Oral Presentations

Oral Presentation 01
Ionic Channel, Receptor (1)

(March 16, 9:00–10:00, Room H2)

1O1H2-1

Inhibitory effects of monoterpenes on human TRPA1 and the structural basis of their activity

Takaishi, Masayuki[1]; Fujita, Fumitaka[1]; Shimizu, Mayumi[1]; Shimada, Tadashi[1]; Urabe, Shun[1]; Matsui, Hiroshi[1]; Uchida, Kunitoshi[2]; Tominaga, Makoto[2]([1]*Technical Development center, Mandom corp., Osaka, Japan,* [2]*Section of Cell Signaling, National Institute for Physiological Sciences, Okazaki Institute for Integrative Bioscience, National Institutes of Natural Sciences*)

Monoterpenes, such as menthol, camphor, and 1,8-cineole, comprise a group of naturally occurring organic compounds that have been used for anesthetic, analgesic and anti-inflammatory purposes. Among monoterpens, camphor and 1,8-cineole, both are well-known components of essential oils, are reported to exert analgesic effects through the inhibition of TRPA1.

In this study, we screened several monoterpene analogs of camphor to identify more effective naturally occurring TRPA1 antagonists. We found that borneol, 2-methylisoborneol, and fenchyl alcohol exhibited higher inhibitory effects on human TRPA1 (hTRPA1) activity than either camphor or 1,8-cineole. Furthermore, sensory irritation tests in vivo showed that borneol conferred an analgesic effect on the sensory irritation produced by menthol. These data suggest that borneol, 2-methylisoborneol and fenchyl alcohol have the potential to be effective analgesic compounds.

In addition, we found that the S873, T874, and Y812 residues of hTRPA1 were involved in the inhibitory effects, suggesting that the hydroxyl group in the six-membered ring of the inhibitors may interact with these amino acids. Further research on borneol, 2-methylisoborneol and fenchyl alcohol could lead to the development of anti-nociceptive agents through TRPA1 inhibition. No COI.

1O1H2-2

ATP-mediated odontoblast-odontoblast communication following TRP channel activation

Sato, M; Tsumura, M; Soya, M; Kawaguchi, A; Nishiyama, A; Ogura, K; Mochizuki, H; Kodama, S; Shibukawa, Y; Tazaki, M(*Tokyo Dental Collge*)

Odontoblasts produce the dentin matrix during tooth formation and control its mineralization under physiological and pathological conditions. These cells localize at the interface between the dental pulp and dentin and are organized as a palisade layer. Although odontoblasts form dentin cooperatively, how these cells communicate remains unclear. In this study, we investigated the chemical signaling that mediates intercellular communication between odontoblasts following mechanical stimulation. Single mouse odontoblast lineage cells (OLCs) were stimulated using a glass pipette filled with standard extracellular solution. We measured the intracellular free-Ca^{2+} concentration ($[Ca^{2+}]_i$) by using fura-2 in stimulated OLCs and in the OLCs located near them. Direct mechanical stimulation of single OLCs increased $[Ca^{2+}]_i$ by activating TRPV1, V2, and V4 channels. Moreover, we observed increases in $[Ca^{2+}]_i$ not only in the stimulated OLCs, but also in nearby OLCs. This increase in $[Ca^{2+}]$i in the nearby OLCs, but not in the stimulated OLC, was blocked by pannexin-1 inhibitor in a concentration- and distance-dependent manner. Moreover, in the presence of PLC inhibitor, the increase in $[Ca^{2+}]_i$ produced in the nearby OLCs after mechanically stimulating single OLCs was abolished. Our results indicate that ATP is released from mechanically stimulated odontoblasts via pannexin-1 because of TRP channel activation and that ATP signals to nearby odontoblasts by activating P2Y receptors. The results also strongly suggest that odontoblasts communicate to drive their cellular functions. No COI.

1O1H2-3

Molecular mechanisms for developmental acquisition of single-spiking property of Mauthner cell among homologous reticulospinal neurons in zebrafish

Shimazaki, Takashi; Watanabe, Takaki; Oda, Yoichi(*Graduate School of Science, Nagoya University, Nagoya, Japan*)

The Mauthner (M) cells, which are bilaterally paired giant reticulospinal neurons (RSNs) located at the fourth segment (r4) in the hindbrain of teleost and known to trigger fast escape. M-cells deliver a single spike at the onset of depolarizing input, whereas their homologs, MiD2cm and MiD3cm, which are located in adjacent segment (r5 and r6) fire repetitively. The unique firing property of zebrafish M-cell is acquired during larval development: immature M-cells exhibit burst firing as their homologs. Here, we show the expression of two different Kv subunits and their modification are a key for the acquisition of the M-cell excitability in larval zebrafish. First, pharmacological studies reveal that two types of Kv channel, Kv1 and Kv7, are necessary for single-spiking of M-cell. Second, *in situ* hybridization showed that two different Kv α-subunits, Kv1.1 and Kv7.4, are expressed in M-cells. Both channels, however, are already expressed before M-cells exhibit single spiking and Kv1.1 is also expressed in MiD2/3cm. Nevertheless, the cell-surface expression of Kv1.1 α-subunits is uniquely enhanced by an auxiliary subunit Kvβ2 and the gating of Kv.7.4 is modified by protein kinase C specifically in the M-cells when they acquire the single-spiking property. Thus, these results show critical steps for developmental acquisition of the unique firing properties of the M-cell among the segmentally homologous neurons. No COI.

1O1H2-4

The interaction of new I_{K1} blocker (PA-6) with intracellular spermine and magnesium.

Takanari, Hiroki[1,2]; Houtman, Marien[2]; Stary-Weinzinger, Anna[3]; Ono, Katsushige[1]; van der Heyden, Marcel[2]([1]*Department of Pathophysiology, Oita University School of Medicine, Yufu, Japan,* [2]*Department of Medical Physiology, University Medical Center Utrecht, Utrecht, The Netherlands,* [3]*Department of Pharmacology and Toxicology, University of Vienna, Vienna, Austria*)

Background: The inward rectifier K^+ current (I_{K1}) contributes to a stable resting membrane potential and action potential repolarization in cardiac cells. We developed a new specific I_{K1} blocker, pentamidine analogue (PA-6). Now we investigated the interplay of PA-6, intracellular spermine and Mg^{2+} on channel inhibition.
Methods: HEK293T cells were transfected with human $K_{IR}2.1$ by lipofectamine, and used for inside-out patch clamp experiment with a ramp protocol from -100 mV to 100 mV in 5 s. Both wild type and mutant (E224A) channels were tested.
Results: PA-6 (200 nM) alone blocked I_{K1} completely. Spermine decreased the outward component of I_{K1} by 70% at 0.1 μM, and the inward component by 5%. In the presence of 0.1 μM spermine, PA-6 provided 41% and 39% of additional block on I_{K1} in inward and outward components, respectively. Mg^{2+} (1 mM) had a small blocking effect on I_{K1} by 7%, and did not interfere with I_{K1} block of PA-6. Both PA-6 and spermine had less effect on E224A-$K_{IR}2.1$ current, and no reciprocal interference was observed, suggesting that the negative residue E224 is essential for PA-6 and spermine to interact with the $K_{IR}2.1$ channel.
Conclusion: Spermine, but not Mg^{2+}, partially relieved I_{K1} block of PA-6 in a dose-dependent manner. PA-6 may have an overlapping binding site on $K_{IR}2.1$ channels with spermine, but not with Mg^{2+}. No COI.

Oral Presentation 02
Motor Function / Sensory Function (1)

(March 16, 11:05–12:05, Room J)

1O2J-1

Hierarchical connectivity among morphologically homologous and repeated reticulospinal neurons in the segmented hindbrain for escape behavior in goldfish and zebrafish

Neki, Daisuke; Oda, Yoichi(*Graduate School of Science, Nagoya University, Nagoya, Japan*)

Segmentation along the neuraxis is a prominent feature of the central nervous system in vertebrates. In wide range of fishes, hindbrain segments contain orderly arranged reticulospinal neurons (RSNs). Individual RSNs in goldfish and zebrafish hindbrain are morphologically identified, and similar RSNs are called segmental homologs. Segmental homologs are repeated in adjacent segments of hindbrain and thought to be functionally related. Here, we investigated the functional relationships of them by examining electrophysiological connectivity between the Mauthner cell (M-cell), paired giant RSNs in segment 4 (r4) and known to trigger fast escape or prey capture of fish, and different series of homologous RSNs in r4 to r6. Paired intracellular recordings in adult goldfish revealed unidirectional connections from the M-cell to RSNs. M-cell connectivity was closely correlated with the morphological homology of repeated RSNs in r4 to r6: Single spike of the M-cell produced IPSPs in dorsal RSNs in r4 to r6 on the ipsilateral side and excitatory depolarization on the contralateral side, whereas strong depolarizations equally in ventral RSNs in r4 to r6 on the both sides. These results suggest that each functional connection works as a functional unit during the M-cell-initiated escape or prey capture. No COI.

1O2J-2

Tectal commissural connections and their functional roles in saccades in relation to the VOR and Listing's law

Takahashi, Mayu; Sugiuchi, Yuriko; Shinoda, Yoshikazu(*Dept of Neurophysiology, Tokyo Medical and Dental Univ, Tokyo, Japan*)

The commissural connection between the superior colliculi (SCs) was known to be inhibitory, but we recently found that strong excitatory commissural connections also exist. Electrophysiological study showed that caudal tectoreticular neurons (TRNs) received only inhibition, whereas rostral TRNs received excitation and inhibition from the opposite rostral SC. TRNs in the medial and lateral SCs received excitation from the contra- medial and lateral SC, respectively, and received inhibition from the contra- lateral and medial SC, respectively. These findings suggest that inhibitory commissural connections exist between the medial (lateral) SC representing upward (downward) oblique saccades on one side and the lateral (medial) SC representing downward (upward) oblique saccades on the other. This pattern of reciprocal inhibition between the up-oblique and down-oblique saccades in the two SCs is very similar to that seen in the anterior and posterior canal-related vestibular neurons. This similarity implies that the SC saccade system may use the same coordinates as the VOR. In contrast, mirror-symmetric excitatory connections between medial-medial and lateral-lateral SCs play a role in conjugate upward and downward vertical saccades, respectively, because coactivation of TRNs in symmetric sites of the two SCs may occur through the commissural excitations, and torsional components of individual eyes seem to cancel each other, leaving mainly vertical components. Therefore, these tectal excitatory commissural connections may contribute to Listing's law. No COI.

1O2J-3

Cytoplasmic Regulation of Channel Opening of Electrical Synapses between Retinal Ganglion Cells.

Hidaka, Soh(*Dept. Physiol., Fujita Health Univ. Sch. of Med., Aichi, Japan*)

Electrical synapses are present in retinal neurons expressing channel subunit, connexin36 (J Neurosci, 2004, 2009). Recent studies revealed channel opening of gap junctions between amacrine cells is regulated by intracellular cyclic AMP as well as intracellular Ca^{2+} concentration (Brain Res, 2012). In the present study, I investigated regulation of channel opening of electrical synapses by application of ligands under dual whole-cell patch clamp recordings between retinal ganglion cells. I measured passage currents through electrical synapses by application of antibodies against connexin36 and by change of intracellular Ca^{2+} concentration. I also studied morphological change of gap junctions under the application by electron microscopy. Chelating intracellular Ca^{2+} led us to observe large passage currents between the retinal cells and transjunctional conductance (Gj) between the cells (2.45 nS). Gj suppressed to 0.23 nS by intracellular application of cyclic AMP in pipette with 5mM concentration, compared with that of control condition. Electron microscopy revealed formation of annular gap junctions. Intracellular application of an antibody against the cytoplasmic domain of connexin36 (J Neurosci, 2004) reduced Gj (0.98 nS). The inhibition of Gj by the cytoplasmic antibody was dose-dependent manner. Cocktail of the antibody and cyclic AMP leaves Gj as in the level by single involvement of the antibody. The annular gap junctions were not found under application of the cocktail. These results demonstrate that channel opening of electrical synapses is associated with cytoplasmic development of gap junctions. No COI.

Oral Presentation 03
CNS Function

(March 17, 11:05–12:05, Room G)

1O2J-4

Functional analysis of Na^+,K^+,2Cl^--cotransporters on the lateral cochlear wall contributing to the endolymphatic potential

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The lateral cochlear wall comprises two epithelial layers; strial marginal cells (MCs) facing endolymph, and the syncytium facing perilymph. These layers sandwich a well-vascularized extracellular compartment, the intrastrial space (IS). K^+-gradients established by unidirectional K^+-transport across the lateral cochlear wall elicit K^+-diffusion potentials across K^+-channels, and as a consequence, endolymph has the endocochlear potential (EP) of +80 mV. Since specific inhibitors greatly reduce the EP, basolateral Na^+,K^+,2Cl-cotransporters (NKCCs) expressed on each layer are generally thought to contribute to the unidirectional K^+-transport. In this study, we examined the lateral cochlear wall of guinea pigs in order to analyze the individual function of NKCCs on each layer. When bumetanide, a specific inhibitor of NKCC, was applied perilymphatically, electrochemical properties of the syncytium was barely affected, whereas [K^+] of the IS was elevated just like when bumetanide was vascularly applied. These results suggested that perilymphatically applied bumetanide seemed to penetrate through the syncytium into the IS and inhibit the MC's NKCCs. The syncytium's NKCCs, which was unlikely to be affected, might be dispensable for the unidirectional K^+-transport and the EP. No COI.

2O3G-1

Plasmalogens diet enhance hippocampal-dependent memory by enhancing neurogenesis in dentate gyrus

Hossain, Shamim Md[1]; Ifuku, Masataka[1]; Ahmed, Youssef Md.Saleh[1]; Miake, Kiyotaka[2]; Fuchuu, Hidetaka[2]; Katafuchi, Toshihiko[1]([1]*Department of Integrative Physiology, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan,* [2]*Center Research Institute, Marudai Food Company Limited, Osaka,Japan*)

The ether phospholipids, Plasmalogens (Pls), are characterized by the presence of a vinyl ether linkage at the sn-1 position. In the Alzheimer's disease (AD) patients, Pls were found to be reduced in the brain tissues. Human brain contains a large amount of Pls, mainly ethanolamine Pls (EthPls), in the hippocampus. Hippocampus controls the memory and usually it is highly damaged in the AD patients. We therefore, hypothesized that the reduction of Pls in the hippocampus of brain might be associated with the neuronal damages and memory loss. In our previous study, we have found that Pls can inhibit hippocampal neuronal cell death. In the present study, we have found that the mice having Pls food performed better than control mice in Morris water task suggesting that Pls enhance memory. In in vitro experiments the cultured hippocampal neurons showed a significant increase in the spine formation after treatment with EthPls ($P<0.01$). In addition, Pls diet significantly increased neurogenesis marked by an increase of doublecortin positive neurons in the DG ($P<0.01$). More interestingly, we have found that Pls diet enhance mRNA expression of BDNF in the hippocampus. We therefore, propose that Pls diet increase neurogenesis and spine formation probably through the expression of BDNF in the hippocampus resulting in the memory formation. No COI.

2O3G-2

Subtypes of nicotinic acetylcholine receptors in nicotine-induced vasodilation in the rat hippocampus

Watanabe, Saori[1,2]; Misawa, Hidemi[2]; Uchida, Sae[1]([1]*Department of Autonomic Neuroscience, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan,* [2]*Department of Pharmacology, Faculty of Pharmacy, Keio University, Tokyo, Japan*)

Previous study in our laboratory has shown that stimulation of cholinergic neurons in the medial septal nucleus increases blood flow in the hippocampus (Hpc-BF) via activation of the nicotinic acetylcholine receptors (nAChRs). Of the several subtypes of nicotinic receptors, the $\alpha4\beta2$ and the $\alpha7$ subtypes are the most abundant in the hippocampus. The present study aimed to determine whether either or both the $\alpha4\beta2$ or the $\alpha7$ subtype is involved in the cholinergic vasodilation in the hippocampus. In urethane-anesthetized rats, Hpc-BF was measured by laser Doppler flowmetry. We examined the response of Hpc-BF induced by administration of nicotine (10–300 μg/kg, i.v.) as a nAChR agonist. Hpc-BF increased dose-dependently following the administration of 30–300 μg/kg nicotine. Mean arterial pressure was not influenced at 10–30 μg/kg nicotine, but was increased at 100–300 μg/kg nicotine. The increase in Hpc-BF by nicotine at 30 μg/kg, without changing mean arterial pressure, was completely abolished by the $\alpha4\beta2$ nAChR antagonist dihydro-β-erythroidine (DHβE, 5 mg/kg, i.v.), while it was not influenced by the $\alpha7$ selective nAChR antagonist methyllycaconitine (MLA, 5mg/kg, i.v.). These findings suggest that cholinergic vasodilation in the hippocampus is mediated via $\alpha4\beta2$ nicotinic receptors while $\alpha7$ nicotinic receptors are not to be involved. No COI.

2O3G-3

Rat thalamic neurons encode complex combinations of facing and movement directions and trajectory route during translocation with sensory conflict

Nyamdavaa, Enkhjargal(*System Emotional Science, Grad Sch of Med and Pharmaceu Sci, Univ of Toyama,Toyama, Japan*)

Previous studies have reported that some thalamic neurons encode the animal's directional heading, and these are referred to as head direction cells. The present study investigated effects of sensory mismatch among ideothetic cues on thalamic neurons. Rats were placed on a treadmill stage that moved in a figure-8-shaped pathway. The anterodorsal and laterodorsal thalamic neurons were recorded under 2 conditions: 1) control sessions, in which both the stage and the treadmill moved forward, and 2) backward sessions, in which the stage was moved backward while the rats ran forward on the treadmill. Of the 222 thalamic neurons recorded, 60 showed differential responses to the south and north directions, along which the animals were translocated in the long axis of the trajectory. Of these 60, 19 showed facing direction-dependent responses regardless of movement direction. Twenty neurons displayed facing and movement direction-dependent responses; activity of 10 and the remaining 10 neurons increased during forward and backward movement, respectively. Twenty one neurons showed movement direction-related responses regardless of facing direction. Furthermore, the activity of some direction-related neurons increased only in a specific trajectory. The results suggested that the activity of these neurons reflects complex combinations of facing direction, movement direction, motor/proprioceptive information, and the past history of movements. No COI.

2O3G-4

Classical conditioning reinforced by visual stimuli after V1 lesion

Takakuwa, Norihiro[1,2]; Kato, Rikako[1]; Redgrave, Peter[3]; Isa, Tadashi[1,2]([1]*Dept Dev. Physiol, Nat'l Inst. Physiol. Sci., Okazaki, Japan,* [2]*The Graduate Univ. for Advanced Studies, Hayama, Japan,* [3]*Dept Psychol, Univ. of Sheffield, Sheffield, U.K.*)

Classical conditioning is when animals learn an association between a predicting conditioned stimulus (CS) and a reward. During conditioning, the CS acquires the properties of a secondary reinforcer so that, subsequently, it can reinforce the acquisition of novel instrumental behavior. We hypothesised that the direct projection from the superior colliculus (SC) to dopamine (DA) neurons in the substantia nigra pars compacta (SNc) plays a key role in associative learning with conditioned visual stimuli. To test this hypothesis we classically conditioned two monkeys with unilateral V1 lesions (an animal model of "blindsight") when visual CSs were presented in the lesion-affected and intact visual fields.After training with two CSs, one predicting high (large/immediate) and the other low (small/delayed) reward, the monkeys exhibited appropriately timed, quantitatively modulated anticipatory licking. These classically conditioned responses were elicited when the visual CSs were presented in either the lesion-affected and intact visual field. After conditioning, we recorded the responses of SNc dopamine neurons when the CSs were presented again in the lesion-affected visual field. We showed clear phasic responses when CSs were presented in the lesion-affected visual field. Together these results show that visual input via the SC has access to basic associative learning circuitry in the basal ganglia. No COI.

Oral Presentation 04
Heart, Circulation (1)

(March 17, 11:05–12:05, Room G)

3O4G-1

Alteration of the arterial baroreflex sensitivity by electrical stimulation of the mesencephalic ventral tegmental area in decerebrate rats

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We have provided evidence that arterial baroreceptor-heart rate (HR) reflex is blunted at the onset of spontaneous motor activity in paralyzed, decerebrate cats (Matsukawa et al. Am J Physiol Heart Circ Physiol 2012). Given that the mesencephalic ventral tegmental area (VTA) plays an important role in the generation of the cardiovascular response during exercise, we hypothesized here that arterial baroreflex sensitivity (ABS) is altered by neuronal activities within the VTA. Rats were decerebrated at the premammillary and precollicular level and then paralyzed. Activation of the VTA was evoked by an electrical stimulation (40–80 μA) lasting for 30 s. A brief occlusion of the abdominal aorta was used to naturally activate arterial baroreceptors. ABS for HR was estimated from the baroreflex ratio between the pressor and bradycardia responses during aortic occlusion and from the slope of the baroreflex curve between changes in the mean arterial pressure (MAP) and HR. As compared to that without VTA stimulation, both baroreflex ratio and slope of the δMAP-δHR curve were blunted when aortic occlusion was given at the onset of VTA stimulation (0–3 s). At 10–25 s from the start of VTA stimulation, surprisingly, the baroreflex ratio and the slope of the curve reverted to the pre-stimulation level and even turned to increase. The present findings suggest that activation of VTA leads to decease of the ABS at the onset of, but not during, the fictive motor activity. No COI.

3O4G-2

β-adrenergic vasodilatation does not contribute to centrally-induced muscle hyperemia at start of voluntary one-legged cycling and during motor imagery

Ishii, Kei[1]; Matsukawa, Kanji[1]; Liang, Nan[1]; Endo, Kana[1]; Idesako, Mitsuhiro[1]; Hamada, Hironobu[2]; Kataoka, Tsuyoshi[3]; Ueno, Kazumi[3]; Watanabe, Tae[3]([1]*Department of Integrative Physiology, Graduate School of Biomedical and Health Sciences, Hiroshima University,* [2]*Department of Physical Analysis and Therapeutic Sciences, Graduate School of Biomedical and Health Sciences, Hiroshima University,* [3]*Department of Health Care for Adults, Graduate School of Biomedical and Health Sciences, Hiroshima University*)

We hypothesized that central command induces sympathetic β-adrenergic vasodilatation at start of exercise, which in turn may contribute to increasing muscle blood flow. To test the hypothesis, the effects of propranolol on the blood flow responses to voluntary one-legged cycling and imagery of the exercise were examined in 9 subjects. The relative concentrations of oxygenated-hemoglobin (Oxy-Hb) in bilateral vastus lateralis muscles were measured as an index of muscle tissue blood flow with near-infrared spectroscopy. Increases in Oxy-Hb of the bilateral muscles observed at start of one-legged cycling and during motor imagery were not blunted by propranolol but were abolished by additional atropine. When femoral blood flow to the non-exercising limb was measured with ultrasound Doppler flowmetry in 5 subjects, both one-legged cycling and motor imagery evoked the increases in femoral blood flow and vascular conductance. The vasodilatation was atropine-sensitive and was not affected by propranolol. Thus we concluded that central command evokes cholinergic, but not β-adrenergic, vasodilatation in skeletal muscles at start of voluntary exercise in humans. No COI.

3O4G-3

Drinking-induced bradyarrhythmias and cerebral injury in Dahl salt sensitive rats with sinoaortic denervation

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

We have demonstrated that a drinking-induced pressor response was larger if the baroreflex did not operate, and the mean arterial pressure reached 163 mmHg in conscious rats with sinoaortic denervation (SAD). Thus, we hypothesized that a drinking behavior became a cardiovascular risk factor, if a basal arterial pressure was high. To clarify this, we analyzed the occurrence of arrhythmias and the accumulation of microglia in Dahl salt sensitive rats (Dahl S) with SAD. We maintained Dahl S and Dahl salt resistant (Dahl R) with high-sodium diet for 5 weeks. After SAD surgery, we measured AP and electrocardiogram (ECG) during water drinking behavior in all rats. Furthermore, we measured TNF-α concentration in the cerebrospinal fluid (CSF) and microglial accumulations around the 3rd and 4th ventricles in rats with programmed drinking at a rapid or slow rate for 7 days. Incidence of drinking-induced bradyarrhythmias and premature ventricular contractions (PVCs) were significantly larger in Dahl S than Dahl R rats. Both bradyarrhythmias and PVCs were completely abolished by atropine administration. Accumulations of microglia around the 3rd ventricle and increase in TNF-α in the CSF were observed in rats that drank water at a rapid rate; these were not seen in rats that drank water slowly. In conclusion, both cardiovascular events and cerebral injury may be increased by drinking in Dahl S rats with SAD. These risks are reduced by modifying drinking behavior such as slowing the drinking rate. No COI.

3O4G-4

The Analysis of the polyunsaturated fatty acids and the incidences of supraventricular arrhythmias of the elderly in Group Home

Todoroki, Kikue[1,2]; Ikeya, Yoshimori[1]; Tanaka, Etsuro[2]; Fukuyama, Naoto[1]; Mori, Hidezo[1]([1]*Dept Physiology, Tokai Univ School of Medicine,* [2]*Dept Nutritional Science, Tokyo Univ of Agriculture*)

We reported that the plasma eicosapentaenoic acid (EPA) concentration in ischemic stroke patients especially in cardioembolism are significantly lower than the control (Ikeya et al, 2013). Atrial fibrillation increases the risk of cardioembolism. However, the polyunsaturated fatty acids and the incidences of supraventricular arrhythmias with reference to cardioembolism of the elderly in Group Home (GH) has not been examined.To analyze EPA and other polyunsaturated fatty acids and the incidences of supraventricular arrhythmias between the 30 elderly in GH and 30 age-matched control subjects, we compared the blood sample data (EPA, arachidonic acid (AA), docosahexiaenoic acid and triglyceride, LDL-cholesterol, HDL-cholesterol and HbA1c) and quantitated the incidences of supraventricular arrhythmias using a non-parametric test and a multiple logistic regression analysis. We also investigated lifestyle habit of fish consumption using a questionnaire.The multiple logistic regression analysis revealed that lower EPA and HbA1c and higher AA were specific in the elderly in Group Home. The incidences of supraventricular arrhythmias were not significantly different between the two groups. This cross-sectional study of the elderly in GH showed that significant lower plasma EPA concentration and fish consumption and higher AA and that no significant difference in the incidences of supraventricular arrhythmias. These results might lead to EPA replacement therapy for the elderly in GH. No COI.

Oral Presentation 05
Cell Physiology, Molecular Physiology (1)

(March 18, 11:05–12:05, Room J)

3O5J-1

Basic fibroblast growth factor-induced neuronal differentiation of canine bone marrow stromal cells: Involvement of FGFR2/PI3K/Akt pathway

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The domestic dog is an optimal model for the pre-human trials in spinal cord injury. However, the capacity and mechanism of neuronal differentiation of canine bone marrow stromal cells (BMSCs) have not well been elucidated. We here investigated the effect of basic fibroblast growth factor (bFGF) on neuronal differentiation of canine BMSCs and the signalling pathway. In canine BMSCs, bFGF (100 ng/ml) induced an increase in mRNA expression of neuron markers, MAP2, NEFL and ENO2, and a decrease of that of neural stem cell and glia markers, NES and GFAP. Immunocytochemical study showed that the cells expressed neuron markers, NF-L and NSE. In the bFGF-treated cells loaded with the Ca^{2+} indicator Fluo3, a high concentration of KCl and L-glutamate induced an increase in intracellular Ca^{2+} levels. In canine BMSCs, cross-linking and immunoprecipitation analysis revealed that bFGF bound to the FGFR2. In the presence of the FGFR inhibitor SU5402, the PI3K inhibitor LY294002 and the Akt inhibitor MK2206, bFGF failed to induce mRNA expression of MAP2. In bFGF-treated cells, Akt was phosphorylated, and the phosphorylation was inhibited by SU5402, LY294002 and MK2206. Taken together, it is most likely that FGFR2/PI3K/Akt pathway is involved in bFGF-induced neuronal differentiation of canine BMSCs. No COI.

3O5J-2

Aloin differentiates 3T3 fibroblasts to osteoblasts through MAPK mediated Wnt dependent BMP signaling cascade

Pengjam, Yutthana; Kumar, Harish; Madhyastha, Radha; Omura, Sayuri; Nakajima, Yuichi; Maruyama, Masugi(*The Department of Applied Physiology, Faculty of Medicine, University of Miyazaki, Miyazaki, Japan*)

Bone mass is maintained by balance between bone forming osteoblast and osteoclast cells. In this study, we investigated the effects of aloin, an anthraquinone glycoside, on 3T3 fibroblast cells and studied the proliferation, differentiation and bone formation parameters like mineralization and associated signaling cascade. Initially we confirmed the action of aloin on the proliferation patterns of 3T3 cells and results confirmed the dose dependent activity. Results showed 0.05 μM of aloin increased ALP activity significantly. The mineralization of the extracellular matrix was determined by Alizarin red S staining and showed aloin 0.05 μM concentrations significantly deposited calcium. We studied different concentration of aloin on fluctuations of osteoblast marker genes. Results revealed the dose dependent fluctuations of marker genes. We also investigated the osteogenesis signaling pathways like MAPK, Wnt dependent bone morphogenetic protein (BMP) signaling pathway. Furthermore, the BMP and Runx2 antagonist noggin and MAPK inhibitors, including p38 inhibitor, SAPK/JNK inhibitor and Akt inhibitor attenuated aloin-promoted protein signaling activity. Taken together, these results indicate that aloin differentiates 3T3 fibroblast cells into osteoblast through MAPK, Wnt dependent BMP signaling pathway. Furthermore, aloin has anabolic effects of bone formation and may be useful for the treatment of osteogenic related diseases. No COI.

3O5J-3

SCF/beta-TRCP Negatively Regulates Cdh1 Stability to Ensure Proper Cell-Cycle Progression

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Cell-cycle, the series of events that take place in a cell leading to its division and duplication, is the fundamental mechanism of development, differentiation and aging. Mutations of the cell cycle components frequently founded in several cancers. Proper cell-cycle transitions is progressed by "Kinase Engine" containing CDK/Cyclin complexes activity and tuned by ubiquitin system controlling protein amount of kinases. Skp1/Cullin/F-boxprotein (SCF) complexes and anaphase-promoting complexes (APC) represent two major classes of ubiquitin ligases whose activities are thought to regulate primarily the G1/S and metaphase/anaphase cell-cycle transitions, respectively. However, transition mechanism between SCF and APC complex activities are still unclear. We previously reported that APC/Cdh1 regulates the SCF component Skp2 for degradation. Here, we continue to report that SCF/beta-TRCP reciprocally controls APC/Cdh1 activity by governing Cdh1 ubiquitination and subsequent degradation. Dis-function of this mechanism cause protraction of G1/S transition and loss of stemness in mouse stem cells. Thus, our work reveals a timely crosstalk between APC and SCF defines an ordered cascade of APC and SCF activity during cell- cycle transitions. No COI.

3O5J-4

Calcineurin B homologous protein 3 (CHP3) modulates Akt-GSK3beta signaling in rat cardiomyocytes

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Calcineurin B homologous protein3 (CHP3) is a 25-kDa EF-hand calcium-binding protein mainly expressed in heart, but its function remains largely unknown. To understand the role of CHP3, we knocked down the gene in rat neonatal ventricular cardiomyocytes by using adenovirus-mediated RNA interference technique. CHP3 knockdown significantly enlarged the size of cardiomyocytes and increased the protein expression level of the hypertrophy marker ANP. In addition, CHP3 knockdown increased the phosphorylation levels of Akt and GSK3beta, key regulators of cardiac hypertrophy. On the other hand, when CHP3 was overexpressed in lung-derived fibroblast cells which do not express the protein, the insulin-induced phosphorylation levels of Akt and GSK3beta was decreased. Furthermore, co-immunoprecipitation experiments demonstrated the interaction of CHP3 with GSK3beta. These results suggest that CHP3 may be a novel regulator for cardiac hypertrophy through modulating Akt-GSK3beta signaling. No COI.

Oral Presentation 06
Ionic Channel, Receptor (2) / Neuron, Synapse

(March 18, 14:00–16:00, Room F)

3O6F-1

Compromised GABAergic maturation causes abnormal network activity in the hippocampus of epileptic Ca^{2+} channel mutant mice, *tottering*

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Cognitive impairments are devastating co-morbidities of epilepsy, but their underlying mechanisms are still elusive. In this study, using 64-electrode array (MED64 system), we investigated network activity in the hippocampus in P/Q-type Ca^{2+} channel mutant *tottering* (*tg*) mice, a well-established model of spontaneous absence epilepsy. Hippocampal slices of *tg* mice displayed muscarinic acetylcholine receptor-induced epileptiform discharges with abnormal high-frequency patterns originating from the CA3 region. These were attributable to a developmental retardation of GABAergic inhibition caused by immature intracellular Cl^- regulation via abnormality in developmentally regulated expression of Cl^- transporters and by $GABA_A$ receptor compositions in hippocampal neurons. Our study suggests that compromised GABAergic maturation causing abnormal network activity in the hippocampus presumably contributes to cognitive co-morbidities in epilepsy due to aberrant P/Q-type channel functions. No COI.

3O6F-2

Genetic and functional analysis of HCN channel mutation in familial febrile seizure patients

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Hyperpolarization-activated cyclic nucleotide-gated (HCN) currents (I_h) are identified in neuron. I_h maintain resting membrane potentials and control neuronal rhythmic activity. HCN channels in the human brain are formed by HCN1 and HCN2 subunits.Recent studies show that mutations in sodium channels and GABA were found in Febrile seizure (FS) patients. However, these were rare and do not explain the common cause of FS. We examined the *HCN2* in 160 FS patients and found the same mutation (S126L) in 2 of them. We performed a whole-cell patch clamp experiment for wildtype (WT), homo-mutant (MT) and heteromeric channels. To replicate physiological human temperature, the bath solution's temperature was set from 35 to 38 °C.At 35 °C and again at 38 °C, there was no significant difference in voltage dependence between each channels. However, when the temperature was set from 25 to 38 °C, the shift of the half maximal activation voltage ($V_{1/2}$) for MT channels significantly increased. No significant differences were found in the response of cyclic AMP.These results indicate that the S126L mutation increases temperature sensitivity, suggesting that this mutation may trigger the onset of FS. No COI.

3O6F-3

Local temperature changes of epileptogenic zone relate to disease progression through enhancement of TRPV4 activity

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Physiological brain temperature is an important determinant for neuronal functions, and it is well established that changes in temperature have dynamic influences on brain neuronal excitabilities. We have clearly revealed that a thermo-sensor TRPV4 (activated above 34°C) is activated by physiological temperature in hippocampal neurons and thereby controls their excitability. Therefore, if local brain temperature can dynamically elevate depending on the neuronal activities, a thermo-sensor TRPV4 can enhance electrical excitability in neurons, and might lead to hyperexcitability. In this study, we focused on epilepsy, since it was caused by hyperexcitability of neurons. We generated a model of partial epilepsy by utilizing kindling stimuli in ventral hippocampus of wild type (WT) or TRPV4KO mice, and measured electroencephalogram (EEG). The frequencies of epileptic EEG in WT mice were significantly larger than those in TRPV4KO mice. These results strongly indicate that TRPV4 activation is involved in disease progression of epilepsy. We expected that the disease progression enhanced hyperexcitability, and lead to hyperthermia in the epileptogenic zones. To confirm it, we developed a new device to measure exact brain temperature only in restricted local area. From the recording results by the new device, we revealed that the brain temperatures in epileptogenic zones were dramatically elevated compared with normal regions. No COI.

3O6F-4

Endocannabinoids shorten seizures presumably by suppressing excitatory synaptic inputs around the inner molecular layer of the dentate gyrus

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In temporal lobe epilepsy, expression of cannabinoid CB_1 receptors around the inner molecular layer (IML) of the dentate gyrus is markedly decreased. However, it is unclear how deficient endocannabinoid (eCB) signaling around the IML affects seizures. The present study aimed at elucidating roles of eCB signaling around the IML of the dentate gyrus during seizures. Stimulus and recording electrodes were implanted into the angular bundle and the dentate gyri, respectively, of adult C57/bl6J mice. Afterdischarges were evoked 30 min after intraperitoneal injection of a CB_1 antagonist, AM251 (10mg/kg), or vehicle. Current source density was calculated from local field potentials. We found that seizures in the dentate gyrus were significantly longer in AM251-treated mice than vehicle-treated mice. Seizures in AM251-treated mice consisted of repeated burst discharges triggered by excitatory inputs around the IML of the dentate gyrus. Furthermore, the duration of each burst discharge was significantly longer in AM251-treated mice due to the sequential activation of the tri-synaptic circuit in the hippocampus. Optogenetic stimulation of the IML projections during seizures significantly increased the number of repetition, but not the duration of each burst discharge in wild-type mice. These results suggest that eCB signaling around the IML of the dentate gyrus specifically suppresses repetition of burst discharges and shortens seizures. No COI.

3O6F-5

On-demand biosynthesis by diacylglycerol lipase*α* is the major source of 2-arachidonoylglycerol, the endocannabinoid that mediates retrograde signaling.

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The endocannabinoid (eCB) 2-arachidonoylglycerol (2-AG) produced by diacylglycerol lipase *α* (DGL*α*) is one of the best-characterized retrograde messengers at central synapses. 2-AG is thought to be produced 'on demand' upon neuronal activity. However, recent studies proposed that 2-AG is presynthesized by DGL*α* and stored in neurons, and that 2-AG is released from such 'pre-formed pools' without contribution of DGL*α*. To address whether the 2-AG source for retrograde signaling is the on-demand biosynthesis by DGL*α* or the mobilization from preformed pools, we examined the effects of acute pharmacological inhibition of DGL by a novel potent DGL inhibitor, OMDM-188, on several forms of eCB signaling. We found that both depolarization-induced suppression of inhibition (DSI), a purely Ca^{2+}-dependent eCB signaling, and group I metabotropic glutamate receptor (I-mGluR)-induced eCB signaling were blocked by OMDM-188 in cultured hippocampal neurons. We also found that OMDM-188 blocked DSI in acute slices from the hippocampus, striatum and cerebellum. Moreover, OMDM-188 blocked synaptic suppression of Purkinje cells induced by burst stimulation of parallel fibers, which is mediated by 2-AG released by combination of Ca^{2+} influx and mGluR1 activation. Our results do not support pre-formed 2-AG hypothesis but strongly suggest that on-demand 2-AG biosynthesis by DGL*α*; initiates retrograde signaling. No COI.

3O6F-6

BDNF derived from Purkinje cell regulates synapse elimination in the developing cerebellum

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Elimination of redundant synapses formed at earlier developmental stage is known as "synapse elimination" and is considered to be an essential process in neural circuit formation. In the neonatal mouse cerebellum, each PC is innervated by multiple CFs. During postnatal development, early-formed surplus CFs are gradually eliminated and most PCs eventually become innervated by single CFs in mature mice. However, molecular mechanisms of CF synapse elimination still remain largely unknown. In this study, we examined whether Brain-derived neurotrophic factor (BDNF) in postsynaptic PCs is involved in CF synapse elimination using conditional knockout mice in which BDNF is deleted in Purkinje cells (BDNF-PC-KO mice). In mutant mice, about 40% of PCs were innervated by multiple (two or three) CFs while only 20% of PCs were innervated by two CFs in control mice from postnatal day 21 (P21). Furthermore, excitatory inputs from parallel fibers (PFs) and inhibitory inputs from molecular layer interneurons were also altered in mutant mice. These results suggest that BDNF signaling from postsynaptic Purkinje cell regulates developmental synapse elimination in the cerebellum either by directly interacting with presynaptic CFs or indirectly affecting PFs or inhibitory synapses. No COI.

3O6F-7

The role of primary somatosensory cortex in causing mirror-image pain

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It has recently been reported that plastic changes of neuronal circuits play important roles in chronic pain. We previously reported that chronic hind paw pain increased the neuronal activity and spine turnover rate in contralateral somatosensory cortex (S1). Among the symptoms of chronic pain syndrome, patients with peripheral nerve injury can suffer from a mirror-image pain that persists at uninjured sites contralateral to the peripheral nerve injury. However, there is no information about the activity of neuronal and glial cell population of ipsilateral S1 (ipsi-S1) with single cell resolution. In the present study, therefore, we investigated the activity of neuronal and glial cells in ipsi-S1 using *in vivo* 2-photon Ca^{2+} imaging under chronic pain conditions. Following peripheral nerve ligation (PSL), we observed increased Ca^{2+} transient currents in the ipsi-S1 for layer 1 inhibitory neurons and astrocytes, but the spine turnover of pyramidal neurons remained unchanged. Interestingly, chronic application of the $GABA_A$ receptor antagonist gabazine (SR95531) to the ipsi-S1 of PSL mice increased spine turnover rate in the ipsi-S1, and decreased the threshold to mechanical stimuli in the intact hind paw contralateral to the PSL site. Synaptic changes in the ipsi-S1 induced additional impairment; thus, an excitation-inhibition balance could be an underlying mechanism for mirror-image pain. No COI.

Oral Presentation 07
Kidney, Urination / Cell Physiology / Sensory Function (2) / Nutrition, Moleculara Physiology (2), Metabolism, Thermoregulation / Absorption / Heart, Circulation (2)

(March 18, 14:00–16:00, Room J)

3O6F-8

Formation of synapses in somatosensory cortex of developing mice: using in vivo two photon imaging

Miyamoto, Akiko[1,2]; Eto, Kei[2]; Murakoshi, Hideji[3]; Shibata, Keisuke[4]; Koizumi, Schuichi[4]; Nabekura, Junichi[1,2]([1]*Dept Physiol, Sch Life Sci, SOKENDAI,* [2]*Dept Homeostatic Develop, Natl Inst Physiol Sci, Okazaki,* [3]*Supportive Center for Brain Research, Natl Inst Physiol Sci, Okazaki,* [4]*Dept Pharmacol, Grad Sch Med and Eng, Yamanashi Univ, Yamanashi*)

It has been recently reported that resting microglia, which are immune cells in the central nervous systems, actively interact with synapses. For example, resting microglia selectively contact onto synapses in intact brain and are also involved in synapse elimination at cortical ischemia, which may contribute to neural circuit reorganization. In addition, activated microglia are known to release several molecules related to synapse formation. In this study, we focused on whether microglia are involved in synapse formation of neocortex at postnatal day 8–10 mice during which excitatory synapses are rapidly increasing in number. Using an in vivo two photon imaging technique, we observed that filopodia was formed at microglial contacted dendrite of L2/3 pyramidal cell, which occurred age specifically. It is known that microglia in immature brain are more likely to be active in morphology. Injection of minocycline, which lessens the activation of microglia, and their ablation induced by genetic manipulation decreased cortical spines in density. Since, microglia-induced filopodia could eventually become to excitatory synapses, we examined miniature EPSC (mEPSC) frequency. mEPSC frequency was significantly reduced in microglia ablated mice. These data indicates that microglia is contributed to functional synapse formation in developing mouse cortex. No COI.

3O7J-1

Overexpression of Leukocyte Kv1.3-Channels Promotes Renal Fibrosis in Rats with Advanced Chronic Renal Failure

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Leukocytes, such as lymphocytes and macrophages, predominantly express delayed rectifier K+-channels (Kv1.3), and the channels play crucial roles in the activation and proliferation of the cells. Since lymphocytes are activated in patients with end-stage renal disease (ESRD), the channels expressed in those cells would contribute to the progression of renal fibrosis in advanced stage chronic renal failure (CRF). In the present study, using a rat model with advanced CRF that underwent 5/6 nephrectomy followed by a 14-week recovery period, we examined the histopathological features of the kidneys and the leukocyte expression of Kv1.3-channels and cell cycle markers. Age-matched sham-operated rats were used as controls. In the cortical interstitium of advanced CRF rat kidneys, leukocytes proliferated in situ and overexpressed Kv1.3-channel protein in their cytoplasm. Treatment with margatoxin, a selective Kv1.3-channel inhibitor, significantly suppressed the number of leukocytes and the progression of renal fibrosis with a significant decrease in the cortical cell cycle marker expression. This study demonstrated for the first time that the number of leukocytes was dramatically increased in rat kidneys with advanced CRF. The overexpression of Kv1.3-channels in the leukocytes was thought to contribute to the progression of renal fibrosis by stimulating cell cycling and promoting cellular proliferation. No COI.

3O7J-2

ERp57 up-regulates gastric H^+,K^+-ATPase activity apart from its chaperoning function

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We found that ERp57, which is an ER chaperone having disulfide isomerase activity, was highly expressed in human gastric parietal cells. To clarify distribution of ERp57 in gastric parietal cells, two types of gastric vesicles, intracellular tubulovesicles (TV) and stimulation-associated vesicles (SAV) containing apical membrane, were prepared from hog gastric mucosa. ERp57 was predominantly expressed in SAV but not in TV. Gastric H^+,K^+-ATPase was expressed both in TV and SAV. ERp57 was co-immunoprecipitated with H^+,K^+-ATPase in the lysate of SAV. Overexpression of ERp57 in the HEK293 cells stably expressing H^+,K^+-ATPase α- and β-subunits (HEK-$\alpha\beta$) significantly increased the ATPase activity. Interestingly, overexpression of a catalytically inactive mutant of ERp57 in the HEK-$\alpha\beta$ cells also stimulated the ATPase activity. In contrast, knockdown of endogenous ERp57 in the HEK-$\alpha\beta$ cells significantly decreased the ATPase activity. It is noted that overexprssion and knockdown of ERp57 had no effects on the expression level of H^+,K^+-ATPase α- and β-subunits. On the other hand, expression and function of endogenous Na^+,K^+-ATPase were not significantly changed by overexpression and knockdown of ERp57 in the cells. These results suggest that ERp57 positively regulates gastric H^+,K^+-ATPase activity apart from its chaperoning function. It may regulate the H^+,K^+-ATPase activity in basal gastric acid secretion. No COI.

3O7J-3

Contribution of developmental changes in ionic systems to myocardial excitation-contraction coupling: a simulation study

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The heart develops and gains new functions while continuously pumping blood, and heart abnormalities during the early developmental stages progress to congenital heart malformations; therefore, the developmental program of the heart, including the expression of the genes responsible for various ionic channels, is likely to be tightly regulated. Here, we integrated developmental changes in 1) ionic systems and 2) energy metabolism on the mathematical models. 1) The quantitative changes in individual ionic systems were represented as relative current densities. We switched the relative current densities of 9 ionic components between early to late embryonic stages in the Kyoto model, and showed that the increase in inward rectifier current before the disappearance of funny current was predicted to result in abnormally high intracellular Ca^{2+} concentrations. 2) The changes in glycolytic enzymatic activities and those in concentrations of glycogen and total creatine were implemented accordingly to the model to represent specific developmental stages. We then simulated effects of hypoxic condition to dynamic changes in contractile force and ATP concentration. As a result, our model showed that the fetal ventricular cells maintained ATP for longer periods of time than the adult ventricular cells, which is consistent with the reported dynamics under hypoxic condition. No COI.

3O7J-4

A novel physiological role of muscle fascia as a nociceptive sensory tissue

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The second skeleton, fascia, is a sheet- or layer-like structure which functionally binds and separates whole body parts from head to toe, and makes it possible for animals to perform smooth and cooperative muscular work. The fascia also protects internal structures that it covers, and forms passageways for blood vessels and nerves. Except for its supportive and biomechanical properties, however, physiological roles of fascia have never been subjected to intensive exploration in the biomedical sciences, even though it has long been considered not only an important source of nociception and pain but also a critical target for clinical treatment of patients with musculoskeletal pain. Here we examined peripheral and spinal mechanisms of fascial nociception in rats, and found that 1) nociceptive nerve fibers with peptidergic and non-peptidergic axons and terminals were distributed in the fascia, 2) peripheral afferents (Aδ- and C-fibers) responding to noxious mechanical, chemical, and thermal stimuli existed in the fascia, 3) nociceptive information from the fascia was projected mainly to laminae I-II of the spinal dorsal horn where the nociceptive input is mainly processed, and 4) spinal dorsal horn neurons receiving input from the fascia existed, and the neurons were definitely sensitized in hyperalgesic condition of the tissue. Taken together, these results strengthen the supposition that muscle fascia is not just a supportive tissue surrounding the muscle, but is a nociceptive sensory tissue/organ, and that it should be a target of treatment in patients with myofascial pain such as a stiff neck and low back pain. COI properly declared.

3O7J-5

Hypothalamic neuropeptide Y-induced GABA inhibition of the rostral medullary raphe to inhibit sympathetic outflow to brown adipose tissue

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Injection of neuropeptide Y (NPY) into the paraventricular hypothalamic nucleus (PVH) or the lateral ventricle lowers metabolism as well as causes strong hyperphagia. However, the neural mechanism of these obesogenic effects of hypothalamic NPY is unknown. We hypothesized that NPY signaling from the hypothalamus may inhibit sympathetic premotor neurons in the rostral medullary raphe (rMR) that control thermogenesis in brown adipose tissue (BAT). In this study, we examined this hypothesis by recording BAT sympathetic nerve activity, BAT temperature and other parameters in anesthetized rats, whose trunk was covered with a water jacket to control the skin temperature. Skin cooling-evoked increases in BAT sympathetic nerve activity and BAT temperature were eliminated by injection of NPY into the lateral ventricle or PVH. NPY injection into the PVH also eliminated BAT thermogenesis evoked by glutamate receptor stimulation in the rMR with an NMDA nanoinjection. In contrast, NPY injection into the PVH did not affect BAT thermogenesis evoked by antagonizing GABA receptors in the rMR with a bicuculline injection. These results suggest that NPY signaling from the PVH activates a GABAergic input to sympathetic premotor neurons in the rMR to inhibit BAT themogenesis, leading to reduced energy expenditure. No COI.

3O7J-6

Preoperative branched-chain amino acids administration improved prognoses after hepatectomy in the elderly rats with diabetes mellitus

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To study whether preoperative branched-chain amino acids (BCAA) administration improves prognoses after hepatectomy in the elderly rats with diabetes mellitus (DM), we did experiments in the elderly rats with DM in which BCAA was administered before hepatectomy. We used 22 elderly male Fischer 344 rats induced DM by streptozotocin. The rats were randomly divided into two groups; 11 rats with BCAA solution (B group) and 11 rats with water (C group) for 7 days before hepatectomy. Then, 70% partial hepatectomy was performed in all rats according to the method of Higgins and Anderson. Survival rate, rate of body weight reduction, blood sample data (blood sugar, AST, ALT, endotoxin, TNF-α and IL-6) and bromodeoxiuridine (BrdU) labeling index between two groups were evaluated on postoperative day 2. Survival rate in B group was significantly greater than that in C group (100 vs 55%, respectively, $p<0.05$). Rate of body weight reduction in B group was significantly smaller than that in C group (8.1±2.4 vs 12.1±1.2 %, respectively, $p<0.05$). There were no significant differences in blood sugar, AST, ALT, endotoxin, TNF-α and IL-6 between two groups. BrdU labeling index in B group was significantly higher than that in C group (14.5±1.7 vs 11.7±0.8%, restrictively, $p<0.05$). Thus, preoperative BCAA administration improved prognoses after hepatectomy in the elderly rats with DM. No COI.

3O7J-7

Severe hypoxia-induced ventilatory depression analyzed by spectrography of EEG and respiratory output

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Although mild hypoxia stimulates breathing, severe hypoxia depresses ventilation and this phenomenon may underlie the pathophysiology of respiratory arrest in patients with severe hypoxia. We hypothesized that severe hypoxia-induced ventilatory depression is accompanied by a decrease of central command from the higher brain to the lower brainstem. We analyzed the responses of higher brain activity and respiratory output to hypoxia in unanesthetized adult mice by EEG and whole body plethysmography, respectively. To test the hypoxic response, mice breathed room air, then hypoxic gas (mild 12% or severe 6% O_2 in N_2) for several minutes, and again room air. The EEG, respiratory flow, and inspired O_2 concentration were measured at 400 Hz sampling. The EEG and flow data were detrended and bandpass filtered with 0.1–100 Hz, and 0.1–40 Hz (6th order Butterworth), respectively. Digital spectrograms were generated by a fast Fourier transform from the filtered waveforms. Mild hypoxia persistently increased ventilation. Severe hypoxia transiently increased ventilation followed by ventilatory depression. Changes in the spectrum pattern of respiratory frequency were found in hypoxia. Severe hypoxia depressively influenced both EEG and the respiratory output pattern. We suggest that severe hypoxia affects the higher brain function and depresses ventilation. No COI.

3O7J-8

Endogenous ATP attenuates hypoxia-induced excitation of the RVLM neurons via P2 purinergic receptors in the *in situ* arterially-perfused preparation of rats.

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It has been known that neurons in the rostral ventrolateral medulla (RVLM neurons) generate the activity of the cardiovascular sympathetic nerve (SNA). Recently, we have reported that the RVLM neurons sense and are excited by central hypoxia. However, it is still unclear how the RVLM neurons sense hypoxia. In this study, we examined participation of endogenous ATP via P2 purinergic receptors in the hypoxia-sensing mechanism of the RVLM neurons in the *in situ* arterially perfused preparation of rats. We systemically applied a P2 purinergic receptor antagonist, PPADS (100 μM), and analyzed the effect on responses of SNA to the application of NaCN into the RVLM (5 mM, 30 nl) and the arterial chemoreceptors (0.03%, 0.1 ml). As a result, administration of PPADS did not change the basal SNA, but completely suppressed the excitation of SNA which was caused by NaCN-induced activation of the arterial chemoreceptors (control: 158.4 ± 35.6%, n = 5; PPADS: -3.1 ± 35.1%, n = 5; $p = 0.027$). Moreover, administration of PPADS significantly enhanced the excitation of SNA which was caused by injection of NaCN into the RVLM (control: 121.8 ± 26.2%, n = 5; PPADS: 161.7 ± 37.3%, n = 5; $p = 0.036$). These results may indicate that activation of P2 purinergic receptors by endogenous ATP is related with hypoxia-sensing mechanism of the arterial chemoreceptors, and also suppressively regulates the excitation of the RVLM neurons to hypoxia. No COI.