Poster Presentations

Day 1

(March 22, 13:00 - 14:00)

1P-001 – 1P-019	Ionic Channel, Receptor (1)
1P-020 – 1P-037	Heart, Circulation (1)
1P-038 – 1P-056	Neuron, Synapse (1)
1P-057 – 1P-073	Sensory Function (1)
1P-074 – 1P-081	Neurochemistry
1P-082 – 1P-100	Autonomic Nervous Systems
1P-101 – 1P-123	Muscle Physiology

1P-001

Analyses of structural rearrangements of P2X2 by voltage-clamp fluorometry using fluorescent unnatural amino acid

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P2X2 is a ligand-gated ion channel activated by extracellular ATP. This receptor has a complex gating depending on both [ATP] and voltage, in spite of the absence of canonical voltage sensor domain. It remains unknown how the structural rearrangements occur during voltage dependent gating. Besides, the detail of the structural rearrangements upon ATP binding in the pore region remains controversial. In the present study, we aim at analyzing the structural rearrangements upon (1) voltage and (2) ATP dependent gating, by voltage-clamp fluorometry (VCF) using fluorescent unnatural amino acid (fUAA) probe. This methodology made it possible to label any residue within the protein including intracellular region which is not accessible by conventional VCF fluorophores. The fUAA used here, named ANAP, is incorporated into protein by using *in vivo* nonsense suppression method where the tRNA ANAP-CUA and tRNA-synthetase pair is used to introduce ANAP in amber nonsense codon mutation. We successfully expressed functional P2X2 using this expression system in *Xenopus* oocytes and recorded simultaneously ATP- and voltage-evoked current as well as ANAP fluorescent signal. We also observed ANAP fluorescent signal change upon ATP binding at W46, a residue in transmembrane region, as well as at D209 and A283 in extracellular region, indicating that conformational changes occur around labeled residues. These results provide us a clue to approach how the structural rearrangements upon ATP binding occur in the pore region. (COI:No)

1P-002

Characterization of voltage-gated proton channel in zebrafish

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Voltage-gated proton channel (VSOP or Voltage-Sensor domain-Only Protein or Hv1) is the membrane protein that mediates the rapid movement of protons (H^+) across the cell membrane. The molecular architecture of VSOP consists of four transmembrane segments (S1 - S4) as found among voltage-gated ion channels, but lacks the pore domain. Thus far, the gene encoding VSOP was identified in several species including zebrafish; however, the functional role of this protein in zebrafish has remained elusive. Electrophysiological recording from the zebrafish VSOP (drVSOP) utilizing heterologous expression system in HEK293 cells showed voltage-dependent proton conductance. drVSOP exhibited similar activation kinetics as mouse VSOP (mVSOP); however, the current-voltage relationship indicated that drVSOP required lower membrane voltage to activate the channel. In addition, while mVSOP is highly sensitive to extracellular zinc ion (Zn^{2+}), drVSOP is resistant. We further studied the functional roles of drVSOP in this animal model beginning with defining the tissue expression pattern in the zebrafish. Also, we are in the process of generating drVSOP knockout zebrafish by means of CRISPR/Cas9 system. (COI:No)

1P-003

Extracellular Ca^{2+} is required in the heat-evoked activation of green anole TRPA1

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Ca^{2+} influx through thermosensitive TRP channels has a crucial role in cellular homeostasis and activity. Transient receptor potential ankyrin 1 (TRPA1) is a Ca^{2+}-permeable nonselective cation channel expressed in nociceptors and activated by irritant compounds such as allyl isothiocyanate (AITC) and temperature. TRPA1 was initially identified as a potential mediator of noxious cold stimuli in rodent nociceptive sensory neurons while TRPA1s from non-mammalian vertebrates (snakes, green anole lizards, frogs and chickens) were recently reported to be activated by heat, but not cold stimulus.A number of studies have shown that intracellular Ca^{2+} is a key regulator of many TRP channels, including TRPA1 In the previous study, we found that extracellular Ca^{2+}, but not intracellular Ca^{2+} plays an important role for heat-evoked activation of green anole TRPA1 (gaTRPA1). In this study, we focus on extracellular Ca^{2+}-dependent heat sensitivity of gaTRPA1 by comparing gaTRPA1 with other heat-activated TRPA1s from rat snake and chicken. It was found that, rat snake and chicken TRPA1s are activated by heat with small inward currents even in the absence of extracellular Ca^{2+}. We found several negatively charged amino acids near the outer pore region, which could be involved in heat-evoked activation of TRPA1 channel. These results suggest that gaTRPA1 channel, but not TRPA1s of rat snake and chicken needs extracellular Ca^{2+} for heat-evoked activation. (COI:No)

1P-004

Role of NHERF1 scaffold protein in expression of exogenously transfected ROMK1 K+ channels to the apical membrane of cultured M-1 cells

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ROMK1 K+ channels are known to be localized in the apical membrane of principal cells in renal collecting duct, and are responsible for urinary K+ excretion to maintain body fluid electrolytes homeostasis. To date, several factors have been reported to play important roles in the channel expression to the cell membrane. PDZ-domain at C-terminus was considered to be one of the important factors for the channel expression. However, our previous studies with the patch-clamp technique applied to the polarized apical membrane of the cultured mouse renal collecting duct (M-1) cells showed that exogenously transfected ROMK1 K+ channels were frequently observed even though PDZ-domain of ROMK1 was deleted. In this study, we further examined the involvement of another factor, NHERF1 which is known as a scaffold protein for the channel, in the apical expression of ROMK1 K+ channel. Confocal microscopy analysis revealed that NHERF1 was mainly localized at the apical membrane of polarized M-1 cells. Moreover, the patch-clamp technique showed that the observed apical ROMK1 K+ channels were significantly decreased in the NHERF1 knockdown cells. These results strongly suggest that NHERF1 is a major candidate for the apical expression of ROMK1 K+ channels in polarized M-1 cells. (COI:No)

1P-005

Analyses of the role of Asp727 in C-linker domain for the modulation of the slow deactivation of hERG channel

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hERG channel which belongs to the voltage-gated K^+ channel family is well known for its very slow kinetics of deactivation. It has been shown that this slow deactivation is modulated by its intracellular domains. Among the C-terminal intracellular domains, there are cyclic-nucleotide binding homology domain (CNBHD) and C-linker domain (CLD) which links S6 segment with CNBHD. Recent studies have shown that CNBHD and CLD contribute to the slow deactivation, but the mechanisms of slow deactivation are not fully understood. In this study, we hypothesized that the interactions between these domains in C-terminus modulate the slow deactivation, and examined this hypothesis by analyzing various mutants under two electrode voltage clamp in *Xenopus* oocytes. When aspartic acid (D) 727 in CLD and D767 in CNBHD were substituted respectively with arginine (R), these mutations (D727R, D767R) accelerated the deactivation speed. In the double mutant (D727R&D767R), the deactivation speed was much faster, and the slope of G-V curve was less steep. However, when R696D mutation in CLD, which did not change the phenotype of WT, was added onto the double mutant, the triple mutant (R696D&D727R&D767R) showed partially rescued phenotype, suggesting electrostatic interaction between these charged amino acid residues. Furthermore, we also obtained data showing the existence of other interacting partners for D727. These results suggest that the interactions between C-terminal domains are important for the modulation of the slow deactivation of hERG channel. (COI:No)

1P-006

Thermosensitive TRPV1 channel activation in single neuronal cells by using surface-engineered gold nanoparticles

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Controlling cell functions using external photo-responsive nanomaterials has enormous potential for the development of cell engineering technologies and therapies for intractable diseases, but currently requires genetic modification of target cells. Here, we present a method using plasma membrane (PM)-targeted gold nanorods (pm-AuNRs), prepared with a genetically cationized form of high-density lipoprotein (HDL), to activate a thermosensitive cation channel, TRPV1. The results indicated; firstly, pm-AnNRs were localized at the PM without cytotoxicity. Secondly, the localized photothermal heating on PM was confirmed by using a fluorescent thermometer. Lastly, highly localized photothermal heat generation mediated by pm-AuNRs induced Ca^{2+} responses in TRPV1 expressing HEK293T cells. The feasibility of this new method for TRPV1 photoactivation was further assessed in primary cultured DRG neurons from mice. Upon illumination after pm-AuNR treatment, DRG neurons from wild type mice exhibited Ca^{2+} responses, whereas DRGs from TRPV1 knock-out mice did not. This clearly demonstrates that pm-AuNRs can exclusively activate endogenous TRPV1 channels under quasi-physiological conditions. The present method provides an unprecedentedly unique optogenetic platform without genetic engineering of target cells and may be useful for future TRPV1-targeted phototherapies. (COI:No)

1P-007

Pharmacological distinction between acid-sensitive outwardly rectifying anion channel (ASOR) and volume-sensitive outwardly rectifying anion channel (VSOR)

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The acid-sensitive outwardly rectifying anion channel (ASOR), which is expressed in a variety of cell types, is known to be activated by extracellular acidification and involved in acidotoxic necrotic cell death. On the other hand, the volume-sensitive outwardly rectifying anion channel (VSOR), which is also ubiquitously expressed in mammalian cells, is activated by osmotic cell swelling or ROS and involved in cell volume regulation and apoptotic cell death. The roles of these anion channels have been studied mainly by pharmacological approaches, because the molecular identities of their pore-forming components have not yet been firmly determined. DIDS and phloretin are known as the blockers for both channels. However, there is no selective inhibitor that can distinguish ASOR from VSOR. In this study, we explored the effects of 12 putative VSOR inhibitors on ASOR-mediated currents in HeLa cells. Among them, eight VSOR blockers including DCPIB and NPPB were found to be totally ineffective to block the ASOR channel activity. Furthermore, the remaining four VSOR inhibitors, suramin, DIOA, arachidonic acid and niflumic acid, were found to inhibit the ASOR currents at one to two orders of magnitude lower concentrations. (COI:No)

1P-008

Bridging ion permeation and ion selectivity through K^+ channel

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Ion channels are membrane proteins selectively transporting ions. The electrophysiological measurement of single channel recording gives the currents through channels, that is, the rates of ion permeation. Meanwhile, the selectivity of channels has been studied by measuring the reversal potential, which provides the ratio of the permeability. In potassium channels, shifts of the reversal potential upon replacement of ion species are undetectable because of their strict selectivity to K^+ over other monovalent cations. Thus, an alternative method to estimate the selectivity named the punch-through experiments has been applied for the KcsA potassium channel, in which single channel currents under K^+ solution with Na^+ were evaluated in a wide voltage range. The result showed unusual S-shape current-voltage curves, however, there is no theoretical or kinetic model that can fit the S-shape curves. Here we developed a kinetic model, which successfully fit the punch-through currents. The model predicts the single-channel current amplitudes when the ratio of intracellular Na^+ increases. Moreover, this model also expects the ion permeation rate and the reversal potential. Thus, it bridges the ion permeation and the ion selectivity. (COI:No)

1P-009

Regulation of KCNK13 channel by Gi/o or Gq coupled receptors

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The KCNK13 channel, a member of the two-pore-domain potassium channel family, is known to be activated by arachidonic acid. In addition, we found that activation of either Gi/o or Gq coupled receptor increases amplitudes of the KCNK13 channel currents when expressed in human embryonic kidney 293T cells. In this study, we have investigated which signaling molecules play roles as regulators of KCNK13 channel in Gi/o and Gq pathway. First, we examined the effect of free $G_{\beta\gamma}$ subunits, the signaling molecules of Gi/o pathway, on the channel. Co-expression of $G_{\beta\gamma}$ significantly increased the current density of wild type KCNK13 channel but not of the S365A/E366A/M367A mutant channel. As the amplitude of the triple mutant channel current was not increased by the activation of the Gi/o coupled receptors, $G_{\beta\gamma}$ was thought to be a regulator of the KCNK13 channel activity. Next, the effect of a signaling molecule in the Gq pathway, diacylglycerol (DAG), was examined. Under the inside-out patch clamp configuration, application of 1-stearoyl-2-arachidonoyl-sn-glycerol, a DAG analogue, increased the amplitude of the KCNK13 channel current in a concentration dependent manner, indicating that DAG is a key molecule to regulate the activity of KCNK13. Taken together, these results suggested that free $G_{\beta\gamma}$ subunits and DAG serve as the positive regulators of the KCNK13 channel upon activation of Gi/o and Gq coupled receptor, respectively. (COI:No)

1P-010

Electrophysiological properties of neurons derived from human iPS cells.

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The phenotypic diversity of neurons derived from induced pluripotent stem (iPS) cells is influenced by culture conditions, iPS cell clones, and so forth. To evaluate the electrophysiological diversity of these cells, human iPS cell-derived neurons from ReproCELL Inc. were cultured according to the manufacturer's protocol and examined by whole-cell patch clamping. All cells examined (n = 66) expressed voltage-gated outwardly rectifying currents (probably K^+ currents), with a mean ± SD current density of 55.4 ± 30.4 pA/pF. Forty-eight cells (72.7%) also expressed transient inward currents (probably Na^+ currents), with a mean ± SD current density of −58.4 ± 81.5 pA/pF. There were no significant differences in current densities among 3−7-, 12−19-, and 21−28-day-old cultures. The correlation between the transient inward and outwardly rectifying current densities revealed that these human iPS cell-derived neurons consisted of three distinct subpopulations: cells with a high density of both current types able to fire action potentials on depolarization (three cells, 4.6%), cells with a low density of both currents (48 cells, 72.7%), and cells with no detectable transient inward current (15 cells, 22.7%). These results suggest that human iPS cell-derived neurons cultured under the same conditions are heterogeneous with respect to membrane excitability. It is thus possible that these neurons are also heterogeneous in other respects such as transmitter phenotype and receptor expression pattern. (COI:No)

1P-012

Identification and functional characterization of a novel inhibitor of transient receptor potential vanilloid 4

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Transient receptor potential vanilloid 4 (TRPV4) is a non-selective calcium-permeable cation channel that shows highly broad expression such as in kidney, skin and vascular endothelium. Here we found that a low molecular weight compound having inhibitory effects on TRPV4 channels heterologously expressed in human embryonic kidney 293T cells. The compound is contained in commercially available creams for topical application on the itchy skin and its molecular target has not been known. The compound inhibited GSK1016790A-activated TRPV4 currents in a dose-dependent manner. In order to examine the inhibition mechanism of the compound more in detail, we examined whether the inhibitory effect is also observed in TRPV4 activated by arachidonate derivatives, warm temperature or low osmolality. Calcium-imaging experiments were conducted as well to confirm the TRPV4 inhibition property by the compound. The inhibition mechanism could lead to the clarification of TRPV4 involvement in itch sensation, and contribute to weakening itch sensation in the skin. (COI:No)

1P-013

The role of voltage-gated proton channel Hv1 in neutrophil chemotaxis

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Neutrophil's chemotactic movement is necessary for inflammatory responses and pathogen elimination. The motility is highly regulated for these events. Chemoattractants such as IL-8, leukotriene B4 and N-formyl peptide fMLF guide neutrophils to eliminate invading pathogens. Voltage-gated proton channel Hv1 discovered in our laboratory regulates intracellular pH and membrane potential. Using Hv1-deficient neutrophils, we have revealed that disruption of its channel function causes reduced ROS production and enhanced degranulation of azurophilic granules. We also found that Hv1 is involved in neutrophil chemotaxis. Using transwell chamber, bone marrow derived neutrophils were stimulated with fMLF. Hv1-deficient neutrophils showed normal response to 10uM fMLF. In the 1uM fMLF, wild-type neutrophils showed only weak response to it, but Hv1-deficient neutrophils exhibited strong response to it. The enhanced response to 1uM fMLF in Hv1-deficient neutrophils was abrogated when NADPH oxidase activity was inhibited by DPI. These results indicate that Hv1 is necessary for proper chemotaxis. We are trying to identify cascades leading to enhanced chemotaxis in Hv1-deficient neutrophils. (COI:No)

1P-014

Contribution of TRPM7 and FYN to Endothelial-Mesenchymal Transition (EndoMT) in human umbilical vein endothelial cell (HUVEC)

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Background and purpose: Endothelial-Mesenchymal Transition (EndoMT) is an important process underlying pathophysiological vascular remodeling. During this process, a number of Ca^{2+}-dependent signaling pathways and molecules are activated, which are often causally associated with inflammatory vascular diseases. We focused on a stretch- and swelling-activated cation channel TRPM7 (transient receptor potential melastatin subfamily member 7) and a non-receptor type tyrosine kinase FYN which are important regulator for vascular remodeling. In this study, we investigated whether TRPM7 and FYN affect EndoMT in HUVEC.
Method: We evaluate the effects of a TRPM7 antagonist FTY720, TRPM7-siRNA, FYN-siRNA on TGF-β2 induced EndoMT in HUVEC by immunocytochemical, real-time RT-PCR and western blot analyses.
Results: We performed immunoblot analysis of HUVECs for mesenchymal markers and endothelial markers. The results suggested that FTY720 and TRPM7-siRNA significantly suppressed TGF-β2 induced EndoMT. Immunocytochemical analysis indicated that FYN-siRNA and a dominant negative form of FYN prevented TGF-β2-induced stress fiber formation. Finally, the phosphorylation of an EndoMT-associated transcription factor STAT3 (Signal Transducer and Activator of Transcription 3) was significantly suppressed by TRPM7 or FYN knock-down.
Summary: The above results suggest that TRPM7 and FYN may contribute to the EndoMT process of vascular endothelial remodeling, and could thus provide novel targets of anti-fibrotic therapy for some inflammatory vascular diseases. (COI:No)

1P-015

Activation mechanisms of an orphan metabotropic receptor Prrt3

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Proline rich transmembrane protein 3 (Prrt3) is an orphan G-protein-coupled receptor which contains a large N-terminal extracellular domain and seven transmembrane domains. There is no publication concerning Prrt3 and its physiological roles and functioning mechanisms are totally unknown. We previously observed: (1) Prrt3 is highly expressed in mouse brain; (2) it is involved in the retention of spatial memory and fear conditioning memory; (3) Prrt3 extracellular domain is partly cleaved from its transmembrane domain in mouse brain. In the present study, we aimed at identification of ligands and examined the effect of various neurotransmitters and their related chemicals. We found that a muscarinic agonist, oxotremorine-M (Oxo-M), but not ACh, slightly activate the $G_{i/o}$ protein-gated inwardly rectifying K^+ (GIRK) current in Prrt3-expressing oocytes. Oxo-M fail to induce G_q-mediated Ca^+-activated Cl^- current in Prrt3-expressing oocytes, suggesting Prrt3 couples with $G_{i/o}$. We also observed that coexpression of Prrt3 with a proprotein convertase furin partly generates the cleaved form of Prrt3 in HEK293 cells and oocytes by immunohistochemical staining. Coexpression of Prrt3 with furin or truncation of the extracellular domain directly by mutagenesis in oocytes showed no significant difference in Oxo-M-induced GIRK current from that of wild-type Prrt3 alone, suggesting Oxo-M binds to the transmembrane domain of Prrt3. Taken together, present data showed Prrt3 couples with $G_{i/o}$ and provided us with a clue toward the identification of the physiological ligand. (COI:No)

1P-016

Reciprocal effects of capsaicin and menthol on thermo-sensation through regulated activities of TRPV1 and TRPM8

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Transient Receptor Potential vanilloid 1 (TRPV1) is activated by elevated temperature (> 42°C) whereas cold temperature reportedly decreases capsaicin-induced TRPV1 activity. On the other hand, TRPM8 is activated by low temperatures and menthol, whereas heat stimulation suppresses menthol-evoked TRPM8 currents. These findings suggest that the effects of specific agents on TRPV1 and TRPM8 channels are intricately related to each other channel. We examined the effects of menthol on human TRPV1 (hTRPV1) and the effects of capsaicin on hTRPM8 using Ca^{2+}-imaging and patch-clamp methods. The hTRPV1-mediated currents induced by capsaicin were inhibited by menthol in a dose-dependent manner. On the other hand, the hTRPM8-mediated currents induced by menthol were inhibited by capsaicin in a dose-dependent manner. Moreover, heat-activated hTRPV1 responses and cold-activated hTRPM8 responses were inhibited by menthol and capsaicin, respectively. An in vivo sensory irritation test showed that menthol conferred an analgesic effect on the sensory irritation produced by vanillyl butyl ether (VBE), a TRPV1 agonist. Our findings suggest that the agonists of TRPV1 and TRPM8 interacted with both of these channels and that the anti-nociceptive effects of menthol could be partially explained by this phenomenon. (COI:No)

1P-017

Modulation of single sodium channel currents by nitric oxide signaling pathway in insect memory neurons isolated from the mushroom body of the cricket brain.

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To understand the role of NO in insect olfactory learning, first, we characterized the electrophysiological properties of single Na^+ channels as a possible target molecule for NO and then examined their modulation by NO signaling pathway. Single Na^+ channel activity was recorded in cell-attached patches of the freshly isolated Kenyon cells from the mushroom bodies of the cricket brain. Upon step depolarization, many channels rapidly open near the beginning of the voltage pulses and appear to close and rapidly re-open with repeated brief opening of channels during the period of 150 ms step depolarizations. Extracellular application of Na^+ channel blocker TTX by the backfill procedure reduced the activity of both rapid and persistent channel currents, confirming that they are passing through TTX-sensitive Na^+ channels. Application of NO donor, GSNO increases the open probability (Po) of both rapid and persistent Na^+ channels. As a result, the amplitude of ensemble current reconstructed by averaging individual current responses increased. The NO-induced increase in the Po of Na^+ channel was diminished by co-application of guanylate cyclase inhibitor ODQ and protein kinase G (PKG) blocker KT5823, respectively. These results indicate that NO increases the activities of both rapid and persistent Na^+ channels via cyclic GMP/PKG signaling pathway. Functional significance of the NO-induced increase in Po of Na^+ channels was discussed in relation to the formation of long term memory in olfactory learning in insect. (COI:No)

1P-018

Purinergic modulation of neuronal activity in the prepositus hypogrossi nucleus

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Adenosine trisphosphate (ATP) acts as excitatory and inhibitory neurotransmitters via ionotropic P2X receptors and metabotropic P2Y receptors even in the central nervous system. It has been suggested that the ATP receptors are expressed in the prepositus hypoglossi nucleus (PHN), which is a brainstem structure involved in controlling horizontal gaze. However, it has not been clarified whether ATP indeed affects the activity of PHN neurons. In the present study, we investigated the effect of ATP on neuronal activities in the PHN using the whole-cell recordings in rat brainstem slice preparations. Bath application of ATP (100-500 μM) increased the spontaneous firing rates in most neuronal types that were classified on the basis of distinct firing patterns in response to depolarizing current pulses. Notably, ATP completely blocked spontaneous firings of neurons that exhibited a low firing rate pattern (LFR neurons). These results indicate that ATP modulates neuronal activity in the PHN. To explore the mechanisms of the blockade of spontaneous firings (SF blockade) by ATP, we investigated current responses to puff application of ATP (1 mM) to the LFR neurons. The application of ATP induced the transient (less than 1 s) inward current followed by long-lasting (more than 5 s) outward current at the holding potential of -70 mV. These results suggest that the long-lasting outward currents induced by ATP participate in the SF blockade in the LFR neurons. Furthermore, the observation of the inward and outward currents suggests that different purinoceptors are expressed in the LFR neurons. (COI:No)

1P-019

IL-1β suppressed activity of exogenously transfected ROMK1 K^+ channel via Ca^{2+}/PKC pathway in cultured M-1 mouse CCD cells

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We previously reported that IL-1β decreased activity of an inwardly rectifying K^+channels in renal proximal tubule cells. However, acute effect of cytokines on ROMK1 K^+ channel in CCD cells is not well known. In this study, we investigated the effect of IL-1β on the exogenously transfected ROMK1 K^+ channel in cultured mouse M-1 cells using the patch-clamp technique and Fura-2 imaging. In cell-attached patches, IL-1β acutely suppressed ROMK1 K^+ channel activity, which was blocked by IL-Ra.Then we investigated the cellular mechanisms for IL-1β to ROMK1 K^+ channel. Since PKC is known to one of major inhibitory factors for ROMK1 K^+ channel, we first examined the effect of PKC inhibitor on channel activity. A PKC specific inhibitor, GF103209X, blocked the suppressive effect of IL-1β to ROMK1 K^+ channel, suggesting that the inhibitory effect of IL-1β was mediated by PKC. In addition, a PLC inhibitor, neomycin, also prevented the effect of IL-1β. In the next, we examined changes in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) using Fura-2AM Ca^{2+} imaging. The results shows that IL-1β increased $[Ca^{2+}]_i$ within a few minutes regardless of the presence or the absence of Ca^{2+} in bath solution. Moreover, IL-1β-induced rise in $[Ca^{2+}]_i$ was inhibited by pretreatment with IL-Ra or neomycin. These findings strongly suggest that IL-1β increases $[Ca^{2+}]_i$, which is derived from intracellular Ca^{2+}-stores, and activates Ca^{2+}-dependent PKC, resulting in inhibition of the transfected ROMK1 K^+ channel activity in M-1 cells. (COI:No)

1P-020

A Novel Nicotinamide Adenine Dinucleotide Correction Method for Mitochondrial Ca2+ Measurement with FURA-2-FF in Single Permeabilized Ventricular Myocytes of Rat

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Fura-2 analogs are ratiometric dyes that are widely used for the quantitative measurement of [Ca^{2+}]. However, the dye usage is intrinsically limited, as the dyes require UV excitation, which can also generate great interference, mainly from NADH autofluorescence. Specifically, this limitation causes serious problems for the quantitative measurement of mitochondrial [Ca^{2+}], as no available ratiometric dyes are excited in the visible range. Thus, NADH interference cannot be avoided during quantitative measurement of [Ca^{2+}] because the majority of NADH is located in the mitochondria. The emission intensity ratio of two different excitation wavelengths must be constant when the fluorescent dye concentration is the same. In accordance with this principle, we developed a novel online method that corrected NADH and Fura-2-FF interference. We simultaneously measured multiple parameters, including NADH, [Ca^{2+}], and pH/ψm; Fura-2-FF for mitochondrial [Ca^{2+}] and TMRE for ψm or carboxy-SNARF-1 for pH were used. With this novel method, we found that the resting mitochondrial [Ca^{2+}] concentration was 1.03 μM. This 1 μM cytosolic Ca^{2+} could theoretically increase to more than 100 mM in mitochondria. However, the mitochondrial [Ca^{2+}] increase was limited to 30 μM in the presence of 1 μM [Ca^{2+}]c. Our method solved the problem of NADH signal contamination during the use of Fura-2 analogs, and therefore the method may be useful when NADH interference is expected. Support:NRF2014M3A9D7034366 (COI:No)

1P-021

A role of miRNA204 in the regulation of T-type calcium channel's expression by aldosterone in cardiomyocytes

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Background: Aldosterone is considered as a cardiovascular risk factor. In the heart, aldosterone alters the expression of ion channels, which might be a cause of arrhythmia. We have shown that, through the activation of mineralocorticoid receptor (MR), aldos-terone stimulates an increase expression and activity of T-type voltage-gated calcium channels (T-channels) resulting in an increase beating frequency of cardiomyocytes. We investigated here a possible role of miRNA in the aldosterone-stimulated expression and activity of T-channels in ventricular cardiomyocytes. Results: A microarray screening identified miRNA204 as the major miRNA ex-pressed after aldosterone stimulation in cardiomyocytes. MiR-204 overexpression in neaonatal rat ventricular myocytes increased T-type calcium currents and both T-channels pore subunits, CaV3.1 and CaV3.2, protein and mRNA. On the contrary, miRNA204 inhibition prevents aldosterone effects on T-channels expression and T-type currents. Discussion: These results suggest that miR-204 regulates the expression of T-channels downstream MR activation by aldosterone. We establish a working hypothetical model where miR-204 suppresses a yet unidentified regulatory protein of T-channel's expres-sion. (COI:No)

1P-022

The optical kymographion: a novel optical device for non-contact recording of muscle contraction

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Measurement of muscle contraction is one of the fundamental techniques in physiological studies. This has long been done with a mechanical device, such as a kymographion for measuring the muscle length, and a strain-gauge for measuring the developed force, using isolated muscle preparations tied to a thread. Here, I present a novel device "optical kymographion" for recording the muscle contraction without a need for isolated preparations or a thread. This device has an infrared LED to illuminate the muscle surface, and a phototransistor to collect and convert the reflected light into the output voltage. This arrangement picks up the displacement of muscle surface that occurs during the muscle contraction. The displacement of muscle surface alters the total length of the light path, thereby changes the light intensity collected by the phototransistor. Accordingly, the output voltage accurately follows the waveform of the muscle contraction. To demonstrate the operation of "optical kymographion", spontaneous contractions were recorded from euthanized bullfrog hearts. This device required no thread tied to an isolated muscle, thus it allowed simultaneous recording of the action potential and the contraction from the heart *in situ*. Also, this device was able to pick up local contractions, enabling concurrent recording of atrial and ventricular contractions from a single heart. In conclusion, "optical kymographion" provides a simple and useful technique for the measurement of muscle contraction, and potentially of general biological motility. (COI:No)

1P-023

Molecular mechanism of diastolic dysfunction in hypertrophic cardiomyopathy caused by troponin T mutation

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We created knock-in mice with a missense mutation S179F in cardiac troponin T (cTnT), which had been found to be associated with human hypertrophic cardiomyopathy (HCM). The knock-in mice suffered from sudden death frequently, with significant displacement fibrosis, hypertrophy, and myocyte disarray in myocardium. Echocardiography showed that left ventricular (LV) end-diastolic dimension was decreased and interventricular septum thickness was increased, with no significant changes in LV ejection fraction. Echocardiography also showed a decrease in E/A ratio, and in vivo cardiac catheter measurements showed an increase in $LVdP/dt_{min}$ with no significant changes in $LVdP/dt_{max}$. Further, we found that cardiac myofilament Ca^{2+} sensitivity was increased, while the expression of sarcoplasmic reticulum Ca^{2+} pump (SERCA2a) and the phosphorylation of phospholamban were decreased. These results suggest that the knock-in mouse model of HCM with S179F mutation in cTnT has severe LV diastolic dysfunction probably due to a heightened myofilament Ca^{2+} sensitivity, which is partially compensated by a diminution of Ca^{2+} transient through SERCA2a downregulation. (COI:No)

1P-024

Role of respiratory chain complexes in myocardial stretch-induced increase in reactive oxygen species

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We have previously reported that myocardial diastolic stretch hyperpolarizes mitochondrial membrane potential and increases NADPH oxidase (NOX)-derived reactive oxygen species (ROS) production. Although mitochondrial hyperpolarization is likely to increase mitochondrial ROS production, relation between mitochondrial hyperpolarization and NOX derived ROS production is not clear. In the present study, we investigated contribution of mitochondrial ROS production in stretch-induced net ROS production. Isolated mouse ventricular myocytes were exposed to 10% axial stretch using computer-controlled piezo-manipulated carbon fibers, attached to both cell ends. Net ROS production, mitochondrial ROS production and mitochondrial membrane potential were studied using DCF, MitoSox and TMRE-loaded cells, respectively. Applying 2.4 μM Rotenone (inhibitor of complex I in electron transport system) or 5 μM Antimycin A (inhibitor of complex III) suppressed the stretch-induced hyperpolarization of mitochondrial membrane potential. Despite the suppressive effects on the response of mitochondrial membrane potential, rotenone did not significantly affect stretch-induced increase in ROS production, while Antimycin A suppressed the response. Despite the stretch-induced hyperpolarization of mitochondrial membrane potential, we could not detect change in MitoSox signal during stretch. These results suggest that mitochondrial complex III contribute to stretch-induced increase in ROS production not by producing ROS, but by stimulating NOX. (COI:No)

1P-026

Periodic hydrodynamic pressurization promotes layered structure of human arterial smooth cells

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Background: Small diameter biological artificial vessels have been desired for coronary artery bypass. In the previous study, we demonstrated that periodic hydrodynamic pressurization promoted assembly of human umbilical artery smooth muscle cells (hUASMCs). Therefore, we aimed to fabricate organized layered structure of hUASMCs using the periodic hydrodynamic pressurization. Methods and Results: hUASMCs were plated on an 8-well chamber slide (2.0×10^5 cells per each well) and cultured with 10% fetal bovine serum/DMEM for 24 h in normal atmospheric pressure. We then cultured the hUASMCs for 24 h under periodic hydrodynamic pressure (110kPa-180kPa 0.002Hz). Next, a second layer of hUASMCs were seeded on the first layer. The cycles of culture in atmospheric pressure and subsequent pressurization were repeated four times. HE stain of paraffin sections of the hUASMCs revealed organized four layers of smooth muscle cells. In contrast, control hUASMC sheets constructed under atmospheric pressure exhibited less than three layers. The hUASMC sheets fabricated by periodic hydrodynamic pressurization were significantly thicker than the control hUASMC sheets (26.5±2.2 μm vs. 12.5±1.2 μm, $p<0.05$, n=3-4). Conclusions: Three-dimensional layered structure of human arterial smooth muscle cells was fabricated when cells were alternately exposed to periodic hydrodynamic and atmospheric pressure. (COI:No)

1P-027

Fabrication of layered elastic fibers in rat arterial constructs by periodic hydrodynamic pressurization

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Background: Elastic fibers provide arterial integrity. However, it has been difficult to synthesize elastic fibers in vitro. Our previous study showed that periodic hydrodynamic pressurization increased mRNA expression of elastic fiber-related genes in vitro. Therefore, we aimed to fabricate organized elastic fibers in arterial constructs composed of rat arterial smooth muscle cells (RASMCs).Methods and Results: RASMCs were plated on a 35mm dish at the density 1×10^6 cells/2mL and cultured with 10% fetal bovine serum/DMEM for 24 h in normal atmospheric pressure. The first layer of RASMCs was then cultured for 24 h under periodic hydrodynamic pressure (110 kPa-180 kPa, 0.002 Hz). Next, a second layer of RASMCs were seeded on the first RASMC layer. The cycles of culture in atmospheric pressure for 24 h and subsequent 24 h pressurization were repeated five times. As a result, we obtained RASMCs exhibited sheet-like structure. Elastica van Gieson stain revealed that the RASMC sheets contained five layers of elastic fibers. The pressurized RASMC sheets were 1.3±0.1-times thicker than the RASMC sheets constructed under atmospheric pressure (n=3). Furthermore, the pressurized RASMC sheets were stretched by 140±10% compared to atmospheric control RASMC sheets (n=3). Conclusions: Periodic hydrodynamic pressurization provided functional layered elastic fibers in RASMC sheets. (COI:No)

1P-028

Presence of prion protein-expressing cardiac progenitor cells in human cardiac ventricles with myocardial infarction

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Functional heart comprises heterogeneous cell lineages including cardiac stem or progenitor cells. We recently discovered a novel subpopulation of adult mouse heart cells that spontaneously develop into beating cardiomyocytes, defined as atypically-shaped cardiomyocytes (ACMs). ACMs are likely to be cardiac progenitor cells that possess more resistance to severe ischemic conditions compared to the ventricular myocytes and survive the long-term post-natal development while preserving the expression of fetal cardiac gene products. In combination with cardiac-specific contractile protein cardiac troponin T (cTnT), cellular prion protein (PrP) was demonstrated to specifically identify native ACMs in the adult mouse heart. According to the data obtained in the mouse heart, we examined the localization of PrP/cTnT-expressing ACMs-like cells in the human heart. A small number of PrP/cTnT-positive cells were observed in the interstitial space among ventricular myocytes from the endo- to the epicardium of the normal heart tissues, indicating that these cells remain in the normal human heart throughout the lifetime. We also found that the PrP/cTnT-positive cells existed in the border zone of acute myocardial infarction, ~7 days after infarction, in which adjacent ventricular myocytes died. These findings suggest the possibility that the PrP/cTnT-positive cardiac progenitor cells can survive under pathophysiological conditions. (COI:No)

1P-029

Effects on hemodynamics in chronic phase in protein direct introduction into the rat nucleus tractus solitarii.

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Efficient delivery of proteins into the cells in vivo could be achieved only when the molecules are very small. Recently, it has been shown that intraperitoneal injection of the β-galactosidase protein, fused to the protein transduction domain from the human immunodeficiency virus TAT protein, results in delivery of the biologically active fusion protein to all tissues in mice, including the brain (Science). There are no reports that protein transduction into the specific restricted brain area have succeeded.We investigated whether in vivo protein direct transduction into the nucleus tractus solitarii (NTS) is possible using microinjection technique and hemagglutinating virus of Japan envelope vector in anesthetized rats. Male Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were used. The arterial cannula was connected to a radio telemetry transmitters and a polygraph system was used for continuous recording of pulsatile ABP (arterial blood pressure), MABP (mean ABP), and HR (heart rate). All rats were given 7 days to recover after surgical procedures. In this study, we examined the effects of nNOS (neuronal nitric oxide synthase) and eNOS (endothelial nitric oxide synthase) on cardiovascular functions at the level of the NTS. After microinjection, we have continued to take a record 7 days. We will report on the results. (COI:No)

1P-030

Effect of aging on the CO_2 reactivity in cerebral and ocular vessels

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Cerebral and ocular vessels are sensitive to a change in partial pressure of arterial CO_2 ($PaCO_2$). It is well known that the resting blood flow in these vessels decreases with age. However, an existence in an age-related change in the cerebral CO_2 reactivity, i.e., the relative response in blood flow to a given increase in $PaCO_2$, is controversial. No studies investigated the age-related change in the ocular CO_2 reactivity. We compared the CO_2 reactivity responses in middle cerebral artery (MCA) and optic nerve head (ONH) to a 3-min inhalation of CO_2-rich air (5%) between 14 young (23 ± 0 yr) and 11 middle-aged healthy subjects (48 ± 2 yr). There were no differences in the CO_2 reactivity in MCA between the young and middle-aged groups (2.2 ± 0.2 %/mmHg and 2.7 ± 0.4 %/mmHg, respectively). In turn, the CO_2 reactivity in ONH was significantly greater in the young group (1.0 ± 0.1 %/mmHg) than the middle-aged group (0.6 ± 0.1 %/mmHg) and was significantly correlated with age ($r=-0.45$, $P<0.05$). These results suggest that the CO_2 reactivity in cerebral vessels is unaffected by aging, while that in ocular circulation decreases with age, implying that there are regional differences in the effect of aging on the CO_2 reactivity. (COI:No)

1P-031

Chronic intermittent hypoxia accelerates coronary microcirculatory dysfunction in young insulin resistant Goto-Kakizaki rats

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Objective: Sleep apnea and intermittent hypoxia (IH) have been shown to induce oxidative stress and inflammation, which impair endothelial function. Long-term insulin resistance also leads to endothelial dysfunction. We hypothesised that the onset of coronary dysfunction in young insulin resistant rats (Goto-Kakizaki, GK) is accelerated by chronic IH. **Method:** The effects of IH (21-4% O_2, normocapnia, 40 cycles/h, 8 hr/d) for 4 weeks was examined in 16 week old male GK rats and Wistar control rats. In vivo coronary endothelial responses were assessed using synchrotron microangiography before and after blockade of NO and prostacylin. **Result:** We found that dilation to acetylcholine after blockade was reduced in the microvessels of Wistar rats and changed to constriction insulin resistant rats after chronic IH. Further, IH treatment increased remodelling of resistance microvessels in GK rats.**Conclusion:** Medium term chronic IH reduced NO-independent dilation in control rats and evoked constriction in insulin resistant rats. Larger resistance arteries and arterioles had the most impaired endothelial function and showed remodelling. (COI:No)

1P-032

Neuronal Ca^{2+} sensor-1 contributes to stress tolerance in cardiomyocytes by activating the mitochondrial detoxification pathway

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Intracellular Ca^{2+} regulates various cellular processes including mitochondrial function and cell death/survival. We previously reported that an EF-hand Ca^{2+}-binding protein neuronal Ca^{2+}-sensor-1 (NCS-1) is a positive regulator of Ca^{2+} handling in cardiomyocytes. In the present study, we aimed to study whether NCS-1 plays beneficial roles in cardiac survival during stress using NCS-1-deficient (KO) mice. KO cardiomyocytes were more susceptible to oxidative (H_2O_2) and metabolic stress, and ischemia-reperfusion injury, compared to wild-type cardiomyocytes. In addition, they had reduced cellular ATP levels and mitochondrial respiration, especially with oxidative stress. Mitochondrial "proton leak" is known to be increased by oxidative stress and inhibit production of reactive oxygen species, thereby plays a protective role. However, this response was considerably diminished in KO cardiomyocytes. Consistently, H_2O_2-induced loss of mitochondrial membrane potential, a critical early event in cell death, was accelerated in KO cardiomyocytes. Furthermore, Ca^{2+}-dependent stress-induced survival pathways including PI3K/Akt and PGC1 pathways, the latter of which promotes mitochondrial biosynthesis, were less activated in KO cardiomyocytes. Taken together, these results suggest that NCS-1 contributes to stress tolerance in cardiomyocytes via activation of Ca^{2+}-dependent mitochondrial detoxification and survival pathway. (COI:No)

1P-033

Prostaglandin E_2 is Involved in Avian Ductus Arteriosus Closure

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Background: The ductus arteriosus (DA) is an essential artery that connects the main pulmonary artery and the descending aorta during a fetal period. Prostaglandin E_2 (PGE_2) has a potent vasodilatory effect, and the decline is a cause of DA closure after birth in mammals. Avian DA also closes after birth although avian species have no placenta that is a main source of circulating PGE_2 in rodents and mammals. Therefore, the effects of PGE_2 in avian DA have been little explored. Aim: The aim is to elucidate the effects of PGE_2 on chicken DA closure. Methods and Results: First, we examined developmental changes in PGE_2 receptors in chicken DA and the aorta by quantitative RT-PCR analyses. At day 19 embryos, the expressions of EP4 receptor mRNA in the DA was significantly higher than those of the aorta. The expressions of EP4 receptor mRNA in the DA after birth significantly decreased compared to those of embryonic day 19^{th}. Next, enzyme immunoassay revealed that blood concentration of PGE_2 in chicken of embryonic day 19^{th} was significantly higher than that of termed rat. These data suggested that vasodilatory effects of PGE_2 contributed to chicken DA closure. Finally, we measured the internal diameter of the DA at 2hrs after *in ovo* injection of indomethacin, which is a nonselective cyclooxygenase inhibitor. Indomethacin constricted the DA at day 19 embryos, but did not constrict the aorta. This data suggested that inhibition of prostanoid production constricted the chicken DA *in vivo*. Conclusion: In chicken, PGE_2 signal via EP4 receptor is involved in DA closure. A source of PGE_2 should be further explored in chicken. (COI:No)

1P-034

Arrhythmogenic properties of rat pulmonary vein cardiomyocytes

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Pulmonary veins (PVs) are the crucial origin of atrial fibrillation (AF), and have potential to generate spontaneous activity. However, cellular and molecular mechanisms underlying the arrhythmogenecity of PVs remain elusive. To investigate molecular basis of the spontaneous excitability, we performed the patch-clamp technique on isolated PV cardiomyocytes. In the cardiomyocytes, application of noradrenaline (NA) evoked the automatic excitability. During the NA-induced automaticity, an oscillatory current was observed under the instantaneous voltage-clamp conditions. The arrhythmogenic current was inward irrespective of the membrane potential, and repetitive increase of intracellular Ca2+ preceded the automaticity. Functional coupling between Na+-Ca2+ exchanger (NCX) and IP3 receptor (IP3R) appeared to deliver the Ca2+ dependent activity, since the NA-induced automaticity was completely suppressed by NCX, PLC or IP3R inhibitors, and since NCX and IP3R were co-localized along T-tubules. Concurrently, we revealed a unique Cl- current that facilitates the arrhythmic automaticity. The current was activated by hyperpolarization, intracellular Cl-, extracellular acidification and hyper-osmotic stress. Cl-channel inhibitors arrested the automaticity by attenuating the diastolic depolarization. These results suggest that NCX, IP3R and the unique Cl- channel may be involved in the NA-induced activity, and have potential to be novel targets for AF therapy. (COI:No)

1P-035

Identification of critical molecule mediating oxygen-dependent cell cycle arrest of cardiomyocytes around birth in mice.

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Mammalian cardiomyocytes (CMs) stop dividing soon after birth, which is a major obstacle for adult heart regeneration. Recently the transition from intrauterine oxygen (O_2)-poor to postnatal O_2-rich milieu has been suggested as a key upstream signal to stop CM division. However critical O_2-responding molecule(s) remains unidentified. Here we hypothesized that if the elevated O_2 tension underlies CM cell cycle arrest around birth, conventional isolation/culture protocol performed under ambient air (21% O_2) would cause inhibition of the proliferative activity of isolated fetal CMs residing in O_2-poor milieu *in utero*. Time-lapse imaging revealed that the activity of fetal CM division was drastically improved when isolation as well as subsequent culture was conducted under strict low O_2 conditions to maintain intrauterine milieu. In this system, even a short-term O_2 exposure during isolation severely inhibited cell division and depressed cell cycle-associated gene expressions, elucidating the O_2-dependent cell cycle arrest. Next, by genome-wide screening, we identified the novel critical O_2-responding and cell cycle-promoting molecule. It was amply expressed in fetal CM nuclei, which was strongly depressed both after birth and by O_2 exposure. Silencing this gene severely inhibited CM division and depressed cell cycle-associated gene expressions. In conclusion, our low-O_2 isolation protocol revealed the O_2-dependent CM cell cycle arrest around birth in mice and identified its critical mediator. (COI:No)

1P-036

The role of intracellular Ca^{2+} on the contraction of the guinea pig hepatic veins

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It has been reported that the increase of resistance in hepatic vessels regulates the blood circulation in the body through the venous return. We examined the intracellular mechanisms of the contraction on the guinea-pig hepatic veins. Tetrodotoxin (3 μM) or phentolamin (3 μM) inhibited the contraction evoked by transmural nerve stimulation (TNS; duration 50 μMs, 30 Hz, 1 s). Nifedipine (1 μM) did not have any effects to the TNS-evoked contraction. 1 - 30 μ M phenylephrine evoked vasoconstriction in a dose response manner. In presence of phenylephrine, 10 μM acetylcholine increased the contraction. CPA (10 μM) inhibited the TNS or phenylephrine evoked contraction. The intensity of the intracellular Ca^{2+} indicator, Cal-520, was transiently increased in the smooth muscle cells when the cells were stimulated with the TNS. This response was inhibited in presence of tetrodotoxin (3 μM) or phentolamin (3 μM). 30 μM phenylephrine or 10 μM acetylcholine also increased the intensity of the Ca^{2+} indicator. 10 μM CPA also increased the intensity but inhibited the response induced by phenylephrine. These results suggest that the adrenergic nerves stimulate the Ca^{2+} dependent mechanisms to evoke the contraction in the smooth muscle cells of the hepatic vein. The intracellular Ca^{2+} store involves in this contraction through the intracellular Ca^{2+} increase. (COI:No)

1P-037

Elastic structures of cardiac connectin in the vertebrate hearts with coronary and sinusoidal circulation

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In vertebrates, there are two kinds of myocardium, compacta and spongiosa, which are associated with blood supply systems i.e., coronary and sinusoidal circulation, respectively. Transmural perfusion occurs mostly during diastole in coronary circulation, because vascular compression limits flow during systole. Therefore, the heart with coronary circulation may have stiffer mechanical property compared to that with sinusoidal circulation to prevent blood flow disturbance. The expandability of the heart is mainly determined by an elastic protein connectin, which connects from Z-line to M-line of the sarcomere in heart muscle cells and generates passive tension during diastole. However, it is not uncovered whether connectin involves in the adaptation of the cardiac stiffness for proper blood supply. In this study, we determined the primary structure of connectin in frog heart with sinusoidal circulation, and compared it with that in human heart with coronary circulation. As a result, we found that the domain structure is conserved between frog and human connectin. However, the length of PEVK region and N2B region of frog connectin, which play a crucial role in the generation of passive tension, were dramatically longer than that of human connectin. These results may indicate that the restriction on the mechanical property of the heart, which was imposed by the existence of coronary circulation system, dramatically shorten the elastic region of connectin and made mammalian heart stiffer and less-extensible during evolution. (COI:No)

1P-038

Cytotoxic effects of silica nanoparticles on hippocampal neuron

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Recently, the utilization of nano-size material has increased significantly. Nanoparticles (NPs) of different material, sizes, shapes, surface modifications, were produced to use in a variety of applications, including engineering, food, medicine, and skin care. Silica (Silicon dioxide, SiO_2) is a major component of glass and sands and found in various living organisms, including hair and nails, and it has been used in the synthesis of NPs. Silica NPs (SiNPs) are generally considered to be nontoxic and inexpensive and easy to produce. SiNPs are being formulated for cellular imaging and for drugs or gene delivery in the central nervous system (CNS), but it is unclear what potential effects SiNPs can elicit once they enter the CNS. Therefore, we examined the effects of SiNPs exposure using primary rat hippocampal cultures. In this report, we analyzed cytotoxicity using luminescent cell viability assay, and reactive oxygen species (ROS) live-cell imaging assay. In this reports, we show that administration of SiNPs increase the productions of intracellular ROS and induce cell death. We also observed the pre-treatment of anti-oxidants significantly decreased of ROS production and cell death. Our findings suggest that SiNPs are capable of altering neuronal functions. (COI:No)

1P-039

Molecular mechanisms of synaptic homeostasis in zebrafish neuromuscular junction

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Homeostasis is an essential mechanism that enables a biological system to work properly, which functions also in synapses. At the neuromuscular junction (NMJ), the suppressed sensitivity of postsynaptic neurotransmitter receptors generally leads to a compensatory increase of synaptic efficacy, which offsets the dysfunctional postsynaptic receptor function and restores the muscle excitation. We previously identified a mutation in the δ subunit of acetylcholine receptor (AChR) in zebrafish that leads to a loss of the AChR expression on the plasma membrane. The AChR-null fish exhibited complete paralysis. We chemically induced the expression of an exogeneously introduced wild-type δ subunit in a developing AChR-null fish and found that the fish recovered from the paralysis. However, electrophysiological studies revealed that the fish lacked miniature End Plate Current (mEPC). We speculate that the lack of postsynaptic responses in paralyzed fish changed the mechanism of synaptic vesicle release on the presynaptic terminal. We performed RNA sequencing on the mEPC-null fish to identify molecules responsible for the loss of mEPC, and identified several up-regulated proteins and a down-regulated protein. We will overexpress or knock down these proteins in wild-type fish and discuss their functional roles. (COI:No)

1P-040

Suppression of Cerebellar Long-Term Depression after Adaptation of Optokinetic Response

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Long-term depression (LTD) at parallel fiber (PF) to Purkinje cell (PC) synapses in the cerebellum has been regarded as a main mechanism for motor learning. Previous studies suggested the necessity of LTD in motor learning by blocking molecular pathways required for LTD and examining ability of motor learning. However, some of motor learning failures reported might be caused not by LTD failure but by other defects in cerebellar functions. In addition, normal motor learning under suppression of LTD was reported. Thus, the necessity or involvement of LTD in motor learning was challenged. Here, we tried to clarify whether LTD occurred during motor learning in a mouse. Adaptation of oculomotor reflexes, vestibulo-ocular reflex (VOR) and optokinetic response (OKR), are widely used as models of cerebellum-dependent motor learning, and the cerebellar flocculus is known as a regulating center for oculomotor reflexes. First, we trained an adult mouse (from 8- to 10-weeks-old) in order to induce OKR adaptation. We applied sinusoidal rotation of vertically-striped screen surrounding a mouse for an hour as a training. Then, we prepared slices from the flocculus of OKR-trained mouse, and examined LTD at PF-PC synapses in the region responsible for horizontal oculomotor regulation. LTD induction was suppressed in the trained mice, whereas LTD occurred in slices prepared from untrained mice. These results suggest that LTD might occur and might be saturated during OKR training. (COI:No)

1P-041

Brain region-dependent differential regulation by monocarboxylate transporters of distinct neuronal functions

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Neuronal activities require high-level energy supply. As various processes essential in maintaining the neuronal functions, such as the firing, vesicular release/recycling and postsynaptic responses, occur at distinct subcellular domains, such as the soma, axonal terminals and dendrites, respectively, each of these is equipped with appointed source of energy. For example, in the nucleus of the solitary tract (NTS), monocarboxylate transporters (MCTs)-mediated astrocytes-to-neurons transfer of lactate is the most predominant source in maintaining the excitatory synaptic transmissions, but not the membrane potentials (Nagase et al, 2014). Here we sought whether this is a common rule also in other brain regions. We recorded membrane potentials and synaptic transmissions in pyramidal cells in the hippocampal CA1 region and the lateral amygdala and cerebellar Purkinje cells and analyzed the effects of a MCT inhibitor, alpha-cyano-4-hydroxycinnamic acid (4-CIN, 1 mM). 4-CIN quickly and markedly attenuated the excitatory synaptic transmission in all of these neurons. In contrast, unlike the neurons in the NTS, 4-CIN significantly hyperpolarized these neurons in a manner sensitive to tolbutamide (0.5 mM), suggesting an involvement of ATP-dependent K+ channels in the maintenance of resting membrane potential. These results suggest that, in addition to maintaining synaptic transmission, MCTs also regulate membrane potentials, in a part of the brain regions, through mechanisms different from those on the synaptic effects. (COI:No)

1P-042

Nogo Receptor Signaling Restricts Adult Neural Plasticity by Limiting Synaptic AMPA Receptor Delivery

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Experience-dependent plasticity is limited in the adult brain, and its molecular and cellular mechanisms are poorly understood. Removal of the myelin-inhibiting signaling protein, Nogo receptor (NgR1), restores adult neural plasticity. Here we found that, in NgR1-deficient mice, whisker experience-driven synaptic α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) insertion in the barrel cortex, which is normally complete by 2 weeks after birth, lasts into adulthood. In vivo live imaging by two-photon microscopy revealed more AMPAR on the surface of spines in the adult barrel cortex of NgR1-deficient than on those of wild-type (WT) mice. Furthermore, we observed that whisker stimulation produced new spines in the adult barrel cortex of mutant but not WT mice, and that the newly synthesized spines contained surface AMPAR. These results suggest that Nogo signaling limits plasticity by restricting synaptic AMPAR delivery in coordination with anatomical plasticity. (COI:No)

1P-043

Accurate estimation of AMPA receptor amount on the cell surface during hippocampal long-term depression

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At excitatory synapses, AMPA receptors dynamically move into and out of synapse, and the increase and decrease in number of receptors on the postsynaptic membrane are thought to play major roles in synaptic plasticity. Super-ecliptic pHluorin (SEP), pH-sensitive GFP variant, has been widely used to observe AMPAR trafficking on the cell surface. It had been thought that most SEP-AMPAR signals came from cell surface. However, recent study showed that there are significant SEP-AMPAR signals inside of a neuron and that the intensity of such signal changes with intracellular acidification caused by neuronal activities. Thus, the SEP signal intensity does not necessarily reflect the amount of cell-surface AMPA receptors.
Previously, we developed methods to visualize individual endocytosis of AMPA receptors around postsynaptic membrane. We formed postsynaptic-like membrane on a glass surface for TIRFM (Total Internal Reflection Fluorescence Microscopy) observation, and detected individual endocytosis of SEP-AMPAR by changing extracellular pH to quench cell-surface other SEP-AMPAR signals.
Here, we applied this pH exchange method to estimate the amount of cell-surface AMPAR. We subtracted the signal intensity at pH 6.0 corresponding to the intracellular signal from that at pH 7.4. We estimated the amount of each AMPA receptor subunit in both post- and extrasynaptic membrane during long-term depression. We also examined individual exo- and endocytosis events which should underlie changes of amount of cell surface AMPA receptor subunits. (COI:No)

1P-044

Visualization of exocytosis and endocytosis around hippocampal presynaptic active zones by total internal reflection fluorescence microscopy

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The detailed molecular mechanisms regulating exocytosis and endocytosis in presynaptic varicosities are not elucidated. To address this issue, we have developed a novel method to observe exocytosis and endocytosis around presynaptic active zones by total internal reflection fluorescence microscopy (TIRFM). TIRFM enables us to observe fluorescent signals with a high signal-to-noise ratio by limiting the depth of excitation volume to approximately 100 nm. To visualize molecular movement around active zones with TIRFM, we induced their formation just on the glass surface with synaptic adhesion protein Neuroligin, which is expressed on the postsynaptic membrane and can induce presynaptic differentiation. We prepared cultured hippocampal neurons on the cover glass coated with Neuroligin. A type of presynaptic scaffold protein CAST tagged with a fluorescent protein was observed just on the glass surface with TIRFM, suggesting that presynaptic structure was formed directly on the cover glass. Then, we visualized exocytosis with SypHy, fusion protein of Synaptophysin and pH-sensitive fluorescent protein Super Ecliptic pHluorin, which gets brighter upon exocytosis. The increase of SypHy signal triggered by single electrical stimulation was detected in the active zones. We also tried to examine endocytosis with Dynamin fused to tagRFPt. Dynamin is a GTPase involved in membrane fission to generate an endocytic vesicle. The increase and decrease of Dynamin signal were recorded around the active zones. (COI:No)

1P-045

Imaging of signal transmission in mouse brain slices including cerebral cortex and hippocampal formation; Visualization of the pathway of signal transmission

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Pathways of signal transmission in mouse brain slices including the cerebral cortex and entorhinal cortex (EC), hippocampal formation and fimbria were visualized using membrane potential and calcium imaging techniques. The brains of male C57BL6J mice aged 7-8 weeks were cut along the plane of the front elevation angle of 30 degrees from the horizontal plane, and slices including the cerebral cortex, EC, hippocampal formation and fimbria/fornix were prepared. The slices were stained with a voltage sensitive dye (di-4-ANEPPS) or a calcium indicator (Rhod-2). Fluorescence changes of the slices evoked by an electrical stimulation to the fimbria or EC were detected every 1.2 ms, and the areas revealing excitation were recorded using an image processor equipped with a high-speed camera. An electrical stimulation to the fimbria evoked transient excitation in the hippocampal CA2 and CA1, followed by propagation to the subiculum. Bicuculline enhanced the excitation propagation reaching to the EC and cerebral cortex. The excitation in the cerebral cortex sustained for 300 ms or longer. A stimulation input to the EC evoked a transient excitatory response in the dentate gyrus. The response increased in the presence of bicuculline, and the excitation propagated to the CA1 and subiculum. Imaging of excitation propagation in mouse brain slices, which were cut along the angled plane, allows for the visualization of the pathways of signal transmission in the regions including the cerebral cortex, EC and hippocampal formation. (COI:No)

1P-046

Roles of biased distribution of synaptic terminals in auditory coincidence detector neurons of birds.

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The interaural time difference (ITD) of low frequency sound is a major cue for sound source localization of horizontal plane. Neurons in nucleus laminaris (NL) of birds are the coincidence detector of binaural inputs and involved in processing of ITDs. NL neurons with low tuning frequency (LF neurons) have prominently longer dendrites than the other frequency neurons have. However, the functional roles of dendrites for the ITD processing still remain elusive. To address this question, we analyzed the distribution of glutamate receptors along the NL dendrites with the focal uncaging of MNI-glutamate in the chicken brain slices. We found that the density of glutamate receptors was biased in the LF neurons and large receptor currents were generated at thin distal dendrites. Furthermore, the properties of focal-evoked mEPSCs showed no clear differences between the proximal and distal dendrites. Therefore, the synaptic terminals of excitatory inputs might concentrate at the distal and thin dendrites of LF neurons. We recorded the voltage responses at the soma and found that the synaptic inputs generated at the distal dendrites were strongly attenuated. Interestingly, the extent of attenuation depended on the input intensity and was decreased with blockade of K^+ channels, suggesting that the local depolarization and following activation of K^+ channels contribute to this attenuation. We will further examine the functional roles of this attenuation for the ITD processing. (COI:No)

1P-047

Microglia display circadian changes in their morphology in the normal mature rat brain

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Microglia are the only glia derived from mesodermal cells displaying macrophage-like nature. Recently, it has been suggested that microglial cells display circadian changes in their morphology. Inspired by the preceding studies, we investigated the changes in the morphology of microglial cells in the frontal or parietal cerebral cortex by fixing the rats (Wistar, male, 8 weeks-old) at 7 (- 8) AM and PM. As a result, CD11b+ microglial cells displayed larger morphology with longer ramified processes at 7 AM than those at 7 PM. CD68 is expressed by phagocytes and located on phagosome membrane. Although macrophages in the peripheral tissue normally express CD68, it has been said that microglial cells in the normal mature brain do not express CD68. However, we found that microglial cells in the cortex at 7 AM bore CD68+ phagosomes in their somata by close observation using confocal laser scan microscopy, although the number of phagosomes were a few. Furthermore, the CD68+ phagosomes included a synapse-specific protein synaptophysin, a suggestive of microglial phagocytosis of synapses at 7 AM. Morphometrical investigation based on double-immunofluorescence staining using antibodies to CD11b and synapsin I, microglial cells significantly engulfed more synapses at 7 AM than at 7 PM. These results suggest that microglial cells are engaged in sleep-wake cycle by their synaptic engulfment. (COI:No)

1P-048

Bumetanide prevents the neonatal sevoflurane induced impairments of the spatial memory and emotional behavior in mice

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Sevoflurane is widely used in clinical anesthesia for infants and has effects on GABAA receptor-mediated tonic inhibition. Recent reports have shown that exposure to sevoflurane at an early age lead to long-term cognitive dysfunction. However, the mechanisms underlying the sevoflurane induced cognitive dysfunction have not been fully understood. Aiming to find the mechanisms, we observed the effects of bumetanide, an NKCC1 inhibitor, on the sevoflurane induced cognitive dysfunction. Neonatal mice (3-5 days old) were received intraperitoneal bumetanide or saline injection. Bumetanide abolishes GABA-induced depolarization. Just after the injection, mice were exposed to 2% sevoflurane in O2 for 4hrs. The control mice received saline injection before O2 inhalation. These mice were examined with behavioral tests from 49 days old. The escape latencies and the quadrant time in the water maze test was measured as indices of spatial memory formation. In the open field test, the total distance of moving and number of standing were measured as indices of emotional levels. Mice exposed to sevoflurane with saline injection showed impaired water maze performance and emotional behaviors as compared with the control mice. On the other hand, mice exposed to sevoflurane with bumetanide injection showed those indices comparable to the control mice. Our results suggest that the sevoflurane induced tonic depolarization in the neonatal brain might lead to the impairment of brain circuits underlying the spatial memory and emotional behaviors. (COI:No)

1P-049

Tonotopic variation in the expression of low-voltage-activated Ca^{2+} channels in avian nucleus laminaris

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Interaural time difference is a cue for sound localization and first determined at nucleus laminaris (NL) in birds. Neurons in NL detect the coincidence of bilateral excitatory inputs. EPSP with rapid kinetics and proper size is required for accurate coincidence detection. NL is tonotopically organized and varies in morphological and biophysical properties corresponding to its characteristic frequency (CF). In this study, we focused on low-voltage-activated (LVA) I_{Ca} and examined its tonotopic variation and contribution to EPSP by whole-cell patch clamp recordings at NL in acute brainstem slices of chicks. In current clamp, transient depolarization after hyperpolarization, a hump, was prominent in low CF neurons. The hump was diminished by LVA I_{Ca} blockers under the blockades of I_{Na} and I_h, indicating the prominent presence of LVA I_{Ca} in low CF neurons. In addition, the hump appeared at physiological resting membrane potential (RMP), implying that LVA I_{Ca} can be activated at RMP. Then, we analyzed the effects of the LVA I_{Ca} blocker on EPSP. While the blocker caused no effect on small (around 10 mV) EPSP, the blocker reduced the amplitude of large (over 20 mV) EPSP. This indicated that large depolarization is required for activation of LVA I_{Ca}. Interestingly, when I_h was inhibited, the blocker reduced the amplitude of small EPSP. This suggested that I_h may inactivate LVA I_{Ca}. Currently, we are investigating the stimulus-intensity dependence of activation of LVA I_{Ca} and whether some physiological conditions inhibit I_h or not. (COI:No)

1P-050

Inhibitory Actions of Pregabalin on NMDA Receptor-mediated Synaptic Transmission and D-serine Content in the Mouse Spinal Cord

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Pregabalin (PGB) is widely used as an analgesic for the treatment of neuropathic pain. The activation of NMDA receptors has been associated with neuropathic pain. In the present study, we investigated the effects of PGB on NMDA receptor-mediated synaptic transmission and the content of NMDA receptor coagonist D-serine in the spinal cord dorsal horn. Experiments were performed on adult male ICR mice with their sciatic nerve ligated partially under halothane anesthesia. PGB was injected intraperitoneally (IP) in doses of 300 mg/kg bw/day for a consecutive 5-6 days. The concentration of D-serine was measured by using liquid chromatography-mass spectrometry. Tight-seal whole-cell recordings of evoked excitatory postsynaptic currents (eEPSCs) were made from neurons in the superficial dorsal horn in a lumbar spinal cord slice preparation. Sciatic nerve-ligated mice showed an increase in the concentration of D-serine in the spinal cord compared to sham-operated mice. IP injection of PGB considerably decreased D-serine concentration of sciatic nerve-ligated mice. Electrophysiological analysis revealed that partial ligation of the sciatic nerve lengthened the decay time course of NMDA-eEPSCs, and that the prolonged decay time course was shortened by IP injection of PGB. These observations suggest that PGB decreases D-serine concentration in the spinal cord, and thereby attenuates the NMDA receptor-mediated sensitization of spinal dorsal horn neurons, which might underlie the mechanisms for analgesic effects of PGB. (COI:No)

1P-051

Activation of lysosomal cathepsin L contribute to the irreversible depolarization induced by oxygen and glucose deprivation in rat hippocampal CA1 neurons

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Intracellular recordings were made from hippocampal CA1 neurons in rat slice preparations. Superfusion with oxygen and glucose deprived medium (OGD) produced a rapid depolarization ~5 min after the onset of the superfusion. Even when oxygen and glucose were reintroduced immediately after rapid depolarization, the membrane depolarized further (persistent depolarization) and reached 0 mV (irreversible depolarization) after 5 min from the reintroduction. The pretreatment of the slice preparation with cathepsin L inhibitors (calpain inhibitor 2, 1-10 nM; cathepsin L inhibitor 1, 0.5-2 nM; cathepsin L inhibitor 4, 1-20 nM) significantly restored the membrane to the pre-exposure potential level in dose-dependent manner after the reintroduction of oxygen and glucose. In the presence of a cathepsin L inhibitor, biocytin-labeled pyramidal neuron after OGD appeared to be as same as the control neuron. These results suggest that the acitivation of cathepsin L and the leakage of that from lysosomes contribute to the irreversible depolarization produced by OGD. (COI:No)

1P-052

Widely-spreading wave activity in the embryonic chick forebrain induced by olfactory nerve stimulation: Optical imaging with a voltage-sensitive dye

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We have applied multiple-site optical recording with a voltage-sensitive dye to the olfactory nerve (N.I)-olfactory bulb-forebrain preparation of the chick embryo, and pursued functional development of the olfactory system. In our previous studies, we showed that optical responses in the olfactory bulb induced by N.I stimulation consisted of three components, viz., a fast spike-like signal, a delayed long-lasting slow signal, and an oscillation. The fast spike-like signal corresponded to the sodium-dependent action potential, and the slow signal included the glutamatergic excitatory postsynaptic potential. In the present study, we found that the slow signal spread into the forebrain as a wave-like activity and distributed widely in the cortex in some conditions. This wave-like activity was elicited from the embryonic 9-day stage in normal physiological solution and the 8-day stage in the Mg^{2+}-free solution. The wave-like activity was inhibited or completely eliminated by various neurotransmitter blockers. We examined fundamental characteristics of the wave-like activity and their developmental dynamics. (COI:No)

1P-053

Calmodulin-Munc 13-1 signaling regulates the coordinated retrieval of synaptic vesicle membrane and protein at the calyx of Held

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At the nerve terminal, neurotransmitter is released by the fusion of synaptic vesicles with plasma membrane. After exocytosis, vesicle components (membrane and proteins) are retrieved by endocytosis, and are recycled for next rounds. To sustain reliable neurotransmission, it is necessary to retrieve the right amount of membrane and proteins. Synaptic vesicle recycling has been studied for decades using membrane capacitance measurements, or imaging of the fluorescent markers for vesicular proteins. However, there are very limited number of studies that apply both techniques at the same, mainly because of the technical difficulty caused by the small size of the nerve terminal. Thus, it remains unclear how membrane and proteins retrieval is coordinated.
Here, we combined capacitance measurements and pH-sensitive vesicular protein marker imaging, and directly compared the kinetics of membrane and vesicular protein exo-endocytosis by taking advantage of the use of a large calyx of Held presynaptic terminal as a preparation. To label vesicular proteins, we used pH-sensitive fluorophore cypHer5E, which has a pH dependence opposite to pHluorin. CypHer was coupled to antibodies against the luminal domains of Synaptotagmin 2 (Syt2). By comparing the time course of membrane and Syt2 uptake, we found that the coordinated retrieval of vesicle membrane and proteins was perturbed by calmodulin inhibition. We also found that synaptic vesicle priming protein Munc 13-1 is involved as the downstream molecule of calmodulin. (COI:No)

1P-054

Muscarine receptor-mediated inhibition of GABAergic transmission onto striatal cholinergic interneurons

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Striatal cholinergic interneurons are connected reciprocally and receive GABAergic input from medium spiny neurons in the striatum. Several studies have revealed that GABAergic transmission onto medium spiny neuron is inhibited by cholinergic agonist in the striatum and nucleus accumbens. However, cholinergic modulation of GABAergic transmission onto striatal cholinergic interneurons are still unknown. In the present study, we examined the cholinergic modulation on the GABAergic transmission onto striatal cholinergic interneurons. Acute coronal slices were obtained from C57BL/6 mice. Striatal cholinergic interneurons were identified by their large somata. Inhibitory postsynaptic currents (IPSCs) were evoked by focal electrical stimulation around the recording cholinergic interneuron in the presence of CNQX (5 μM), D-AP5 (25 μM) and strychnine (0.5 μM) to block glutamatergic and glycinergic components. A muscarine receptor agonist, carbachol, was bath applied at a concentration of 1 or 10 μM. The amplitude of electrically evoked IPSCs was inhibited in a dose dependent manner (1 μM: to 53.2 ± 10.0% of baseline, 10 μM: to 17.3 ± 5.9% of baseline). These results suggest that GABAergic transmission onto striatal cholinergic interneurons is modulated by muscarine receptors. (COI:No)

1P-055

Tonotopic differentiation of nucleus magnocellularis in organotypic culture

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Nucleus Magnocellularis (NM) is an avian auditory nucleus, and NM neurons are tuned to a specific frequency of sounds (characteristic frequency, CF). These neurons are differentiated in membrane properties depending on the CF; neurons with high-CF have lower resting potential and higher threshold voltage for action potential than those with low-CF. These are attributable to larger potassium current and smaller sodium current in high-CF neurons. Interestingly, high-CF neurons receive a large synaptic input from an end-bulb terminal, while low-CF neurons receive small inputs from multiple bouton terminals. These observations may suggest the involvement of auditory input in the tonotopic differentiation of NM neurons. In this study, we established organotypic slice culture of chick brainstem that includes NM, and examined the possibility.
In the slice culture, NM neurons had almost similar membrane properties, but showed significant differences in half-width and number of spikes between high- and low-CF. Furthermore the amplitudes of potassium current were almost same between high- and low-CF, while the composition of Kv channels calculated by activation curve of the Kv tail currents were slightly different. These results indicate that, in slice culture, NM neurons showed smaller potassium conductance than that of corresponding stage in vivo, and the functional tonotopic differentiation is attenuated. Given that auditory inputs are absent in the slice culture, the results may indicate that the auditory inputs are important in the tonotopic differentiation of potassium current during development. (COI:No)

1P-056

Nanoscale organization of synaptic proteins in dendritic spines and shafts

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Dendritic spines are postsynaptic responsive regions of excitatory synapses and play an important role in synaptic transmission and plasticity. Drebrin is an actin-binding protein in dendritic spines and regulates spine morphology and function. It is known that immunoreactivity of drebrin increased after long-term potentiation (LTP) induction or NMDA receptor activation induces a shift in subcellular distribution of drebrin. These facts indicate that a shift in subcellular distribution of drebrin is one of important factors of synaptic plasticity. In conventional fluorescence microscope images, it was hard to detect in which part of spines drebrin exist, although the EM-ICH indicated that drebrin is localized far from postsynaptic membrane or postsynaptic density in mature spines. To reveal the shift of drebrin in nanoscale, we used stochastic optical reconstruction microscopy (STORM). Relatively small numbers of fluorophores are activated randomly and it allows temporal separation of individual molecules, resulting in super-resolution images. For STORM analysis, we fixed cultured hippocampal neurons and immunolabeled drebrin with Alexa 647. In the STORM images, the accumulated drebrin located in the center of the spine heads, and the drebrin localized in dendrites tended to be scattered along the shafts. It has been proposed that at least two pools of actin filaments comprise the spine head: a stable actin pool and a dynamic actin pool. Using this technique, it is possible to explain the existence of a stable core of F-actin which usually colocalized with drebrin in the central region of the spine head. (COI:No)

1P-057

Acute changes in the propagating excitation wave pattern on the rat somatosensory cortex after ulnar nerve crush.

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Using our original optical recording system, we have reported that neural excitation spreads over the somatosensory cortex as a wave which was induced by a somatic stimulation and appeared first on the somatotopically corresponding site. In this study, we examined acute changes after the ulnar nerve crush (UC) in the spatiotemporal pattern of the wave. The left somatosensory cortex of the anesthetized rat was exposed and stained with a voltage-sensitive dye (RH-414). The optical recordings were performed before (PRE), soon after (t0) and 30 min after (t30) UC. After UC, the optical signal induced by electrical stimulation at the right hypothenar pad survived. The latency at the initiation site of the response was significantly longer at t0 and t30 than that at PRE. The amplitude of the optical signal at the initiation site was significantly larger at t0, but decreased at t30 than that at PRE. The propagating velocity, compared at the circumference of the initiation site, was significantly smaller at t0 and t30 than that at PRE, only in the medial-posterior direction. Survival of the cortical response after UC is probably ascribed to the overlapping innervation of the skin by the ulnar and median nerves. Ascending pathway of these two afferents would undergo mutual inhibition at some stage, so that these changes may reflect possible elimination of the inhibition. Thus, the propagating excitation wave pattern was readily altered even at the earliest stage after UC. (COI:No)

1P-058

Voice dependent formation of associative memory circuit in mouse primary auditory cortex

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Chirps of animals are emitted including multiples of the fundamental frequency. We hypothesize that the primary auditory cortex (AI) includes associative memory circuits for combining the fundamental frequency and its multiples to be stored by passive learning, in which neurons with the best frequency corresponding to those components reinforce connectivity with each other. Mice frequently emit chirps around 5 kHz accompanied by its multiples, which would make 5, 10, 15, 20, 25...kHz circuitry units according to the Hebbian rule. Using flavoprotein fluorescence imaging, we found that harmonic sounds of simultaneously presented 20 kHz and 25 kHz activated the AI 5 kHz area, while inharmonic sounds of simultaneously presented 19 kHz and 26 kHz did not. These findings suggest that simultaneous presentation of 20 kHz and 25 kHz activates circuits to the neurons existing in the low frequency area. This expectation was confirmed by two-photon imaging of neuronal activites. Furthermore, simultaneous presentation of 4 kHz and 8 kHz activates high frequency areas up to 20 or 24 kHz, while inharmonic sounds of simultaneously presented 5 kHz and 7 kHz did not. These data suggest that neuronal groups responding to 4+8+12+16+20...kHz were activated as a whole, so that responses reached the high frequency area. These circuits were not formed in AI of a mouse strain with few spontaneous chirps. These associative memory circuits will contribute to recalling the whole view of the stored original vocal sounds from a piece of the sound components, with minimal efforts. (COI:No).

1P-059

Spatio-temporal pattern discrimination of whisker-barrel inputs

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The rodent whisker-barrel system has been a model to study somatosensory representation in the cortex. To investigate how temporal patterns are discriminated in the brain, we used a transgenic rat (W-TChR2V4), in which channelrhodopsin-2 (ChR2) is expressed in the mechanoreceptive nerve endings around whisker follicles, as a model system. Each whisker was optogenetically stimulated using an LED flash or a multi optical fiber system of 3x3 matrix with high spatio-temporal resolutions. The rat trained by Go-task protocol associating the LED flash on its whisker pad with conditioned reward also responded to the 3x3 matrix with high success rate when every fiber was irradiated synchronously. However, the success rate was less than chance level when irradiated asynchoronously. The trained rat also responded with high success rate to the LED flash applied on the contralateral barrel cortex in which neurons are expressing ChR2. It is suggested that the synchronous whisker input is significantly discriminated from asynchronous inputs and that the non-selective enhancement of cortical neuronal activity would be involved in this interpretation. (COI:No)

1P-060

Modulation by off-flavors of the olfactory transduction channel

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Olfactory transduction starts at the cilia that display a nano-scale structure (100 nm diameter). Binding of odorants to the receptor protein triggers the activation of G-protein and adenylyl cyclase, which leads to sequential openings of two transduction channels (CNG and $Cl_{(Ca)}$ channel). We have examined the effect of off-flavors that are generated in varieties of foods/beverages. Current responses were obtained from the newt isolated olfactory cells using photolysis of caged cAMP under the whole-cell recording. One of the most powerful off-flavors is 2, 4, 6-trichloroanisole (TCA), which is known for inducing the cork taint in wines. Generally, it has been thought that off-flavor substances induce unpleasant smells exogenously. However, it was shown that TCA suppressed potently the current. The order of potency of CNG suppression by TCA analogues TBA or TCP were identical to that of the human detection, suggesting that the cork taint is related to TCA suppression. Furthermore, current suppression by TCA was detected even with atto-molar (10^{-18} M). To explain such super efficiency, the TCA effect showed the time-integration and slow recovery, representing the integration of substance with the hydrophobic site of the ciliary membrane. We saw the positive correlation between LogD of TCA derivatives and suppression ratio of CNG channels. These findings not only reveal a likely mechanism of flavor loss, but also may suggest certain molecular structures for possible olfactory masking agents and powerful channel blockers. Furthermore, we validated new candidate substance which is possible to suppress the current. (COI:No)

1P-061

Creation of novel model mice for reversible sensorineural hearing loss with optogenetics

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More than ten percent of world population currently suffer from hearing loss, which is primarily caused by disorders in the inner ear. Some cases that show reversibility at the early stage become irreversible and finally result in refractory hearing loss. To develop new therapeutic strategies for the disease, creation of model animals that mimic reversible sensorineural hearing loss (SNHL) in human is crucial. In the present study, we have accessed this issue by using optogenetics. There are three target tissues for SNHL in the cochlea; spiral ganglion neurons, hair cells and stria vascularis. Of these, the stria vascularis maintains a highly potential of +80 ~ +120 mV, endocochlear potential (EP). The EP enhances the sensitivity of the hair cells. The loss of the EP induces deafness. Therefore, we have optogenetically dysfunctioned the stria. We used a bigenic approach to obtain mice expressing channelrhodopsin-2 (ChR2), a blue light-gated, nonselective, cation channel (Tanaka KF, et al : Cell Rep. 2012). Reversible hearing loss accompanied by EP reduction arose from these mice by exposing their cochlea to the light. (COI:No)

1P-062

Expression pattern of voltage-gated sodium channel in dopaminergic amacrine cells.

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In retinal ganglion cells (RGCs), various types of $Na_v\alpha$ (voltage-gated sodium channel α subunit) mRNAs ($Na_v1.1$ - 1.3, and $Na_v1.6$) are expressed [Fjell et al., 1997]. And isoform-specific sodium channel targeting in RGCs has been reported [Boiko et al., 2001, 2003; Van Wart et al. 2007]. On the other hand, AII amacrine cells mainly express $Na_v1.1$ mRNA [Kaneko & Watanabe, 2007] and have an axon initial segment-like process where $Na_v1.1$ localized [Wu et al., 2011]. Difference in expression pattern of $Na_v\alpha$ subtypes might reflex functional difference of action potentials between amacrine and ganglion cells. Difference in expression pattern of $Na_v\alpha$ subtypes is also found between AII amacrine and dopaminergic amacrine (DA) cells. We reported that DA cells identified by immunostaining did not express $Na_v1.1$ mRNA [90nd Annual Meeting]. In this study, to examine the specific $Na_v\alpha$ subtypes expressed in DA cells, we applied in situ hybridization and immunostaining. We found that some of DA cells expressed $Na_v1.2$. Small number of DA cells expressed $Na_v1.6$ and $Na_v1.3$. Furthermore, we found that DA cells had a single pan-specific anti-$Na_v\alpha$ antibody-labeled process. Our results suggest that DA cells are not characterized by expression of single subtype of $Na_v\alpha$ (like AII amacrine cells). It might be possible that there are specific combinations of more than one subtype of $Na_v\alpha$ in one process of each DA cell. This work was supported by Saitama Medical University Internal Grants no. 27-B-1-18 and no. SMU-FHMC Grant 15-001. (COI:No)

1P-063

Effect of odors on masking sour taste as measured by salivary hemodynamic responses

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Salivary secretion occurs not only by the instruction of the forebrain on the detection of food cue stimuli but also by reflex pathways originating from sour taste cells in the mouth. To assess the effect of odors on masking a sour taste, we monitored salivary hemodynamic responses of the parotid glands to sour stimuli using near-infrared spectroscopy (NIRS). We focused on the masking effect of (4*Z*,7*Z*)-trideca-4,7-dienal (TDD), which is the key aroma-active compound of dried bonito, on sour taste because dried bonito soup stock, also called "*katsuo-dashi*," has been known to effectively mask the sour tastes. The magnitude of salivary responses positively correlated with the concentration of acetic acid solution. We found that the magnitude of salivary responses to 1% acetic acid solution with TDD was smaller than that to 1% acetic acid without TDD. In sensory evaluation, TDD addition to 1% acetic acid solution decreased the intensities of sourness and aversiveness to the acidic stimulus. These results indicated that the decrease in salivary hemodynamic response to acetic acid solution positively correlated with the decrease in subjective sourness and aversiveness, suggesting that a salivary hemodynamic signal, as measured by NIRS, is a useful indicator to evaluate aversiveness to sour stimuli. Because one of the important functions of saliva secretion is to buffer the acidity of a sour solution, we speculate that TDD addition decreases the evaluated sourness of the solution and thus decreases the salivary hemodynamic response to the sour stimuli. (COI:No)

1P-064

The ability to identify sweet and salty tastes in Japanese schoolchildren: a comparison with adults

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We performed whole mouth gustatory test and examined the identification thresholds of sweet and salty tastes in public elementary schoolchildren (n=408, aged 9-11) in 2014, as a part of "Healthy School Project"promoted by Ichikawa City, Chiba Prefecture, Japan, and those in adults (n= 177; mean age 68), who voluntarily attended to the test in 2012 and 2014. These surveys were performed in cooperate with oral-check-ups by Dental Association of Ichikawa City.We used two tastants, i.e. sucrose and sodium chloride (NaCl), and the test solutions were made with mineral water. These ingredients were all purchased at grocery stores. Three-stepped concentrations (mM) of sucrose (4.6, 18.3, 36.5) and NaCl (3.2, 12.8, 25.7) solutions were examined at the whole mouth gustatory test.Among the children, who met our criteria for evaluating the tastes identification thresholds, 48 and 63 % of them detected each taste at 36.5 mM (sweet) and 12.8 mM (salty), respectively. In contrast, 15-16% of them did not meet the criteria, because they declared wrong answer at the highest concentration and therefore were excluded from the evaluation. Due to the same reason, 15% of adult subjects were excluded from the evaluation. Interestingly, in the excluded cases, confusion of sweet and salty tastes was observed more in children (mean 43 %) than in adults (mean 24 %). Our results suggest that differences between the two age groups reflect the changes in the taste perception through the development and/or aging. (COI:No)

1P-065

Functional roles of electrical synapses between retinal amacrine cells in chemical synapses onto retinal ganglion cells.

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Electrical synapses are present in many types of retinal neurons expressing channel subunit, connexins (Hidaka et al., J Neurosci, 2004). Electrical current spread through gap junctions between presynaptic neurons is expected to modulate chemical synapses from these neurons onto postsynaptic neurons. Our studies revealed that individual amacrine cells show specific coupling patterns (Brain Res, 2012). Based on dendritic and coupling morphology, six different classes of homotypic lateral connections between amacrine cells of the same cell subtype were identified. Functional segregation of gap-junctionally connected amacrine cells is evident in individual synaptic contacts between retinal bipolar cells and retinal ganglion cells, when our laser scanning confocal and electron microscopy were performed. Our recent studies demonstrated that channel opening of gap junctions between tip-contact interstitial amacrine cells is regulated by intracellular cyclic AMP as well as intracellular Ca^{2+} concentration (Brain Res, 2012). In the present study, I investigated relationship between electrical synapses and chemical synapses of amacrine cells under dual whole-cell patch clamp recordings. Ultrastructural studies showed that interstitial amacrine cells make GABAergic synapses onto retinal ganglion cells. Physiological experiments demonstrated that depolarizing responses of these amacrine cells increased through cells' electrical synapses. These results suggest that inhibitory synapses onto retinal ganglion cells appear to increase through electrical synapses between interstitial amacrine cells. (COI:No)

1P-066

Quantitative imaging of JNK and p38 MAPKs for understanding of inflammatory adaptation in peripheral nervous system.

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Stress-MAPK family kinases JNK and p38 are involved in normal development of peripheral nervous system as well as adaptive responses to noxious stimuli. Previous studies suggested that stress-MAPKs play causative roles in neuronal remodeling by either directly controlling neuronal plasticity or by indirectly functioning in surrounding microglia or astrocytes, in which MAPK signaling mediates synthesis of inflammatory factors or the transporters of transmitters that may alter neuronal transmission. Thus, abnormal regulation of these MAPKs are potentially relevant to unusual neuropathic pain sensation, such as hyperalgesia and allodynia. It is now necessary to clarify the signaling mechanism of MAPKs. However, our knowledge on MAPK regulation in living organisms are limited because of the lack of tools to determine spatial and temporal dynamics of endogenous MAPK activity in the nervous system. Here we report the development and use of novel indicators for JNK and p38 based on FRET (Foerster resonance energy transfer). We observed activation dynamics of p38 and JNK in living cells and demonstrated the previously undescribed heterogeneity among cells in response to pro-inflammatory cytokine stimulation. We are investigating MAPK dynamics in living neurons and glial cells using these probes and will discuss on how dynamics of MAPK signaling contributes to physiological and pathophysiological remodeling in nervous system. (COI:No)

1P-067

How is the surround response of ON-bipolar cells formed in the mouse retina?

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Surround responses of bipolar cells (BCs) are explained by the hypothesis that the difference of the reversal potential of GABA-mediated chloride currents (gabaR) reverse the polarity between ON- and OFF-BCs. We examined whether this hypothesis is sufficient explanation from two points in isolated ON-BC preparation. First, we examined whether the permeability of bicarbonate to $GABA_A$ and $GABA_C$ receptors affects gabaR by the patch clamp technique. gabaRs of $GABA_A$ and $GABA_C$ receptors without extracellular bicarbonate were -5.1±3.4 mV (n=10) and -3.8±4.5 mV (n=8), and gabaRs of $GABA_A$ and $GABA_C$ receptors with extracellular bicarbonate (24 mM) were -4.5±5.7 mV (n=10) and -2.6±3.4 mV (n=8), respectively. There was no significant difference of the permeability of bicarbonate between two receptors. Second, we examined whether the distribution or the activity of chloride transporters (NKCC1 and KCC2) produces the intracellular chloride concentration ([Cl]i) gradient sufficient to explain the surround responses by the perforated patch clamp techniques. gabaR of soma (-18±7.5 mV, n=10) was higher than that of axon (-42±6.2 mV, n=10). When 50 μM bumetanide, a NKCC1 antagonist, were applied, gabaR of soma shifted to negative direction (-47±4.3 mV, n=14) and [Cl]i gradient between soma and axon disappeared. Application of 50 μM VU0255011, a KCC2 antagonist, did not shift gabaR of soma (-23±4.7 mV, n=3). Immunoreactivity to both NKCC1 and KCC2 distributed evenly in the dendrite and the axon in ON-BCs. Our results suggest that the current hypothesis of the surround responses in ON-BCs needs to be refined. (COI:Properly Declared)

1P-068

Dry eye sensitizes cold cells to capsaicin-mediated inhibition via TRPV1

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Previous studies found that cold sensitive-primary afferents from ocular surface (cold cell) contribute to the regulation of ocular fluid status, especially basal tear production. We recently showed that dry eye has modulatory effects on cold cell activity through TRPM8 mechanisms. In this study, we tested if cold cell activity is affected by TRPV1 agonist, capsaicin (CAP) to determine possible mechanisms underlying dry eye modulation on cold sensitivity in the eye. Unilateral dry eye was created by excision of the exorbital and infraorbital lacrimal glands. Cold responsive single-unit recordings were recorded from the trigeminal ganglion a week after dry eye and aged-matched control. Despite minor roles of CAP (300nM) on ongoing and cold-evoked neural activity of cold cell in aged-matched control animal, 300 nM of CAP had facilitatory effects on ongoing activity with decreases in cold response under dry eye condition. > 300nM of CAP reduced ongoing and cold-evoked neural activity of cold cell regardless of ocular fluid status, suggesting that TRPV1 activation had modulatory roles of cold cell activity. We also tested if modulatory role of CAP on cold cell activity is mediated by TRPV1 and found that TRPV1 antagonist, capsazepine eliminated CAP-related neural modulation of cold cell. These findings support our hypothesis that ocular fluid conditions affect cold sensitivity of ocular afferents that can be mediated by TRPV1 mechanisms in the eye. (COI:No)

1P-069

Enhancement of Information Processing by Rhythm-Excitability Integration in Olfactory Receptor Neurons of Mice.

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Respiratory rhythm associate with accuracy of odor discrimination in humans and rodents. Mechanisms of olfactory information processing linked to the respiratory rhythm remain unknown. It is proposed that the information processing in olfactory receptor neurons is modulated by the rhythm. To test this idea, action potentials generated by olfactory receptor neurons were measured with patch clamp techniques and information amount carried by the action potentials was analyzed on a basis of information theory. The action potentials were elicited by injecting sine wave currents to olfactory receptor neurons under a whole-cell patch-clamp condition. The information amount was quantified by estimating mutual information which indicates non-linear interdependence between the number of the action potentials and the amplitude of the sine wave currents. We compared the information amount for different cycles of the sine wave stimuli. The information amount was increased with the short cycle stimuli (< 200 ms) rather than with the long cycle stimuli (~ 1 sec). This result suggests olfactory information processing is modulated by the cycle of the rhythmic stimuli, which may explain at least a part of mechanisms of respiratory rhythm enhancing the odor discrimination. Rhythm-excitability integration in olfactory receptor neurons contributes to the enhancement of peripheral information processing and should be an essential part of the active sensing in exploratory behaviors of animals. (COI:No).

1P-070

Effects of external Ca^{2+} on the electroolfactogram of the goldfish olfactory epithelium submerged in Ringer's solution

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In a single olfactory epithelium (OE), teleost fish has ciliated olfactory receptor neurons (cORNs) and microvillus ORNs (mORNs) that bear, respectively, cilia and microvilli at their dendrite tips. This feature allows us to compare cORNs and mORNs in a same preparation. In cORNs, odorants activate cyclic nucleotide-gated (CNG) channels via receptors, G protein and adenylate cyclase. Responses of cORNs are suppressed by the external Ca^{2+}, partly because of the direct block of CNG channels by Ca^{2+} and the feedback by its influx. Effects of Ca^{2+} on mORNs, in which phospholipase C pathway has been assumed, are less understood. To compare effects of Ca^{2+} on cORNs and mORNs, we tested effects of additional Ca^{2+} on electroolfactogram (EOG) of the goldfish OE submerged in Ringer's solution. We recorded the responses to 250 μM IBMX (activator of cORNs) and 1 mM serine (odorant) in Ringer's solutions containing 1 or 10 mM Ca^{2+}. While the peak of the IBMX response in 10 mM Ca^{2+} was reduced to 69±8% (SD, n=7) of the peak in 1 mM Ca^{2+}, the peak response to serine in 10 mM Ca^{2+} was 101±12% (n=11) of in 1 mM Ca^{2+}. These results suggest that mORNs mainly contribute to the serine response peak and the transduction channels of mORNs activated at the peak are not practically suppressed by 10 mM Ca^{2+}. The serine response reached the peak within 1 s after the stimulation onset and declined. At 5 s after the stimulation onset, the serine response in 10 mM Ca^{2+} was 70±13% (n=11) of in 1 mM Ca^{2+}, suggesting the suppression by Ca^{2+} in the falling phase. (COI:No)

1P-071

Difference in relative amplitudes of IBMX response recorded from the olfactory tissues of the newt and the goldfish

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A phosphodiesterase (PDE) inhibitor, IBMX, can induce a response in ciliated olfactory receptor neurons (cORNs), even with no odorant. This IBMX response can be explained by assuming that an odorant receptor (OR) with a high basal activity activate G_{olf} and adenylate cyclase (AC) that synthesizes cAMP. And, by inhibiting cAMP hydrolysis, IBMX induces cAMP accumulation underlying the response. Different ORs can have different basal activity. Reisert (2010) reported, in fact, different ORs of mice have varying degrees of basal activity. Do different animal species have different basal activity of ORs? We tested, here, whether animals genetically far distant from each other have different general tendencies in basal activity of ORs. Electroolfactogram (EOG) is a method to measure the summed response of a large population of ORNs. We compared the amplitudes of the IBMX response in EOG recordings of the newt and the goldfish. The olfactory tissues of the animals were isolated and submerged in Ringer's solution. To take a near-maximal response of cORNs as a standard response in each animal, we stimulated the olfactory tissues with a mixture of 10 μM forskolin, AC activator, and 250 μM IBMX (FK+IBMX). Compared to the amplitudes of the response to 5 s application of FK+IBMX, the response amplitudes to 250 μM IBMX in the newt and the goldfish were 97±7% (S.D., n=3) and 48±3% (n=3), respectively. These results suggest that the ORs of the goldfish tend to have lower basal activity than the ORs of the newt in general. (COI:No)

1P-072

Functional connectivity from the piriform cortex during olfactory stimulation

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Functional magnetic resonance imaging (fMRI) makes considerable advances in understanding the central mechanism of olfaction in human. In the present study, we examined how odorants and odorless air discriminated in the brain, utilizing an ultrahigh-field (7 tesla) MRI. Taking the principal advantages of its high spatiotemporal resolution as well as its capacity to demonstrate the entire network of brain area, we optimized echo- planar imaging (EPI) to minimize inevitably geometric distortion and signal loss due to susceptibility artifacts. Nineteen healthy participants were scanned. By sniffing odorants (isovaleric acid, peppermint and coffee) and odorless air, activation was detected in the piriform cortex, amygadalae, hippocampus, thalamus, cingulated cortex, insula and orbitofrontal cortex. In the piriform cortex, odorants stimuli activated both anterior and posterior region, whereas odorless air stimuli activated posterior region. Examining effective connectivity by psychophysiological interaction (PPI) analysis showed that 1) both the anterior and posterior piriform cortex have connections with amygdala, medial orbitofrontal cortex, anterior cingulate cortex and insula, and 2) the posterior piriform cortex has unique connections with the lateral orbitofrontal cortex and the dorsomedial thalamus whereas the anterior piriform cotex has an unique connection with the nucleus accumbens. These results suggested that odorants and odorless air are functionally dissociable in the piriform cortex, and odorants and odorless air could be processed through different neural pathway. (COI:No)

1P-073

Expression of $G\alpha_{olf}$ in the olfactory epithelium of larval and adult newt.

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Olfaction is an important sense in many animal species for reproduction and predation. Amphibians including Japanese fire belly newt (*Cynops pyrrhogaster*) are fully aquatic in larval stage, whereas semi-aquatic in adults. Adult newts that habitat on land respond to volatile odorants, while larvae are not exposed to volatile odorants because they are fully aquatic. It raises the question whether olfactory receptors in larval stage can respond to volatile odorants other than water soluble odorants. In this study, we have focused on volatile odorant detection to find out the difference of odorant receptor (OR) and G protein expression between larvae and adults. To elucidate the expression of ORs, we have analyzed gene expression of $G\alpha_{olf}$. Gene expression of $G\alpha_{olf}$ using RT-PCR showed that PCR products had no difference between larvae and adults. However, anti-$G\alpha_{olf}$ antibodies detected a single band of ~40kDa in larvae, and a band of ~45kDa in adults by western blotting. We found two $G\alpha_{olf}$s in the sequence data of adult newt having different 5' ends. The molecular weights of these $G\alpha_{olf}$s were almost the same, which were estimated from the results of western blot analysis. The sequence data of larval newt is not available at present, but the results of western blot analysis suggest that larvae and adults have different $G\alpha_{olf}$s. In addition, hematoxylin-eosin (HE) staining revealed that larva had no vomeronasal organs (VNOs). It is also shown that an expression pattern of $G\alpha_{olf}$ in the larval olfactory epithelium was considerably localized to olfactory cilia, unlike an expression pattern in adults. (COI:No)

1P-074

Extracellular p38 MAP kinase reactivates the function of endogenous microglia.

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Fibrotic scar tissue was formed after severe damage to prevent regeneration as a barrier during the chronic phase of spinal cord injury (SCI). We hypothesized that reactivation of resting microglia might eliminate such a structural barrier by phagocytotic activity and growth factor-secreting activity. The addition of recombinant p38 MAP kinase protein (p38) to the culture medium promoted the following microglial activation in vitro: morphological change, expression of growth factors, phagocytotic clearance of spinal cord debris, rapid phosphorylation of membrane protein. Furthermore, continuous infusion of p38 protein into cisterna magna for 7 days increased the microglial activation. To ascertain the effect of p38 protein on the chronic phase of SCI, p38 protein injection into spinal cord was started three months after the contusion injury, and intrathecal injection was continued for further 2.5 months. Surprisingly, a significant reduction of scar tissue formation was observed by immunohistochemical analysis with anti-collagen antibody, and locomotor function was significantly improved in mice with p38 protein compared to the mice injected with p38 protein which lost kinase activity by using rota rod test and BMS score. These results suggest that extracellular p38 protein is a reactivation factor for microglia to promote functional recovery from chronic SCI. (COI:No)

1P-075

Activation of TRP channels by stereoisomers in the rat spinal substantia gelatinosa - actions of carvacrol, thymol and carvone

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TRPs, which are expressed in the spinal dorsal horn lamina II (substantia gelatinosa; SG) and involved in the modulation of nociceptive transmission, are activated by various plant-derived chemicals. Carvacrol and thymol, the chemical structures of which are different in the position of -OH in the benzene ring, activated TRPA1 in the SG, albeit with different affinities. On the other hand, stereoisomers are known to modulate ion channels in a manner different from each other. In order to know whether there is a difference in TRP activation among stereoisomers, we examined the effects of (-)- and (+)-carvone on glutamatergic spontaneous excitatory transmission in SG neurons of adult rat spinal cord slices with a focus on TRP activation. The experiment was performed by using the blind whole-cell patch-clamp technique. (-)- and (+)-carvone increased spontaneous EPSC frequency in a reversible and concentration-dependent manner. EC_{50} values for the (-)- and (+)-carvone activities were 0.70 and 0.72 mM, respectively. The (-)- but not (+)-carvone effect was inhibited by a TRPV1 antagonist capsazepine. On the other hand, the (+)- but not (-)-carvone effect was inhibited by a TRPA1 antagonist HC-030031. These results indicate that (-)- and (+)-carvone activate TRPV1 and TRPA1, respectively, resulting in an increase in spontaneous L-glutamate release onto SG neurons, with almost the same efficacy. Such a difference in TRP activation between the stereoisomers may serve to know the properties of TRPs in the SG. (COI:No)

1P-076

Separation of hyper-phosphorylated tau proteins using inverse-gradient polyacrylamide gel electrophoresis

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One of the major pathological deposits in the brains of Alzheimer's disease (AD) patients is abnormally hyper-phosphorylated tau (PHF-tau) proteins. It's well known that degrees of phosphorylation levels of PHF-tau proteins correlate with the severity of dementia. The six isoforms of PHF-tau proteins had been identified, but no phosphorylation status of each isoforms has been reported.

SDS-PAGE is a widely used technique for separating protein mixtures. We had shown novel separation method using inverse-gradient polyacrylamide gel electrophoresis (2011, Electrophoresis 55:1-3). This modified SDS-PAGE separation traps proteins with high molecular size at the upper part of the gel, which contains a higher concentration of acrylamide. On the other hand, small sizes of proteins are separated at the bottom of the gel containing lower concentration of acrylamide. The inverse-gradient gel (15-5%) showed much better separation for the proteins smaller than 100 KDa of molecular mass than 10% standard gel, which I use to use for separating phospho-tau proteins.

Here we investigate the separation of the hyper-phosphorylated tau proteins using inverse-gradient gel. Recombinant human tau protein (441aa) was phosphorylated by cyclin-dependent protein kinase 5 and phospho-tau mixture was separated using the inverse-gradient gel (15-5%) or 10% standard gel electrophoresis and immunoblotted with anti-tau antibody (TAU-5). Unexpectedly standard gel (10%) shows much better separation of phospho-tau proteins than inverse-gradient gel. Farther investigation will need for better separation of phospho-tau proteins. (COI:No)

1P-077

Analysis of brain monoamine biosynthesis in Zinc Finger Protein 521 knockout mice

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ZFP521 is a nuclear protein and regulates the differentiation of several kind of stem cells in a wide range of tissue, such as osteoblast formation and adipose differentiation. In the field of neurobiology, it is reported *ZFP521* is an essential factor for transition of epiblast stem cells into neural progenitors in vitro. To elucidate the role of *ZFP521* in the mouse brain, we generated *ZFP521* knockout (*ZFP521*$^{-/-}$) mice and analyzed them in detail. They displayed abnormal behavior, such as hyper-locomotion, lower anxiety, impaired learning and deficits in prepulse inhibition, which correspond to the symptoms of schizophrenia. The monoamine neurotransmitter such as dopamine, noradrenaline and serotonin plays a key role in motor behavior. In the presence study, we performed the analysis of monoamine in the each part of a *ZFP521*$^{-/-}$ mouse brain. *ZFP521*$^{-/-}$ mouse exhibited an increase in the dopamine level and a decrease in the noradrenaline in prefrontal cortex, striatum, hippocampus and midbrain. Next, we performed real-time PCR to measure the mRNA levels of the enzyme in the monoamine biosynthesis. Dopamine-beta-hydroxylase, which converts dopamine to noradrenaline, was increased in the whole brain of *ZFP521*$^{-/-}$ mouse. These results suggest that ZFP521 might be a regulator of the catecholamine biosynthesis and represses the transcription of the dopamine-beta-hydroxylase. (COI:No)

1P-078

Inhibitory actions of antidepressants on compound action potentials in the frog sciatic nerve

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Although antidepressants and anticonvulsants have been used as adjuvant analgesics to alleviate neuropathic pain, a part of their effects is due to action potential inhibition, as indicated by the fact that local anesthetics are also used as adjuvants. We have previously reported the effects of a variety of anticonvulsants and local anesthetics on voltage-gated Na^+-channel blocker tetrodotoxin-sensitive compound action potential (CAP) recorded from the frog sciatic nerve by using the air-gap method. The aim of this study was to examine the effect of various antidepressants on frog CAP and to compare their effects obtained with those of anticonvulsants and local anesthetics. Duloxetine (SNRI), amitriptyline and desipramine (tricyclic ones; tertiary and secondary amines, respectively) reduced the peak amplitude of the CAP with the IC_{50} values of 0.37, 0.16 and 1.4 mM, respectively. The duloxetine value was similar to those of lamotrigine, carbamazepine and ropivacaine (0.44, 0.50 and 0.34 mM, respectively) while the amitriptyline value was close to that of levobupivacaine (0.23 mM). The desipramine value was similar to those of lidocaine and cocaine (0.74 and 0.80 mM, respectively). They were larger than that of tetracaine (0.013 mM). These results indicate that the three antidepressants inhibit CAPs with efficacies comparable to those of some anticonvulsants and local anesthetics. These antidepressants are suggested to have an ability comparable to those of some anticonvulsants and local anesthetics in inhibiting nerve conduction. (COI:No)

1P-079

Modulation by oxytocin of synaptic transmission in rat spinal substantia gelatinosa neurons exhibits a developmental change and sexual difference

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We previously reported in adult male rats that oxytocin produces an inward current at -70 mV without a change in spontaneous excitatory transmission while enhancing spontaneous GABAergic and glycinergic inhibitory transmissions in spinal dorsal horn lamina II (substantia gelatinosa; SG) neurons which play a pivotal role in regulating nociceptive transmission. These oxytocin responses were mimicked by an oxytocin-receptor agonist TGOT and inhibited by its antagonist dVOT. In order to know a developmental change and also sexual difference in oxytocin actions, we examined the effect of oxytocin on synaptic transmission in SG neurons of young male and adult female rat spinal cord slices by using the whole-cell patch-clamp technique. In young male rat SG neurons, oxytocin produced not only inward but also outward current, enhanced or suppressed spontaneous excitatory transmission while enhancing spontaneous GABAergic and glycinergic inhibitory transmissions. In adult female rat SG neurons, oxytocin produced an inward current, and transiently enhanced spontaneous GABAergic and glycinergic inhibitory transmissions without a change in spontaneous excitatory transmission. The inhibitory transmission enhancements in adult female and young male rats disappeared in the presence of a voltage-gated Na^+-channel blocker tetrodotoxin. It is suggested that cellular mechanisms for the oxytocin-induced antinociception in the SG may exhibit a developmental change and sexual difference. (COI:No)

1P-080

Action of orexin B on glutamatergic spontaneous excitatory transmission in adult rat spinal substantia gelatinosa neurons

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Hypothalamic neurons containing oxytocin or orexin B project to the spinal dorsal horn, particularly lamina II (substantia gelatinosa, SG), and play a role in regulating nociceptive transmission from the periphery. We have reported that oxytocin produces an inward current at -70 mV in 67 % of the SG neurons examined without a change in glutamatergic spontaneous excitatory transmission. In order to know how orexin B modulates nociceptive transmission in the dorsal horn, we examined the effect of orexin B (0.05 μM) on glutamatergic spontaneous excitatory transmission by applying the blind whole-cell patch-clamp technique to the SG neurons of adult rat spinal cord slices. In 60 % of the neurons tested, orexin B superfused for 2 min produced an inward current at -70 mV with the peak amplitude of about 7 pA. On the other hand, in 40 % of the neurons tested, orexin B presynaptically enhanced spontaneous excitatory transmission with a spontaneous excitatory postsynaptic current (EPSC) frequency increase of about 80 % around 2 min after the onset of its superfusion. The inward current and spontaneous EPSC frequency increase were repeated at a time interval of 20 min. The other neurons did not affect excitatory transmission. It is concluded that in SG neurons orexin B produces a membrane depolarization and spontaneous L-glutamate release increase from nerve terminals; the former action is similar to that of oxytocin. Such orexin B actions may play a role in regulating nociceptive transmission in the spinal dorsal horn. (COI:No)

1P-081

Expression of the *c-fos*-enhanced green fluorescent protein fusion gene in the spinal cord and the hypothalamus in transgenic rats after nociceptive stimuli

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Purpose: We have succeeded in *c-fos*-enhanced green fluorescent protein (eGFP) transgenic rats. In this study, we examined eGFP expression in the spinal cord and the hypothalamus after nociceptive stimuli, using the rats.
Materials and methods: We counted eGFP fluorescence cells in lamina I and II of the L5 spinal cord, the supraoptic nucleus (SON) and the paraventricular nucleus (PVN) of the hypothalamus after subcutaneous (s.c.) injections of capsaicin and formalin in both rat hind paws in the rats. Control rats were non-trearted, saline and ethanol s.c. injected.
Results: In the capsaicin and formalin groups, eGFP fluorescence cells in lamina I at 1.5 h, in lamina I, the SON and the PVN at 3 h after s.c. injections were significantly increased. The cells in lamina II at 6 h after s.c. injection of capsaicin, in lamina I and II and the PVN at 6 h after s.c. injection of formalin were significantly increased.
Discussion: We were able to visualize and quantitatively evaluate neuronal activations after nociceptive stimuli, using *c-fos*-eGFP transgenic rats. (COI:No)

1P-082

Involvement of the autonomic vasomotor responses on the thermoregulation in the orofacial area

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Our previous studies indicate that blood vessels in the orofacial tissues are innervated by parasympathetic vasodilator and sympathetic vasoconstrictor fibers, and their vasomotor responses and interactions are important for the regulation of hemodynamics in the orofacial area. Hemodynamics is well known to be one of the important factors for the regulation of the surface temperature in the skin. Although the precise mechanisms underlying thermoregulation in the orofacial area remain largely unclear, autonomic vasomotor responses are particularly appealing because of the speed and magnitude of changes in the orofacial blood flow. The present study was designed to examine the role of autonomic vasomotor responses on the thermoregulation in the orofacial area in urethane-anesthetized rats. Parasympathetic vasodilation evoked by electrical stimulation of the central cut end of the lingual nerve (LN) elicited significant increase of the surface temperature of the lower lip. On the other hand, the vasoconstriction evoked by activation of the superior cervical sympathetic trunk (CST) induced significant decrease of the lower lip temperature. Decrease in the lower lip temperature evoked by CST stimulation was almost abolished by LN-induced parasympathetic reflex vasodilation. Our results indicate that vasomotor responses evoked by autonomic nervous system are involved in the thermoregulation in the facial skin, and suggest that trigeminal-parasympathetic vasodilation may play an important role in the maintenance of the temperature in the orofacial area. (COI:No)

1P-083

Assessment of sympathetic nervous activity by power spectral analysis of digital plethysmography

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In recent years, power spectral analysis of digital plethysmography (DPG) has been applied for the assessment of autonomic nervous function due to its simple applicability. The purpose of this study was to examine whether the low frequency fluctuations of DPG (PW-LF) reflects sympathetic nervous activity (SNA). Ten healthy young Japanese females (group Y; 23.9±3.45 yrs) and ten healthy middle-aged Japanese females (group E; 64.4±6.92 yrs) participated in this study. The DPG, electrocardiography (ECG), instantaneous lung volume (ILV) by the inductance method, and tonometric blood pressure in the semi-supine position were monitored for 5 mins. Power spectral and coherence analyses were performed by the FFT technique. Both of LF and HF values of HRV, DPG, and LF value of diastolic blood pressure (DBP) were significantly higher in the group Y ($p<0.01$). In the ILV spectrum, a single peak was observed at 0.22 Hz in the group Y, whereas in the group E several peaks were showed widely between 0.15-0.40 Hz. Coherence between PW-LF and DBP-LF reached up to 0.7 in the group Y, and 0.5 in the group E, suggesting that, taken together into consideration the fact that coherence between MSNA and DBP-LF is very high (Goso et.al., 1999), PW-LF reflects SNA. Coherence between PW-HF and ILV-HF reached up to 0.7 in the group Y and E indicating that PW-HF was caused by mechanical motion of the thorax. These results suggest strongly that PW-LF is applicable for the assessment of SNA. (COI:No)

1P-084

Fluorescence imaging guided identification of autonomic nerve fibers and the measurement of their activities in vivo by using Thy1-YFP transgenic mice

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Both sympathetic and parasympathetic nervous functions have been studied by direct measurement of their electrical activities in many species of experimental animals. While the measurement techniques of autonomic nerve activities have been established at various organs in large and medium-sized experimental animals, the small body size makes it difficult to identify peripheral autonomic nerve fibers in small rodents, especially in mice. To overcome this problem, this study investigated whether the peripheral autonomic nerve fibers/bundles were visualized by using Thy1-YFP transgenic mice, where yellow fluorescent protein is expressed in neuronal cells. With the aid of a fluorescence stereomicroscope (Leica AF6000), several autonomic nerve fibers/bundles which innervate the kidneys, the stomach, the adrenal glands, and the lungs were easily visualized in urethane/chloralose anaesthetized mice. Several branches from the lumbar sympathetic chain and the cervicothoracic (stellate) ganglion were also visualized. Moreover, sympathetic nerve activities, which were characterized by grouped discharges synchronous with cardiac and respiratory cycles, were successfully recorded from these nerve bundles. In conclusion, visualization of autonomic nerve fibers/bundles by using transgenic mice expressing a fluorescent protein in a broad neuronal population helps to measure their electrical activities in mice. (COI:No)

1P-085

Olfactory stimulation with grapefruit and lavender essential oils affects on pancreatic sympathetic nerve activity and plasma glucose in rats

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In the present study, we examined the effects of olfactory stimulation with the scent of grapefruit and lavender oils on the efferent pancreatic sympathetic nerve activity (pancreatic-SNA) in urethane-anesthetized rats, hyperglycemia induced by intracranial injection of 2-deoxy-D-glucose (2DG) in rats and oral glucose tolerance test in streptozotocin (STZ) diabetic rats. We found the olfactory stimulation with grapefruit oil markedly elevated pancreatic-SNA and significantly increased the plasma glucose level after the 2DG injection, while olfactory stimulation with SLVO markedly inhibited pancreatic-SNA and significantly reduced the plasma glucose level after the 2DG injection. Moreover, Olfactory stimulation with limonene, a major component of grapefruit oil, also elicited elevates in pancreatic-SNA and plasma glucose level after the 2DG injection, while Olfactory stimulation with linalool, a component of lavender oil, also elicited inhibits in pancreatic-SNA and the plasma glucose level after the 2DG injection. In addition, we examined the oral glucose tolerance test of olfactory stimulation with these scents in STZ-diabetic rats, and found the changes of same above the plasma glucose level. These findings suggest that scent stimulation with grapefruit and lavender oils may affect the plasma glucose level via changes in autonomic neurotransmission in rats. (COI:No)

1P-086

Comparison of respiratory sinus arrhythmia in the two groups of young and middle-aged Japanese healthy women in response to randomized breathing: Transfer function and coherence analysis for human health care.

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The purpose of this study was to investigate age-related changes in heart rate variability (HRV) in healthy Japanese women in response to random interval breathing. Ten subjects each in the two groups (20s and 60s) participated in this study. In the control task (CT), subjects were instructed to relax and breathe naturally during a 6-min period, and in the test task (TT) with randomized breathing, to start their breathing upon hearing a digital voice. Electrocardiography, instantaneous lung volume, and tonometric blood pressure in the semi-supine position were monitored simultaneously by a 16-bit A/D converter over 500 Hz sampling. After detected QRS peaks, these data were resampled over 4 Hz. The TT was necessary in checking for mental stress. There was no significant difference in baroreceptor sensitivity, mean HR, SBP, and DBP between the CT and TT in the two groups. Mean values in the HF area of CT-HRV in the two groups were 588±90 and 120±10 bpm^2/Hz ($p<0.05$), and those of TT-HRV were 1164±261 and 201±54 bpm^2/Hz ($p<0.05$). Mean transfer gain values in the TT-HF area were 352±72 and 94±14 bpm/liter ($p<0.05$). Mean cross-spectrum values in the TT-HF area were 57±10 and 39±10 liter×bpm^2/Hz ($p<0.05$). Mean phase in the TT were zero and 30 degrees in the two groups ($p<0.05$). Thus, transfer function is a useful for evaluating autonomic regulation and might be provided human health care. (COI:No)

1P-087

Involvement of brain corticotrophin-releasing factor in the responses of arterial pressure and heart rate to noxious cutaneous stimulation in rats

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In addition to the well-known hormonal action of stimulating adrenocorticotropin and adrenal corticoid secretion, corticotrophin-releasing factor (CRF) acts as a neuronal transmitter in the brain, altering various autonomic functions such as arterial pressure (AP) and heart rate (HR). We have previously reported in the anesthetized rats that AP is tonically augmented by brain CRF. Present study aimed to clarify the involvement of CRF in the responses of AP and HR to noxious cutaneous stimulation in the anesthetized rats. For this purpose α-helical CRF(9-41), a non-selective CRF receptor antagonist, was intracerebroventricularly administered. AP was recorded continuously from the right carotid artery, and HR was counted from the number of arterial pulse wave. Noxious cutaneous stimulation was applied by pinching the unilateral hindpaw. Intracerebroventricular administration of α-helical CRF(9-41), but not its vehicle, significantly decreased the responses of AP and HR to pinching. The present results indicate that brain CRF is involved in the reflex responses of AP and HR to noxious cutaneous stimulation. (COI:No)

1P-088

Multi-electrode recording of mesenteric nerve activity in rats for analysis of afferent and efferent activity

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We previously demonstrated that a recording of afferent and efferent mesenteric nerve activity (MNA) before and after cutting the proximal or distal side of the mesenteric nerve in rats, to evaluate a relationship between afferent activity and efferent activity for blood pressure control. We found efferent MNA after cutting the distal side fluctuated than afferent MNA after cutting the proximal side. The aim of this study was to elucidate the fluctuation of efferent MNA. Using intraperitoneal urethane (1.2 g/kg) in Sprague-Dawley rats, the small intestine was exteriorized through a midline abdominal incision and placed on a dish. The mesenteric nerve was exposed between the mesenteric artery and vein under a dissecting microscope. Two bipolar stainless steel electrodes were put under the nerve which diverged in parallel. Cooling evoked changes in MNA to the distal side of the nerve was recorded with a power lab system. MNA by cooling stimulation to the distal side of the nerve increased or decreased, that was fluctuated in some cases. When one MNA increased by cooling stimulation to the nerve, the other efferent MNA increased on the other nerve. These results suggest that efferent MNA increased by cooling stimulation not only to the distal side but to the circumference of the nerve, indicating that whole efferent nerve activity increased by a part of stimulation in the mesentery. (COI:No)

1P-089

Contribution of the central motor process to skin sympathetic nerve activation in a brisk voluntary contraction

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Voluntary muscle contraction is followed by a burst of skin sympathetic nerve activity (SSNA) in a reaction time situation. We hypothesized that SSNA bursts involved in voluntary muscle contraction are generated by central motor processes rather than autonomic arousal processes. In this study, the transcranial magnetic stimulation (TMS) on the primary motor cortex (M1) or the premotor area (PMA) was utilized in place of descending motor commands in voluntary muscle contraction for the purpose of comparison with voluntary muscle contraction. Stimulation inducing no central motor outflow (peripherally evoked twitch contraction and acoustic artefact of the TMS stimulating coil) was also applied. Subjects were ten healthy volunteers. The SSNA was recorded by microneurography from the tibial nerve at the popliteal fossa, with corresponding sympathetic skin response (SSR) and sympathetic flow response (SFR). The SSNA burst incidence was 71-97% for voluntary muscle contraction. The incidence of TMS evoked SSNA bursts was 53-90% and 50-100% for M1 TMS and PMA TMS, respectively. There was no significant difference in SSNA burst incidence between voluntary contraction and TMS. On the other hand, burst incidence in muscle twitches and acoustic artefacts was less than in voluntary contraction. It is suggested that the central motor process includes activation of sympathetic outflows to glabrous skins. (COI:No)

1P-090

Changes in the sympathetic regulation of ovarian estradiol secretion following long-term estradiol treatment in rats

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Our previous study showed that the activation of the sympathetic nerve to the ovary [superior ovarian nerve (SON)] decreases ovarian blood flow and estradiol secretion in rats in the estrous phase. Furthermore, we showed that the ovarian vasoconstrictor response to SON stimulation at a low frequency (2 Hz) is attenuated by long-term estradiol treatment. The present study examined the effects of estradiol treatment on the sympathetic regulation of ovarian estradiol secretion. Non-pregnant Wistar rats received either sustained subcutaneous estradiol (5 μg/day) or saline for 4 weeks. Under urethane anesthesia, changes in ovarian estradiol secretion were assessed by the electrical stimulation of the SON at different frequencies (2, 5, and 20 Hz). The basal secretion rate of ovarian estradiol was distributed within a wide range (3-192 pg/min) in the saline-treated control rats at different phase of estrous cycle, whereas it was distributed within low and narrow range (4-34 pg/min) in estradiol-treated rats, indicating estrous cycle disruption. In the control rats, no significant changes in the rate of estradiol secretion were observed at any SON stimulation frequency. In the estradiol-treated rats, SON stimulation at 5 and 20 Hz but not at 2 Hz decreased the rate of estradiol secretion significantly. These results suggest that the inhibition of ovarian estradiol secretion by SON stimulation at a high frequency is augmented when the hypothalamic-pituitary axis is eliminated by estradiol treatment. (COI:No)

1P-091

Factors affecting the changes in the salivary Chromogranin A levels before and after night sleep in the adult humans

Taniguchi Kentaro[1,2]、Shimouchi Akito[2]、Jinno Naoya[2]、Shirai Mikiyasu[2]、Seiyama Akitoshi[1]
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Factors affecting the changes in the salivary Chromogranin A levels before and after night sleep in the adult humansTaniguchi, Kentaro[1,2]; Shimouchi, Akito[2] ; Jinno, Naoya[2] ; Shirai, Mikiyasu[2] ; Seiyama, Akitoshi[1] ([1] Human Sci. Sch. Med. Kyoto Univ. Kyoto, Japan; [2] Dept. Cardiac Physiol. National Cerebral and Cardiovascular Research Center. Osaka, Japan)Chromogranin A (CgA) is a member of the granin family of secretory protein, regarded as biochemical markers of stress. The aim of this study was to explore factors relating to the fluctuation of salivary CgA levels before and after night sleep. Eighty seven adult subjects participated in this study. Their RR-intervals and physical activities were monitored for 24 hours during normal daily life including night sleep, in addition to their mental stress-related questionnaires such as SDS, GHQ28 and CMI. Salivary samples were collected before and after night sleep. Salivary CgA levels were determined by enzyme-linked immunosorbent assay. Subjects with higher ratios of CgA levels before and after night sleep indicated higher changes of HF powers during night sleep and after wake up. The level of CgA after night sleep significantly correlated with the scores of mental stress. These results suggested that the changes in the level of CgA before and after sleep be an available index to speculate the qualities of night sleep and mental conditions. (COI:No)

1P-092

Developmennt of a new apparatus for evaluating human emotional changes using its palmer sweating

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Development of a new apparatus for evaluating human emotional changes using its palmar sweatingMasao Sakaguchi1, Hideya Momose2, Nobuharu Hama3, Norimasa Morimitsu4 and Toshio Ohhashi51 Labo. of SKINOS Tohmi, 2 Nishizawa Electric Meters Co.,Ltd., 3 Tamagawa Seiki Co.,Ltd., 4 Rubycon Corp., 5 Dept. of Innovation of Med. and Health Sci. Research, Shinshu univ. Sch. of Med.The palmar sweating responses are related to emotional reactions mainly related to the amygdala. We developed an analyzable apparatus for evaluating emotional changes which may be related with the palmar sweating, with simultaneous recordings of heart rate, respiration and electrodermal activity (SPL). It consists of the following four parts: (1) a palmar sweating rate meter, (2) a heat rate meter, (3) a respirometer consisted of the thermistor, (4) a SPL meter consisted of the differential amplifier. All measured data are taken into personal computer. Under the daily life, these all data are recorded using a new apparatus. Deep breath, mental calculation and breath holding, are utilized as the stimulation to produce emotional changes, and then are analyzed using the new developed apparatus. In conclusion, we have obtained with these apparatus the interesting finding that when the subjects feel drowsy, the SPL changes quickly to positive direction but the palmar sweating produces no significant change. (COI:No)

1P-093

Functional mapping of visceral sympathetic outflow and skeletal muscle blood flow in the hypothalamus of rats

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The lateral hypothalamic area (LHA), including dorsomedial hypothalamic area (DMH), has been proposed to play a critical role in the defensive cardiovascular response to various stressors. In the typical defense response, it is recognized that blood flow redistribution is sympathetically evoked by a decrease in abdominal blood flow and an increase in skeletal muscle blood flow (SMF) and that is evoked in the LHA. In this study, we tested that localization of neurons within the DMH evokes the patterns of the defensive cardiovascular response. Blood pressure (BP), heart rate (HR), renal sympathetic nerve activity (RSNA), SMF and renal blood flow (RBF) were recorded simultaneously in urethane-anesthetized rats. Microinjections of bicuculline (BIC, 2.5pmol in 5nl) were made within sites of the DMH. Two different patterns of RSNA and SMF were observed in the DMH and the marginal areas. The typical pattern of the cardiovascular defense response, which was increases in BP, HR, RSNA and SMF, was observed in the caudal and dorsal region of the DMH. In contrast, microinjection of BIC in some points of the DMH caused an increase in RSNA and a decrease in SMF. However, distinct localization of neurons cased above 2 patterns was not observed. Additionally, almost sites of BIC injection in the DMH evoked increases in BP, HR and RSNA, whereas decreases in RBF were observed in several sites of all BIC injections. These results suggest that there are not clear localizations of neuronal population in the DMH to regulate blood flows of the vascular beds during stress condition. (COI:No)

1P-094

Roles of hypothalamus and midbrain on the cardiovascular response during social defeat stress in the rat

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Stress-induced cardiovascular response is based on a defensive autonomic response. It is known that 2 defense areas, the hypothalamus and the midbrain, participate in the cardiovascular response in mammals, but roles of these 2 defense areas on the cardiovascular response to psychological stress, as a social-defeat stress, are not clear. In the present study, we investigated distributions of expression of c-Fos (a marker of neuronal activation) in the hypothalamus and the midbrain during social defeat stress in conscious Wistar rats. The Wistar rat (an intruder and the stressed rat) was moved into a home cage of a Long Evans rat (a resident). After the social-defeated relationship was established between the intruder and the resident, the rats were separated with a wire-mesh in the same cage for 55min. In the stress group, blood pressure and heart rate were maintained at higher level than non-stress control group. In the intruders, c-Fos immuno-reactive (IR) neurons were increased in the midbrain periaqueductal grey (PAG), but significantly difference was not observed compare to the control animals. In contrast, the c-Fos IR neurons in the hypothalamus, especially in the dorsomedial and perifornical areas (DMH and PeF), were significantly increased in the stress group. These results suggest that the DMH and PeF in the hypothalamus play a critical role on the cardiovascular response evoked by the social defeat stress and that the PAG in the midbrain is involved at least partially in the response. (COI:No)

1P-095

Activation of 5-hydroxytryptamine-1A receptors in the spinal cord suppresses cardiovascular response evoked from the dorsomedial hypothalamus

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The psychological stress such as air-jet stress causes the pressor response and tachycardia. The stress-induced cardiovascular response is mediated via the hypothalamic dorsomedial area (DMH). In addition, central activation of serotonin 5-hydroxytryptamine-1A (5-HT1A) receptors suppress the stress-induced autonomic response, whereas the central descending pathway of the response is still unclear. In this study, we investigated that effect of injection of 5-HT1A receptor agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) into the spinal cord on the cardiovasular response evoked by disinhibition (activation) of the DMH. Microinjection of bicuculline (BIC), GABAa receptor antagonist, in the DMH caused significant increases in blood pressure (BP), heart rate (HR) and renal sympathetic nerve activity (RSNA). After administration of 8-OH-DPAT in the spinal cord (T11 level), the baseline levels of BP, HR and RSNA were slightly decreased or unchanged. The pressor and sympathoexcitatory responses evoked by the DMH activation were suppressed after the 8-OH-DPAT administration in the spinal cord. In contrast, the tachycardic response to the DMH activation was not affected after the 8-OH-DPAT administration. The results suggest that 5-HT1A receptors in the spinal cord may be involved in the descending pathway of the pressor and sympathoexcitatory responses evoked by the DMH activation. (COI:No)

1P-096

Gustatory and thermoregulatory sudomotor pathways differ: An interpretation from a case of hemifacial gustatory sweating deficit

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It is unclear whether gustatory and thermoregulatory sudomotor pathways differ. We examined the lesion in a case of hemifacial gustatory sweating deficit without thermoregulatory sweat impairment, and tried to solve this issue. A 25-year-old man experienced right hemifacial hyperhidrosis upon eating spicy or oily food without definite onset. He did not have a history of dysgeusia or Horner's syndrome. We examined his sweating function using Minor's method, iodine starch test, and skin temperature distributions using infrared thermography under the following two independent tests: 1) application of Tabasco™, a gustatory stimulator, on the lingual apex at a room temperature of 25°C, and 2) whole-body heat exposure at 40°C. Tabasco™ application induced facial sweating and flushing, and skin temperature decrease only on the right side of his face whereas whole-body heat exposure induced facial sweating and flushing symmetrical. Brainstem magnetic resonance imaging revealed no abnormality. These results suggest that gustatory sweating pathways upon stimulation of transient receptor potential vanilloid 1 (TRPV1), a capsaicin receptor, on the intraoral trigeminal nerve are different from thermoregulatory sweating pathways. We speculate that the facial efferent nerve may conduct gustatory sweating reflexes upon the trigeminal afferent stimulation, while the trigeminal efferent nerve may conduct thermoregulatory facial sweating. (COI:No)

1P-098

Does voluntary exercise uniformly increase Fos protein expression within paraventricular hypothalamic nucleus subdivisions in rats?

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During voluntary exercise, sympathetic nervous system is activated, and heart rate and blood pressure are elevated. Paraventricular hypothalamic nucleus (PVN) contains not only endocrine neurons but also sympathetic premotor neurons. The PVN is activated by exercise, thereby playing an important role in sympathoexcitation seen during exercise. The domain of the PVN is coronally divided into four subdivisions including dorsal, ventrolateral, medial, and posterior regions. The purpose of this study was to determine if these PVN subdivisions are uniformly or regionally activated by exercise in rats. Immunohistochemical staining was conducted on coronal brain sections (1.80~1.92 mm caudal to the bregma) of treadmill exercised (16 m/min, 40 min, 0-6°) rats (N=6) to investigate regional distributions within the PVN of Fos protein expression, a marker of neuronal activation. Experiments revealed that the density of Fos-like immunoreactive neurons in the dorsal PVN (1300 ± 293 /mm^2, mean ± SE) was not significantly (P>0.05) different from that in the medial (1841 ± 337 /mm^2), posterior (1658 ± 451 /mm^2), or ventrolateral (1591 ± 249 /mm^2) region of the PVN. These results suggest that voluntary exercise uniformly increases Fos protein expression within the PVN subdivisions in rats. (COI:No)

1P-099

Evaluation of time-dependent changes in autonomic response and cardiovascular dynamics from a decrease to an increase in circulating blood volume

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The assessment of autonomic regulation as an initial compensatory mechanism is vital for monitoring the patient's condition during blood volume fluctuation. However, the time-dependent changes in autonomic response are unclear. We examined the changes in autonomic response and cardiovascular dynamics from decreased to increased circulating blood volume states. We recorded the blood pressure (BP) and electrocardiogram data of 39 patients who were maintained in the supine position throughout autologous blood donation (300-400 mL; ~10% of circulating blood volume) as a model of blood volume decrease and subsequently administered fluid therapy with lactated Ringer's solution equivalent to the hemorrhage amount as a model of blood volume increase. We analyzed heart rate variability parameters, including high frequency (HF) power spectra and the ratio of low frequency (LF) power to HF power (LF/HF) to estimate parasympathetic and sympathetic nerve activity. LF/HF and HF increased and significantly decreased during a blood volume decrease. During a blood volume increase, LF/HF remained constant and HF increased. The heart rate and BP remained within the clinically normal range. Thus, sympathetic and parasympathetic nerve activity instantaneously changed from decreased to increased circulating blood volume states at the very initial stage, when circulating blood volume was changed by 10%, and the vital signs were accordingly maintained. (COI:No)

1P-100

The contribution of cardiac sympathetic efferent nerve activity to heart rate responses to muscle mechanical pressure stimulation in isoflurane-anesthetized rats

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Stimulation of skeletal muscles such as contraction and stretch regulates autonomic nervous functions and induces cardiovascular responses. In contrast, the detail of neural mechanisms for cardiovascular responses to muscle mechanical pressure stimulation appears to be unclear. The present study was aimed to examine the contribution of cardiac autonomic nerves to heart rate (HR) changes induced by mechanical pressure stimulation of skeletal muscle. Wistar male rats were anesthetized using isoflurane. A catheter was implanted into the carotid artery for measuring arterial pressure and HR was calculated. Mass discharges were recorded from cardiac sympathetic efferent nerve. Mechanical pressure was applied to the center of the calf with a force of 10 N/cm^2 for 30 sec with a stimulation probe (6 mm in diameter). The muscle mechanical pressure stimulation increased HR (45% of trials; ranged from +3.6 to +9.5 bpm), decreased (50% of trials; ranged from -4.7 to -55.6 bpm) or did not change (5 % of trials). The HR changes were negatively related with basal HR before stimulation ($r = -0.64$, $p = 0.0025$). The frequency of cardiac sympathetic efferent nerve activity increased or decreased in response to the muscle stimulation. This activity changes coincided with HR changes and these were positively correlated ($r = 0.87$, $p = 0.0001$). The present study results suggest that cardiac sympathetic efferent nerve activity predominantly contributes to HR changes induced by muscle mechanical pressure stimulation. (COI:No)

1P-101

Mechanisms of accelerating effect of protein phosphatase 2A inhibitors on smooth muscle relaxation

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Inhibition of protein phosphatase 2A 2(PP2A) activity by PP2A inhibitors, such as okadaic acid, is known to inhibit smooth muscle contraction (i.e, Watanabe and Takano-Ohmuro; 2002). To clarify the mechanisms of the accelerating effects of PP2A inhibitors on smooth muscle relaxation, the effects of rubratoxin A, a specific inhibitor of PP2A, on the relaxation time course was examined using beta escin skinned taenia cecum from guinea pig. When a maximally contracting preparation with $10^{-5.0}$ M Ca^{2+} was transferred to the relaxing solution in the absence of Ca^{2+}, after a short time lag (about 1-3 sec), the mechanical stress fell in a biphasic manner, an initial rapid phase and the following slow phase. In the presence of rubratoxin A at several micro molar, the relaxation by Ca^{2+} removal was much faster than the control (in the presence of 1% DMSO). Analysis of relaxation time course indicated that rubratoxin A had no effects on the dissociation of fast-cycling cross-bridges, but inhibited slow cycling "latch"bridge formation and/or accelerated latch bridge dissociation. Since latch bridge formation and dissociation are thought to be independent of myosin regulatory light chain phosphorylation/dephosohorylation, PP2A seems to directly affect smooth muscle contractile filament through modulation of the filaments organization and/or their ATPase activity. (COI:No)

1P-102

Effects of calcineurin activators on expression levels of myosin heavy chain type I mRNA in C2C12 skeletal myocytes

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Our previous study using differentiated C2C12 cells indicated that myosin heavy chain type I (MyHC I) mRNA expression levels were significantly increased by the application of La^{3+} to the culture medium. The upregulation of the mRNA levels by La^{3+} were abolished by the co-administration of cyclosporine A. Thus, the effects of La^{3+} on the mRNA levels are considered as a result of calcineurin activation. In the present study, we examined the effects of calcineurin activators such as chlorogenic acid or unsaturated fatty acids including oleic acid and linoleic acid on expression levels of MyHC I mRNA in C2C12 cells. C2C12 cells were induced to differentiate to myotubes by medium exchange to D-MEM containing 2%FBS. The cells were incubated in D-MEM containing 2%FBS with calcineurin activators, with or without cyclosporine A at the beginning of differentiation and removed after 24hr, and were maintained in differentiation medium for 3 days. Our results are as follows: (1) The MyHC I mRNA expression were significantly increased by the application of chlorogenic acid, but were decreased by the application of cyclosporine A with or without chlorogenic acid. (2) The MyHC I mRNA expression were significantly increased by the application of oleic acid or linoleic acid. These results indicate that the application of calcineurin activators upregulates MyHC I mRNA in a Ca^{2+}-independent manner. Further experiments need to be done to clarify the role of calcineurin on the upregulation of MyHC I mRNA. (COI:No)

1P-103

Role of unsaturated fatty acids on expression of myosin heavy chain type II_b mRNA in mouse myocytes

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Oleic acid and linoleic acid are unsaturated long chain fatty acids, and have been reported the biological activity as calcineurin activator, but the effects on skeletal muscle hypertrophy still remains to be determined. Previously, We reported that IL-6-and/or calcineurin-dependent augmentation of myosin heavy chain type I (MyHC I) mRNA expression and skeletal muscle modulators, such as IL-6 and HSP70, mRNAs expression.In the present study, we examined the effects of oleic acid, linoleic acid, and chlorogenic acid as calcineurin activator on mRNA expression of myosin heavy chain type II_b (MyHC II_b) in C2C12 cells. Then our results are as follow: (1) MyHC II_b mRNA were significantly upregulated by IL-6, but not by La^{3+} which was known activator of calcineurin. (2) These mRNA expression were increased by chlorogenic acid and were decreased by calcineurin inhibitor, cyclosporine A, with or without chlorogenic acid. (3) Oleic acid and linoleic acid enhanced MyHC II_b mRNA expression. (4) IL-6 mRNA were significantly upregulated by chlorogenic acid, oleic acid and linoleic acid. These results suggested that production of IL-6 induced by calcineurin acitivation might enhance MyHC II_b mRNA in C2C12 myotube. (COI:No)

1P-104

A new mouse model for myopathy associated with nuclear envelopathy

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Mutations in the genes encoding nuclear envelope proteins cause several diseases, so called nuclear envelopathy. Deficiency of emerin, an inner nuclear membrane protein, cause Emery-Dreifuss muscular dystrophy (EDMD), which is clinically characterized by slowly progressive muscular dystrophy, cardiomyopathy with conduction defects, and early joint contractures. Mutations in the LMNA gene, which encodes A-type lamins, cause variable diseases including EDMD, limb girdle muscular dystrophy, partial lipodystrophy, and premature aging. To date, several mouse models for nuclear envelopathy have been developed, but there is no good model mimicking myopathy observed in EDMD.In this study, we produced a new mouse model (EH) by crossing Emd knockout (Emd) and Lmna H222P knock-in (H222P) mice.The EH mice can survive and fertile, but show progeroid features after 6 months of age and died around 7-8 months after body weight loss. Prominent degeneration of skeletal muscle is seen together with cardiac fibrosis in EH mice. Surprisingly, cardiac fibrosis in EH mice is milder than H222P mice, which died by prominent dilation of heart with plural effusion around 7-8 months of age.This new animal model is useful to elucidate the different roles of nuclear envelope proteins in both skeletal and cardiac muscles. (COI:No)

1P-105

Beta-adrenergic stimulation induced cardiac fibrosis in Tric-a-knockout mice

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The TRIC (trimeric intracellular cation) channel subtypes, namely TRIC-A and TRIC-B, form homo-trimeric complexes to function as intracellular monovalent cation-specific channels. TRIC-A channels are predominantly expressed in muscle and brain, while TRIC-B channels are ubiquitously detected throughout tissues. Based on our observations in knockout mice, TRIC channels seem to mediate, in part, counter-ion movements to support efficient Ca^{2+} release from the sarco/endoplasmic reticulum.Decreased Ca^{2+} spark frequency and larger Ca^{2+} transient amplitude were observed in *Tric-a*-knockout cardiomyocytes. Thus, it was suggested that Iso induced abnormal Ca^{2+} signaling in mutant cardiomyocytes. To investigate the response to Iso-induced cardiac injury in *Tric-a*-knockout mice, Iso was administered by osmotic pump for 14 days. In Massons trichrome staining, cardiac fibrosis was significantly enhanced in mutant heart. Moreover, we identified elevation of serum cardiac troponin T and increased evans blue positive cardiomyocytes after 3 days Iso administration, suggesting cardiac necrosis. Hyperadrenergic condition would lead to catecholamine-mediated excessive intracellular Ca^{2+} accumulation, particularly involving cardiac mitochondria. Therefore, TRIC-A may protect cardiomyocytes from Iso-induced cellular and mitochondrial Ca^{2+} overloading mediate counter-ion movements. (COI:No)

1P-106

Comparison of slow waves between greater and lesser curvature of the guinea pig gastric antrum

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Spontaneous phasic contractions of gastric smooth muscle result from the generation of slow waves (SWs) that are driven by interstitial cells of *Cajal* (ICC). In guinea pig antrum, ICC distributed in the myenteric layer (ICC-MY) contribute to the initial components of SWs, and thus play a predominant role in their generation. Since the density of ICC-MY is reported to be lower in the lesser curvature than the greater curvature, we compared the properties of SWs within the two regions. Whole muscle layer preparations were prepared from the greater and lesser curvature of the guinea pigs gastric antrum, and changes in the membrane potential were recorded using intracellular recording techniques. In the great curvature, caffeine (1mM), a blocker for IP_3 - mediated Ca^{2+} release, decreased the frequency (from 3.8 min^{-1} to 2.4 min^{-1}) and abolished the secondary component of SWs. ML218 (3 μM), a blocker of T-type Ca^{2+} channels, reduced the rate of rise of the initial component without affecting the secondary component. In the lesser curvature of the same bundle, caffeine alone abolished SWs. ML218 had no effect to the rate of rise. These results suggested that both IP_3 - mediated Ca^{2+} release and T-type Ca^{2+} channels are involved in the generation of SWs at the greater curvature, while SWs generation at the lesser curvature exclusively depends on IP_3 - mediated Ca^{2+} release. Such difference of SWs properties appears to be attributed to the regional difference in density of ICC-MY. (COI:No)

1P-107

Detection of Orai1 proteins in bovine ciliary muscle cells prepared by a Percoll density-gradient centrifugation method

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In bovine ciliary muscle (BCM), stimulation of M_3-muscarinic receptors (M_3R) opens two types of non-selective cation channel with different unitary conductances (35 pS and 100 fS) which serve as major pathways for Ca^{2+} entry during sustained contraction. The molecular entities of these channels are still unknown, mainly because of the technical difficulty of obtaining BCM cells with sufficient purity. Recently we developed a new method to obtain BCM cells with unprecedented quality and amount and applied it to examine the existence and localization of TRPCs and Ora1. The ciliary body dissected from bovine eye were treated with collagenase, and the dispersed cells were subjected centrifugation through discontinuous Percoll density-gradient of 1.050 and 1.060 g/mL. Cells were then collected from the 1.050/1.060 interface and cultured for 1-3 days before use. In the cultured BCM cells, carbachol (2 μM) evoked a phasic and tonic increase of $[Ca^{2+}]_i$ monitored with fluo-4 fluorophore Caffeine (20 mM) caused a phasic rise of $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}. These responses were clearly observed in 5×10^6 cells obtained by the single-step centrifugation procedure. Immunological staining revealed abundant expression of TRPC1, TRPC3, TRPC4, TRPC6 and Orai1 (as well as of M_3R) in the plasma and endoplasmic membranes. (COI:No)

1P-108

Differential Epac1-dependent hypertrophic effect of β_2-adrenoceptor stimulation between slow- and fast-twitch muscles

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Clenbuterol (CB), a selective β_2-adrenoceptor (AR) agonist, induces muscle hypertrophy and counteracts muscle atrophy. However, it is paradoxically less effective in slow-twitch muscle than in fast-twitch muscle, though slow-twitch muscle has a greater density of β_2-AR. To elucidate the role of Epac (exchange protein activated by cAMP) in the differential hypertrophic effect of CB between slow- and fast-twitch muscles, we examined the effects of CB treatment (i.p., 2mg/kg/day for 3 weeks) on myofiber cross-sectional area and activities of signaling molecules in tibialis anterior (TA; a typical fast-twitch muscle) and soleus (SOL; a typical slow-twitch muscle) muscles of wild-type (WT) and Epac1-null (Epac1KO) mice. In TA muscle, the CB treatment induced hypertrophy through activation of Akt/mTOR and CaMKII/HDAC4 signaling pathways in WT, but not in Epac1KO. On the other hand, in SOL muscle, the CB treatment did not induce hypertrophy as well as activation of Akt/mTOR and CaMKII/HDAC4 signaling pathways in either WT or Epac1KO. Expression level of phosphodiesterase 4 (PDE4), which activity negatively regulates cAMP level, was about 12-fold greater in SOL than in TA. These results suggest that cAMP/Epac1 plays important roles in the β_2-AR-mediated hypertrophy potentially through subsequent activation of both Akt/mTOR and CaMKII/HDAC4 signaling pathways in fast-twitch muscles rather than in slow-twitch muscles, accounting for the differential hypertrophic effect of CB between slow- and fast-twitch muscles. (COI:No)

1P-109

Characteristics of gene expressions related to muscle blood flow and muscle atrophy in animal model.

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Decreased muscle strength affects Activities of Daily Life of human beings, which induces social negative impact on our society. It is well known that muscle strength is strongly correlated to muscle volume. In this study, we revealed characteristics of gene expression dynamics in Type-1 Fiber by microarray, focusing on the interaction of muscle atrophy and muscle blood flow.Male Fisher rats were used in this study. We used our original experimental model where the right hind-limbs were cast immobilized (CI), with the opposite side as controls. In order to measure muscle blood flow we used Microsphere Method. The target muscle was Soleus Muscle, which is abundant in Type-1 Fiber.We analyzed muscle volume, histology, muscle blood flow change, and gene expressions at the time points of 6 hours, 1, 2, 4, and 10 days of CI model rats. Blood flow decrease was observed rapidly after CI started by 50% and continued the same level. Muscle volume was significantly decreased after four days.By encyclopedic microarray analysis of muscle atrophy related genes, we analyzed genes relevant not only to muscle atrophy, but also to muscle blood flow, and muscle volume.The results showed that among genes related to muscle blood flow, those related to metabolism, and among muscle volume related genes, those related to structure changed significantly. (COI:No)

1P-110

Antioxidant treatment prevents pulmonary hypertension-induced diaphragm dysfunction

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Pulmonary hypertension (PH) induces inspiratory insufficiency, which is associated with intrinsic contractile dysfunction in diaphragm muscle. We here examined the role of reactive oxygen species (ROS) in PH-induced diaphragm weakness. Wistar rats were divided into control (CNT) and monocrotaline-induced PH groups. PH was induced by a single intraperitoneal injection of monocrotaline (60 mg/kg body weight). After 4 weeks of injection, diaphragm muscles were excised for mechanical and biochemical analyses. Tetanic force per cross-sectional area was decreased in diaphragm bundles from PH group. This dysfunction was prevented by the daily intraperitoneal administration of antioxidant EUK-134, a cell permeable mimetic of superoxide dismutase (SOD) and catalase. PH diaphragm showed significant reduction in GSH: GSSG ratio and increase in NADPH oxidase 2/gp91phox, SOD2, and catalase expression. On the other hand, there was no change in malondialdehyde, methionine sulfoxide, and 3-nitrotyrosine content in myofibrillar proteins from PH group. Moreover, compared to CNT group, the expression levels of myofibrillar and Ca2+ handling proteins were unaltered in PH group. These data indicate an important role of ROS in PH-induced diaphragm weakness. (COI:No)

1P-111

STAT6 signaling controls satellite cell differentiation and fusion.

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Skeletal muscle formation requires the satellite cells (i.e., myoblasts) fusion. It is well known that interleukin-4 (IL-4) is necessary for myoblast fusion. Signal Transducers and Activator of Transcription 6 (STAT6) is activated by IL-4. Although STAT6 may play an important role in regulating myoblast fusion, the role of STAT6 is still unknown. The aim of this study was to determine the role of STAT6 signaling on myoblast fusion. Isolated mouse myoblasts were transfected with STAT6 or control short hairpin RNA (shRNA) and maintained in undifferentiated growth medium conditions (GM) for 48 h or differentiation medium (DM) for 48, 72 and 96 h. We observed that protein expression of differentiation maker (myogenin) was significantly increased in STAT6 knockdown cells compared with control shRNA cells in GM. We also found that fusion index (percentage of nuclei inside the myotubes) and myotube diameter were significantly increased in STAT6 knockdown cells compared with control shRNA cells in DM at 48 and 72 h. Interestingly, fusion index and myotube diameter in STAT6 knockdown cells were impaired by incubation of DM for 96 h. The QRT-PCR results indicated that the mRNA levels of pro-apoptosis marker was significantly increased in STAT6 knockdown cells compared with control shRNA cells in DM at 72 h. Collectively, these results suggest that STAT6 signaling controls myoblast differentiation and fusion. (COI:No)

1P-112

Expression of Homer 2 proteins as novelty skeletal muscle regeneration factor

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The calcineurin-NFAT pathway regulates the skeletal muscle regeneration. Homer2 protein that modulates nervous activity has been reported to directly bind to NFATc1. However its role is not descriptively understood in the skeletal muscle. We aimed to investigate the change of Homer 2 protein levels and expression patterns during muscle regeneration. The male ICR mice (12 weeks) were used in the experiment (n=6/group). Their left tibialis anterior (TA) muscle was damaged via intramuscular injection of 0.5% bupivacaine hydrochloride (100μl). The TA muscles of both legs were dissected at 2, 4, 6 days post-injection and performed immunofluorescence staining with Homer 2, NFATc1 and NFATc3, muscle regeneration markers [Pax7,myogenin]. We observed Homer 2 immunoreactivity in TA muscles at 2, 4 and 6 days post-injection ($p<0.001$ vs. control). Homer 2 and Pax7, the satellite cells marker, were co-localized mononuclear cells also in regenerating TA muscles. Many Homer 2-positive mononuclear cells possessed expression of myogenin. The frequency of Homer 2 and NFATc1 positive cells significantly increases in 4 and 6 days rather than 2 days post injection ($p<0.05$). However co-localization with NFATc3 was not observed. In conclusion, we demonstrated that expression of Homer 2 protein increases in TA muscle regeneration. Homer 2 can expect to work in the muscle regeneration via the calcineurin-NFAT pathway (mainly differentiation). (COI:No)

1P-113

Involvement of early growth response 3 (Egr3) in myoblast proliferation

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A zinc-finger transcription factor Egr3 regulates multiple cellular events. Recent study has shown that Egr3 reinforces the transcriptional activity of nuclear factor-kappa B (NF-κB), which is important for the function of muscle specific stem cell, called satellite cell. Therefore, Egr3 might play a role in myogenesis. To address this issue, we firstly injected 10μM cardiotoxin into TA muscle of male mice (8-wks old) to induce muscle injury and examined mRNA level of Egr3. The results indicated that mRNA level of Egr3 was significantly increased in injured muscle compared to vehicle-injected control muscle, suggesting participation of Egr3 in adult myogenesis. Hence, we studied the effect of shRNA-mediated knockdown (KD) of Egr3 on C2C12 myoblast (a model of activated satellite cell). While no obvious phenotype was found in differentiation, the results indicated that Egr3 KD caused significant reduction of a fraction of BrdU incorporated cells. We then examined the effect of cell cycling on Egr3 mRNA and protein levels, and found that both levels were gradually and significantly increased from M to G2 phase. Intriguingly, NF-κB p65 protein level was also increased significantly from M to G2 phase, whereas it was reduced in Egr3 KD cells compared to control cells. Moreover, NF-κB transcriptional activity was decreased in Egr3 KD cells. Collectively, these results suggest that Egr3 is involved in regenerative myogenesis through regulating division of satellite cells. Our results also suggest that NF-κB is engaged in the Egr3-linked myogenic processes. (COI:No)

1P-114

The MLC20 monophosphorylation-dependent and -independent Ca^{2+}-sensitization, respectively at the early and late phase of vascular smooth muscle abnormal contraction

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Rho-kinase (ROK)-mediated Ca^{2+}-sensitization of vascular smooth muscle (VSM) plays a critical role for abnormal VSM contractions such as vasospasm. Previously we identified sphingosylphosphorylcholine (SPC) and Fyn, a member of Src family tyrosine kinase, as a novel signaling molecule to induce the ROK-mediated Ca^{2+}-sensitization of VSM contractions. In the present study, we investigated the time courses of the tension development and myosin regulatory light chain (MLC20) phosphorylation in the VSM strips of porcine coronary arteries after the stimulation of SPC. MLC20 phosphorylation was analyzed by glycerol-PAGE and western blot analysis of MLC20. SPC gradually increased the tension which reached to the maximum after 60 min. On the other hand, the SPC-induced MLC phosphorylation reached to the maximum during 5 min and 30 min after SPC stimulation, with increase of monophosphorylated MLC20, but no detection of diphosphorylated MLC20. SPC induced ROK activation after 5 min, and this time course is consistent with the hypothesis that SPC might induce MLC20 phosphorylation via the activation of ROK. After 30 min of SPC stimulation, MLC20 phosphorylation level turned to decline, in spite of the tension continue to increase, suggesting that the MLC20 monophosphorylation-independent mechanism may be involved in the late phase of SPC-induced abnormal VSM contraction, in contrast to the early phase which depends on MLC phosphorylation. (COI:No)

1P-115

Mechanism of stretch-induced glucose uptake in skeletal muscle of mice

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Muscle contraction is accompanied by mechanical stimuli such as passive stretching of cells and tissues. The stretch stimulates glucose uptake in skeletal muscle. However, the signalling mechanism regulating stretch-stimulated glucose uptake is not well understood. In this study, we investigated the mechanism of stretch-stimulated glucose uptake in skeletal muscle. Passive stretch induced glucose uptake in soleus muscle isolated from mice. This glucose uptake was inhibited by an integrin inhibitor RGD peptide, a focal adhesion kinase (FAK) inhibitor PF-573228, and a NOS inhibitor N^G-nitro-L-arginine methylester (L-NAME). The stretch-induced glucose uptake was also inhibited by dantrolene, an inhibitor of Ca^{2+} release from sarcoplasmic reticulum (SR), a SR Ca^{2+}-ATPase inhibitor cyclopiazonic acid (CPA), and KN-93, an inhibitor of Ca^{2+}/calmodulin-dependent protein kinase II (CaMK-II). But the depletion of extracellular Ca^{2+} did not affect the glucose uptake induced by the stretch. The stretch increased phosphorylation of FAK but not of Akt and AMP-activated protein kinase (AMPK). These results suggest that passive stretch-induced glucose uptake in skeletal muscle is mediated by NO- and Ca^{2+}/CaMK-II-dependent mechanism that appears to be Akt- and AMPK-independent. In addition, integrin/FAK signalling seems to locate upstream of the mechanotransduction cascade. (COI:No)

1P-116

Interaction between water and myoproteins revealed by calorimetry

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Magnetic resonance (MR) images reflect not only water content, but also water states in the tissue. Details of water state are, however, not clarified yet. Interaction between water and macromolecules such as myoproteins in skeletal muscle is considered to restrict their mutual motional freedom. From this it follows that water and macromolecules would gain additional motional freedom absorbing extra enthalpy (heat) with temperature similarly to the melting of ice. With differential scanning calorimetry (DSC) on skinned fibers prepared from sartorius muscle of *Rana Catesbeiana*, we observed extra enthalpy absorption at -25, -22, 0, 45, 63°C. After the heat-denaturation represented by the peak at 45°C, peaks at -25 and -22°C increased. Further heat-denaturation at 63°C decreased the integrated enthalpy (from -80°C to +20°C), which is an index of overall enthalpy capacity. The peaks at <-10°C were preserved after the removal of thick filaments from the fibers by immersing them for 40 min in 450mM KCl solution containing 3.5mM MgATP. The removal of thick filament had no significant effect on the denaturation-induced increase of the peak at -25°C and the integrated enthalpy, but had decreasing effect on the peak at -22°C. These results suggest that 1) DSC effectively explore the interaction between water and surrounding macromolecules, 2) the heat-denaturation at 45 and 63°C selectively affects the peaks at <-10°C and the integrated enthalpy, and 3) the presence of thick filament specifically contribute to one of the two peaks at <-10°C. (COI:No)

1P-117

Novel zebrafish models of Neuromuscular disorders

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Zebrafish disease models are useful to analyze the mechanism of various diseases, including neuromuscular disorders. They have a number of genes that are orthologs of muscle disease causative genes, including SIL1, a gene known to be causative for Marinesco-Sjogren syndrome (MSS). MSS is an autosomal recessive disease characterized by progressive myopathy, cerebellar ataxia, mental retardation, and congenital cataracts. We analyzed zebrafish sil1 function using antisense morpholino oligos. To create MSS model fish, we have started to create MSS knocked-out fish by CRISPR-Cas9 system.Two different morpholinos were injected at 1-2 cell stage. At 4 days post-fertilization (dpf), thirty percent of fish injected with morpholino 1 and 2 showed reduced birefringence compared to embryos injected with a control morpholino and embryos not injected at all. Moreover, co-injection of zebrafish sil1 mRNA along with the morpholino restored normal development the morphants. Additionally, they were found to have up-regulated expression of BiP (a ER stress marker protein) and LC3 (an autophagy marker protein) in these morphants. With CRISPR/Cas9 system, we successfully identified founders that could pass mutations in sil1 gene through the germline. Their offspring carried indel mutations, suggesting that these founders carrying heritable mutations. These findings seem to suggest that it may be feasible to create a MSS model fish for a therapeutic chemical screening. (COI:No)

1P-118

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

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The dynamic changes of water molecules structure surrounding contractile proteins might play an important role in cross-bridge cycling during contraction. The spin-spin relaxation process of 1H-NMR signals from suspension of myosin filaments prepared from rabbit could be well represented by the summation of several exponentials indicating that water molecules in the suspension could be conveniently grouped into several components based on the relaxation time constant (T2). The slowest two components (T2 around 0.4s and 0.15s) dominated over faster relaxation components. This may suggest that the potential of the water molecules existing around myosin filaments is high. (COI:No)

1P-119

Reconstitution of long nerve-gap injury using human skeletal muscle-derived stem cells (Sk-SCs)

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Tetsuro Tamaki, Maki Hirata, Shuichi Soeda. Muscle Physiol. Cell Biol. Unit, Tokai Univ. Sch. of Med.Autologous nerve grafts have been used to bridge the long gap peripheral nerve injury as the gold standard, despite the sacrifice of healthy functions being necessary. Here, we demonstrate that the human Sk-SCs reconstitutes this injury. Sk-SCs were sorted as CD34+/45- (Sk-34) and CD34-/45-/29+ (Sk-DN/29+) cells, were optimally/separately cultured and expanded for 2 weeks with 1 passage. Cells were then injected into the nude mice sciatic nerve long-gap model (7-mm) bridging an acellular conduit. After 8-12 weeks, active cell engraftment was observed only in the Sk-34 cells, showing preferential differentiation into both Schwann cells and perineurial/endoneurial cells, as well as formation of myelin sheath and perineurium/endoneurium surrounding regenerated axons, resulting in 85% numerical recovery. Favorable contributions to vascular formation were also observed. Tetanic tension output of downstream muscles (plantaris, soleus and gastrocnemius) via electrical stimulation from the proximal to conduit, was also measured as an indicator of functional reconstitution; over 90% recovery was achieved. In addition, engrafted Sk-34 cells expressed key trophic, tropic and growth factor mRNAs for the nerve/vascular system in vivo even after 6 weeks of transplantation. Thus, prolonged endocrine/paracrine effects of the donor/recipient cells are also expected. Therefore, Sk-SCs is a practical cell source for the therapy of severe peripheral nerve injury. (COI:No)

1P-120

Effects of electrical stimulation training on muscle atrophy in a mouse model of cancer cachexia

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A hallmark of cancer cachexia is a loss of skeletal muscle mass that is a powerful independent predictor of disease mortality. Glucocorticoid signaling was recently shown to play a critical role in cancer cachexia. Here we investigated the effects of neuromuscular electrical stimulation (ES) training on mass and glucocorticoid signaling in skeletal muscle from a mouse model of cancer cachexia. CD2F1 mice were divided into 4 groups: control (CNT), CNT+ES, C-26, and C-26+ES. Cancer cachexia was induced by a subcutaneous injection of colon 26 (C-26) cells. The ES training (60% of maximum torque, 50 Hz, 2 s on/4 s off, ~30 times) was performed every other day starting 1 day after injection of C-26. After 28 days of C-26 injection, the weight of fast-twitch gastrocnemius (Gas) and slow-twitch soleus (SOL) muscles was decreased in C-26 group. These changes were accompanied by a marked increase in the expression of glutamine synthetase (GS), a downstream regulator of glucocorticoid pathway. ES training prevented the loss of muscle weight in SOL, but not Gas, in C-26 mice. Moreover, ES training inhibited the increase in GS expression in Gas from C-26 mice, whereas the GS level in Gas from C-26+ES group was still higher than CNT group. In contrast, GS expression of SOL in C-26 mice was not affected by ES training. Thus, these findings suggest that ES training is less effective on muscle atrophy induced by glucocorticoid signaling than that induced by disuse, which is preferentially observed in slow-twitch muscle. (COI:No)

1P-121

Contribution of calstabin1 binding to impaired ryanodine receptor with prolonged low-frequency force depression.

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Prolonged low-frequency force depression (PLFFD) that occurs with vigorous muscle contraction is partially caused by impaired function of ryanodine receptor 1 (RyR1), a sarcoplasmic reticulum Ca^{2+} release channel. Calstabin1, a binding protein of RyR1, stabilizes a closed state of RyR1 and some studies showed that the amounts of calstabin1 binding to RyR1 are altered after muscle contractions. The purpose of this study was to investigate whether calstabin1 binding is associated with PLFFD-induced RyR1 impairment. Intact rat gastrocnemius muscles were fatigued in vivo and excised 0.5 h after fatigue induction. The RyR function was evaluated by Ca^{2+} release rate induced by 4-chloro-m-cresol (CMC). $[CMC]_{50}$, a CMC concentration required for half-maximum Ca^{2+} release rate, was increased in fatigued muscle. Western blotting indicated that there were no changes in binding of calstabin1 to RyR1 and in protein kinase A-dependent phosphorylation of Ser2844 in RyR1 whereas dephosphorylated RyR1 was increased in fatigued muscle. These results suggest that i) calstabin1 binding may not contribute to PLFFD-induced impairment of RyR1 and ii) impaired RyR1 function may stem from dephosphorylation of the site other than Ser2844 in RyR1. (COI:No)

1P-122

Effects of glycogen loading on expression of Ca^{2+}-ATPase and ryanodine receptor of sarcoplasmic reticulum in rat skeletal muscle

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Glycogen loading enables to build up storage of muscle glycogen, leading to an increase in work time. The glycogen-related potentiation of exercise performance has been shown to stem, at least in part, from the improved Ca^{2+}-handling function of sarcoplasmic reticulum (SR). The aim of this study was to investigate the effects of glycogen loading on the expression of Ca^{2+}-ATPase and ryanodine receptor (RyR) of SR. Wistar rats were fasted for 24 h, and then randomly assigned to a control (C) and an exercise (E) group. All animals of E group were run on the treadmill for 90 min. After exercise, the E rats were subdivided into an exercise-glycogen-loading (EG) group or an exercise-control (EC) group and allowed to rest for 24 h. During the rest periods, the EG rats were given 5% sucrose in water, whereas the EC rats were given water only. Following the rest period, the superficial region of gastrocnemius muscle was excised and used for biochemical analyses. The EG muscles exhibited an approximately 2-fold higher glycogen concentration compared to the C and the EC muscles. The amounts of SR Ca^{2+}-ATPase and RyR were significantly higher for the EG than for the C muscles. The increased amounts were accompanied by elevations in SR Ca^{2+}-uptake rate but not in release rate. These results suggest that glycogen loading may result in increased synthesis of SR Ca^{2+}-ATPase and RyR. (COI:No)

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Changes of ion channel currents and vascular tone regulation in the hindlimb arteries from exercise-trained and sciatic nerve injured rats

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K^+ channel currents determine the plasma membrane potential of vascular myocytes, thereby regulating arterial tone. Voltage-gated K^+ channel current (I_{Kv}) counterbalances the depolarization and voltage-operated Ca^{2+} channel (VOCC) activation. Moderate increase in extracellular $[K^+]$ ($[K^+]e$) induces relaxation of small arteries (K^+-induced vasodilation) via augmenting inwardly rectifying K^+ channel current (I_{Kir}) and membrane hyperpolarization. In the rats underwent endurance exercise training (ET, rodent treadmill running) for two weeks, both I_{Kv} and I_{Kir} are increased by two fold in the skeletal arterial (SkASMC) and cerebral arterial smooth muscle cells (CASMCs). Same responses were observed in the hypertensive rats where both I_{Kv} and I_{Kir} had been attenuated. However,nonselective cationic currents (INSC) showed opposite directions of changes between the CASMCs and SkASMCs, which refleted differential hemodynamic situations between the two organs. Unilateral sciatic nerve injury and paralysis of the hindlimb induced I_{Kv} increase in the counter lateral part SkASMCs and I_{Kir} decrease in injured side. When two weeks of ET was combined with the sciatic nerve injury model, I_{Kv} was increased in both injured and intact legs. The recovery of I_{Kir} in the injured leg was observed albeit not consistent. The recovery of I_{Kir} and I_{Kv} by ET might be one of the mechanisms for the beneficial effects of regular exercise on the rehabilitation of motor nerve injury. (COI:No)