such as modulation of social behavior and emotional status. These actions of AVP are mediated by three distinct receptor subtypes: V1aR, V1bR, and V2R. Although the antidiuretic action of V2R is relatively well understood, recent years have seen an increasing understanding of the physiological roles of V1aR and V1bR in various peripheral tissues and the central nervous system (CNS). Deletion of V1aR or V1bR genes in mice revealed that the contributions of these receptors extend far beyond peripheral functions. In particular studies on species-specific localization of central AVP receptors and genetic manipulation of the receptor expression in model animals have greatly advanced our understanding on the behavioral effects of AVP. In addition, our recent study with transgenic and neuronal cell models has revealed that AVP plays a crucial role in protecting the CNS against some neural damages, which is mediated by V1aR and V1bR. Thus, together with in vitro studies, genetically altered rodent models have advanced the understanding of a variety of AVP systems especially in the CNS. In this symposium, we review the findings in this important field by covering a wide range of research and discuss recent findings about AVP functions in the CNS. (COI:Properly Declared)

## S40-3

### Evaluation of potentially developmentally neurotoxic chemicals using behavioral testing and neuroendocrinological analysis

Maekawa Fumihiko、Sano Kazuhiro、Suzuki Go、Nakayama Shoji、Isobe Tomohiko、Tin-tin Win-shwe、Hashimoto Shunji、Kawashima Takaharu

*(National Institute for Environmental Studies, Tsukuba, Japan)*

Mounting evidence shows that increasing numbers of children are being diagnosed with neurodevelopmental disorders. This increase is believed to be due to the impact of not only genetic backgrounds but also environmental factors. A number of epidemiological studies have found the association between brain development and gestational and/or childhood exposure to certain chemicals such as pesticides, endocrine disruptors including specific flame retardants, and heavy metals. It is important to elucidate mechanisms underlying the neurodevelopmental toxicities that these chemicals pose using animal models. Behavioral tests and neuroendocrinological analysis are critical tools to assess the causal link between chemical exposure and neurodevelopmental disorders. We examined the effects of developmental exposure to a neonicotinoid pesticide and a phosphate ester flame retardant on behavioral and physiological profiles in mammalian and avian models. Both chemicals were found to impact socio-sexual behaviors in mouse model in different fashions depending on chemical species. In addition, the effects of the neonicotinoid appeared mainly in male while those of the phosphate ester in female. The result indicates that we may need to focus on sex-dependent mechanisms when testing potential neurodevelopmental toxicicants. (COI:No)

## S40-4

### Region-specific actions of sex steroids on the formation of morphological sex difference in the brain

Tsukahara Shinji、Kanaya Moeko

*(Grad Sch Sci Engin, Saitama Univ, Saitama, Japan)*

The brain contains sexually dimorphic nuclei (SDNs). The principal nucleus of the bed nucleus of the stria terminalis (BNSTp) is a male-biased SDN, while the anteroventral periventricular nucleus (AVPV) is a female-biased SDN. SDN formation involves sex steroid actions in the perinatal and pubertal periods, although the action mechanisms remain to be determined fully. Analyses of mice lacking the genes of aromatase, estrogen receptor-α (ERα), ERβ, and androgen receptor (AR) showed that aromatase, ERα, and AR were required to masculinize the BNSTp, while aromatase and ERα were sufficient to defeminize the AVPV. We next examined molecular expression involved in SDN formation. In the BNSTp, aromatase and ERα were expressed from the prenatal to pubertal period, whereas AR was emerged from the neonatal period. Pubertal males had higher AR expression and lower ERα expression in the BNSTp compared to pubertal females. These findings suggest that aromatized testosterone signaling via ERα and testosterone signaling via AR are required for masculinization of the BNSTp. The former may mainly exert in the perinatal period, and the later may become active between the neonatal and pubertal periods. In the AVPV, ERα was expressed in the perinatal and pubertal periods, while aromatase was expressed only in the perinatal period, suggesting that the AVPV is defeminized by aromatized testosterone signaling via ERα in the perinatal period. Thus, there may be a regional difference in sex steroid actions on SDN formation. (COI:No)

## S40-5

### Role of Estrogen Receptor β in the Regulation of Social Behavior

Ogawa Sonoko

*(Lab Behav Neuroendo, Univ Tsukuba, Tsukuba, Japan)*

Two types of estrogen receptors, ERα and ERβ, are differentially involved in the regulation of social behavior by gonadal steroids such as testosterone (after being aromatized) and estradiol. Studies in knockout mice revealed that disruption of ERα greatly reduced the levels of sex-typical social behavior in both sexes of mice whereas that of ERβ might increase the levels of these behaviors depending on age and/or testing conditions (Ogawa et al., IIS, 2015 for review). Recently, in a series of studies using adeno-associated viral vector mediated RNA interference methods, we have identified brain site(s) and time in development responsible for ERα action in the expression of sex-typical social behavior (Musatov et al., PNAS, 2006; Sano et al., EJN, 2012 and SfN meeting, 2015). On the other hand, it is largely unknown about mechanisms of hormonal actions through ERβ. In this talk, we will first present our most recent findings in the effects of brain-site ERβ knockdown on male-typical sexual and aggressive behavior as well as female-typical lordosis behavior and postpartum aggression. We will then discuss possible role of ERβ localized in a number of brain regions in the regulation of social behavior by gonadal steroids in sex-, time-, and behavior-dependent manners. (Supported by KAKEN #23240057 and #15H05724)(COI:No)

# Symposium 41

Joint Symposium with the Japanese Society of Pathophysiology
Role of Novel Regulatory Factors on Organ Remodeling and their Possibilities for the Therapeutic Targets

March 23 (Wed), 9:00 – 10:30, Room I

## S41-1

### Vidarabine, a cardiac adenylyl cyclase inhibitor, prevents catecholamine-induced arrhythmias in mice.

Fujita Takayuki、Suita Kenji、Cai Wenqian、Jin Hui-lin、Hidaka Yuko、Ishikawa Yoshihiro
*(Cardiovascular Research Institute, Yokohama City Univ. Yokohama, Japan)*

Catecholamine-induced activation of signaling molecules including type 5 adenylyl cyclase (AC5), one of the major cardiac AC, have been reported to play an important role in cardiac remodeling. AC5 disruption protected the heart against the development of heart failure accompanied by attenuation of cardiac fibrosis and apoptosis of cardiomyocytes in mice. Recently, we found that deficiency of AC5 results in shorter duration of sympathetic activation-induced atrial fibrillation (AF). Consistently, vidarabine, a cardiac adenylyl cyclase inhibitor, decreased the AF duration and reduced the incidence of sympathetic activation-induced ventricular arrhythmias in mice. We demonstrated that vidarabine inhibits adrenergic receptor stimulation-induced SR Ca2+ leak, spontaneous Ca2+ release and reactive oxygen species production which have been considered as potential arrhythmogenic trigger. The pivotal role of the anti-oxidative effect in its anti-arrhythmic property was also indicated in animal study. Additionally, cardiac function was not affected by the amount of vidarabine which is sufficient to exert anti-arrhythmic effect.These findings indicate that vidarabine inhibits development of AF and ventricular arrhythmia without suppressing cardiac function in mice. Vidarabine, an anti-herpesvirus agent, may be a novel useful agent for preventing and treatment of arrhythmias. (COI:No)

## S41-2

### Subtype-specific role of a new cyclic AMP sensor Epac in the pathogenesis of heart failure and arrhythmia

Okumura Satoshi
*(Dept Physiol, Tsurumi Univ, Yokohama, Japan)*

β-Adrenergic receptor (β-AR) signaling is well established as a primary defense mechanisms against acute stress or changes in hemodynamic load; however, its role in cardiac pathogenesis, although extensively, remains poorly understood. β-AR blockade, rather than stimulation, is beneficial in patients with heart failure. Recently, however, we have developed a mouse model in which type 5 adenylyl cyclase (AC), a major cardiac subtype, is disrupted (AC5KO), and we found that AC5KO showed resistance to the development of heart failure and exhibited increased longevity, indicating that inhibition of cyclic AMP (cAMP) signaling at the level of AC, not β-AR, may also result in cardiac protection. Protein kinase A (PKA) is not the only molecule activated by cAMP. Exchange protein activated by cAMP was recently identified as a new target of cAMP signaling that is activated independently of PKA. Epac has two isoforms (Epac1 and Epac2) and both isoforms are expressed in the heart, even if Epac1 is predominantly expressed in the heart. We show that Epac1, in an additive and independent manner with respect to PKA, phosphorylates phospholamban and ryanodine receptor (RyR2) to regulate cardiac function. Loss of Epac1 slightly decreased basal cardiac function, but afforded greater protection against various stresses, including arrhythmogenic stress, whereas loss of Epac2 did not show protective effects. Accordingly, selective inhibition of Epac1 may prevent hyperphosphorylation of PLN and RyR2, and this may be an alternative strategy to current β-AR blocker therapy for the treatment of heart failure and arrhythmia. (COI:No)

## S41-3

### The Roles of Secretoglobin 3A2 in Respiratory Disease

Kurotani Reiko
*(Bioch Eng, Grad Sch Sci Eng, Yamagata Univ, Yamagata, Japan)*

Secretoglobin (SCGB) is a downstream target of NKX2-1. This was originally demonstrated using the suppressive subtractive library screening of mRNAs isolated from the fetal lungs of *Nkx2-1*-null versus wild-type mice lines. SCGB3A2 is a new member of the SCGB gene superfamily and is predominantly expressed in lung airways. The SCGB gene superfamily consists of cytokine-like molecules of secreted proteins of approximately 10 kDa. SCGB3A2 was first found to play a role in the suppression of lung inflammation using a mouse model for allergic airway inflammation. Since the lungs of *Nkx2-1*-null mice exhibited a sac-like structure, we hypothesized that SCGB3A2 is involved in fetal lung development. SCGB3A2 was found to promote the branching and maturation of fetal lungs using an ex vivo lung culture and the administration of SCGB3A2 to pregnant wild-type and *Nkx2-1*-null mice. SCGB3A2 was also found to be important for the suppression of pulmonary fibrosis in a bleomycin-induced mouse model. Because SCGB3A2 has multiple functions in the lung, we investigated the effect of SCGB3A2 in chronic obstructive pulmonary disease (COPD) using a cigarette smoke (CS)-induced COPD mouse model with wild-type, *Scgb3a2*-null, and *Scgb3a2*-tg mice. The mean linear intercept (Lm) of the lungs was significantly increased by CS-exposure in wild-type and *Scgb3a2*-null mice compared with in the non-exposed mice. Although CS exposure did not increase the Lm of *Scgb3a2*-tg mice, data suggested that SCGB3A2 may suppress emphysema or resist damage by CS. We discuss our recent data in the context of known mechanisms of SCGB3A2 on emphysema. (COI:No)

## S41-4

### The role of tissue thrombin on the myocardial remodeling

Hongo Kenichi
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Introduction: Thrombin is a final and key product of coagulation cascade. Recent observations revealed that thrombin also has multiple roles other than coagulation such as vascular repair and gastric movement. In patients with heart failure, coagulability is significantly enhanced compared with healthy subjects.Purpose: We hypothesized that tissue thrombin expression is enhanced and has significant role on the pathogenesis of cardiac remodeling in diseased heart.Methods: We examined the expression level of thrombin by immunohistochemical analysis in the ventricular tissues obtained from surgical resection during Batista operation in patients with dilated cardiomyopathy (DCM) and those from autopsy in patients died without heart diseases. In mouse model of DCM which carries a deletion mutant of troponin T (DCM mouse), we determined the expression level of coagulation factors including thrombin. We further evaluated the effect of the direct thrombin inhibitor, dabigatran, on cardiac function and survival of DCM mouse.Results: Thrombin expression was significantly enhanced in ventricular tissues in DCM patients compared with those without heart diseases. Thrombin expression was also enhanced in heart tissue of DCM mouse. DCM mouse shows typical cardiac remodeling including left ventricular dilatation and severely depressed cardiac function. DCM mouse also died within 6 months after birth. Application of dabigatran significantly improved left ventricular function and survival of DCM mouse.Conclusion: Tissue thrombin may be a new target for the treatment of the cardiac remodeling in DCM. (COI:No)