Poster Presentations

Day 1

March 28 (Tue) , 13:10 – 14:10

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1P-022 – 1P-043	Heart, Circulation (1)
1P-044 – 1P-060	Neuron, Synapse (1)
1P-061 – 1P-076	Sensory Function (1)
1P-077 – 1P-087	Neurochemistry
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1P-001

Characteristics of Ca^{2+} influx pathways activated by hypotonic stimulation in the principal cells of rat cortical collecting duct

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The apical membrane of cortical collecting duct (CCD) is occasionally exposed to the hypotonic tubular fluid. We previously reported that severe hypotonicity (195 mOsm) evoked a transient increase in $[Ca^{2+}]_i$ of CCD cells, which was inhibited by Ca^{2+} channel inhibitor, nicardipine, but insensitive to a TRPV4 channel inhibitor, Ruthenium Red. Moreover, we demonstrated that an increase in $[Ca^{2+}]_i$ induced by moderate hypotonicity was sensitive to TRPC3 channel inhibitor, Pyr3. In this study, using fura-2AM, we further examined properties of the hypotonicity-induced Ca^{2+} entry pathway in the principal cells of isolated rat CCDs. Although an increase in $[Ca^{2+}]_i$ of principle cells induced by moderate hypotonicity (245 mOsm) was largely reduced by application of a selective TRPC3 channel inhibitor, Pyr3 (10 μM), severe hypotonicty (195 mOsm) induced a gradual and prolonged increase in $[Ca^{2+}]_i$ even in the presence of Pyr3. The gradual increase in $[Ca^{2+}]_i$ observed in the presence of Pyr3 was not influenced by application of nicardipine, but was significantly attenuated by the application of Ruthenium Red. These results suggest that inhibition of TRPC3 channel by Pyr3 induces the compensatory activation of TRPV4 channel under the severe hypotonicity in the principal cells of rat CCDs. (COI:No)

1P-002

Electrical stimulations do not affect the expression patterns of TRPC6 and other effectors regulating bone marrow stromal cell (BMSC) proliferation

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Bioelectrical factors such as membrane potential may control stem cell's fates, proliferation and differentiation. We previously found that depolarization mediated by TRPC6 channels regulates the cell cycle progression of BMSCs, and periodical electrical manipulation of resting membrane potential (RMP) using platinum electrodes affects cell migration and proliferation. Here we studied how electrical stimulation affects the expression of TRPC6 protein, to explore whether there is any interplay between RMP and TRPC6 activity. Immunohistochemical study showed dot-like expression pattern of TRPC6 protein at the surface of all cells tested. This pattern was unchanged by the polarity of pulses. Similarly, Orai1, a store-operated Ca^{2+} channel which also regulates the cell cycle progression of BMSCs together with TRPC6 underwent negligible effects. We also studied the effects of electrical stimulation on the expression of β-catenin, a protein which acts as a transcription factor after nuclear translocation and stimulates proliferation of BMSCs, but the translocation of β-catenin to nuclei was not induced. These results suggest that the distribution of proteins described above may not be influenced by electrical stimulation alone under the condition we tested. (COI:No)

1P-003

Functional and structural divergence in human TRPV1 channel subunits by oxidative cysteine modification

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Transient receptor potential vanilloid 1 (TRPV1) channel is a tetrameric protein that acts as a sensor for noxious stimuli such as heat, and for diverse inflammatory mediators such as oxidative stress to mediate nociception in a subset of sensory neurons. In TRPV1 oxidation sensing, cysteine (Cys) oxidation has been considered as the principle mechanism; however, its biochemical basis remains elusive. Here, we characterize the oxidative status of Cys residues in differential redox environments. Through employing a combination of non-reducing SDS-PAGE, electrophysiology and mass spectrometry, we have identified the formation of subunit dimers carrying a stable inter-subunit disulfide bond between Cys258 and Cys742 of human TRPV1 (hTRPV1). C258S and C742S hTRPV1 mutants have a decreased protein half-life, reflecting the role of the inter-subunit disulfide bond in supporting channel stability. Mass spectrometric analysis of Cys residues of hTRPV1 treated with hydrogen peroxide shows that Cys258 is highly sensitive to oxidation. Our results suggest that Cys258 residues are heterogeneously modified in the hTRPV1 tetrameric complex and comprise Cys258 with free thiol for oxidation sensing and Cys258, which is involved in the disulfide bond for assisting subunit dimerization. (COI:No)

1P-004

Evolutionary trajectory of a heat sensor TRPV1 in clawed frogs inferred from multispecies comparison and ancestral protein reconstruction

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Functional changes of thermal sensors could directly influence thermal perception, thus may have played crucial roles in thermal adaptation. Here we compared thermal responses of two species of clawed frogs (*Xenopus laevis* and *Xenopus tropicalis*) inhabiting different thermal niches. *X. laevis* was much more sensitive to heat stimulation than *X. tropicalis* at behavioral and sensory levels. Then, we compared thermal responses of an ion channel TRPV1 which serves as a heat sensor. Clear species difference in TRPV1 was observed with repeated heat stimulation (sensitization vs desensitization). In order to estimate the evolutionary trajectory of TRPV1 channel property, we then compared thermal responses of TRPV1 from three additional clawed frog species. In addition, ancestral TRPV1 was reconstructed and its thermal responses were examined. The results suggested that the TRPV1 thermal responses changed from desensitization to sensitization in the lineages leading to *X. tropicalis*. Moreover, we identified three amino acid substitutions that are largely responsible for the species difference of TRPV1 heat responses. These results suggest that subtle amino acid substitutions can cause functional changes in thermal sensors and may have served as a driving force for the evolutionary changes in thermal perception. (COI:No)

1P-005

Protective effects of the Transient Receptor Potential Ankyrin 1 Ion Channel in the colon against inflammation and fibrosis

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Background and purpose: Fibroblasts/myofibroblasts play important roles during the processes of intestinal inflammation and tissue remodeling. In this study, we analyzed the functional significance of TRPA1 in an intestinal myofibroblast cell line InMyoFibs, in particular with respect to its contribution to intestinal fibrosis in vitro and vivo.Methods: InMyoFibs was used for in vitro experiments. Trpa1 knockout mice were generated by using the CRISPR/Cas9 system. A murine model of chronic colitis was established by weekly intrarectal administration of trinitrobenzene sulfonic acid (TNBS). Histopathological measurement was performed to detect the inflammation and fibrosis level.Results: In InMyoFibs, TRPA1 agonists counteracted TGF-β1-induced expression of Type 1 collagen, α-SMA, and N-cadherin, being accompanied by reduction of the phosphorylation of Smad-2, p38-MAPK and of the expression of myocardin, a well-known master transcription factor regulating fibrosis signaling at the downstream of TGF-β1 receptor. In TNBS chronic colitis model mice, multiple proliferation centers of mononuclear cell occurred and collagen fiber was significantly increased at lamina propria mucosae of TRPA1-/- KO mice, compared with wild-type mice. Conclusion: TRPA1 could act protectively against intestinal inflammation and fibrosis and would serve to exploit an unprecedentedly unique treatment for highly intractable inflammatory/fibrotic disorders with greatly compromised quality of life, like IBD. (COI:No)

1P-006

Requirement of extracellular Ca^{2+} binding to specific amino acids for heat-evoked activation of TRPA1

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Transient receptor potential ankyrin 1 (TRPA1) is a homotetrameric nonselective cation-permeable channel that has six transmembrane domains and cytoplasmic N- and C-termini. The N-terminus is characterized by an unusually large number of ankyrin repeats. Although the 3-dimensional structure of human TRPA1 has been determined and TRPA1 channels from insects to birds are known to be activated by heat stimulus, the mechanism for temperature-dependent TRPA1 activation is unclear. We previously reported that extracellular Ca^{2+}, but not intracellular Ca^{2+}, plays an important role in heat-evoked TRPA1 activation in green anole lizards (gaTRPA1). Here we focus on extracellular Ca^{2+}-dependent heat sensitivity of gaTRPA1 by comparing gaTRPA1 with heat-activated TRPA1 channels from rat snake (rsTRPA1) and chicken (chTRPA1). In the absence of extracellular Ca^{2+}, rsTRPA1 and chTRPA1 are activated by heat and generate small inward currents. A comparison of extracellular amino acids in TRPA1 identified three negatively charged amino acid residues (glutamate and aspartate) near the outer pore vestibule that are involved in heat-evoked TRPA1 activation in the presence of extracellular Ca^{2+}. These results suggest that neutralization of acidic amino acids by extracellular Ca^{2+} is important for heat-evoked activation of gaTRPA1, chTRPA1, and rsTRPA1, which could lead to thee clarification of mechanisms for heat-evoked channel activation. (COI:No)

1P-007

The TRPM7 kinase limits cellular calcium release by regulating heterotrimeric G-proteins

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The Melastatin-related Transient Receptor Potential member 7 (TRPM7) is a unique fusion protein with both ion channel function and enzymatic α-kinase activity. TRPM7 is essential for cellular systemic magnesium homeostasis and early embryogenesis, it promotes calcium transport during global brain ischemia and emerges as a key player in cancer cell proliferation. TRPM7 channels are negatively regulated through G-protein-coupled receptor-stimulation, either by reducing cellular cyclic adenosine monophosphate (cAMP) or depleting phosphatidylinositol bisphosphate (PIP_2) levels in the plasma membrane. We here identify how TRPM7 regulates G protein receptor-coupled calcium release. We find that heterologous overexpression of human TRPM7 will lead to disruption of thrombin-induced calcium release unless an adequate intracellular supply of adenosine triphosphate (Mg-ATP) is guaranteed. The disruption occurs at the level of G_q, which requires intact TRPM7 kinase phosphorylation activity for orderly downstream signal transduction to activate phospholipase (PLC) β and cause calcium release. We propose that this mechanism may limit receptor-mediated calcium signaling under metabolic stress. (COI:No)

1P-008

Possible involvement of TRPM7-mediated cation influx in K562 hematopoietic cell growth and differentiation

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A human leukemia cell line K562 has been used as a model of hematopoietic cell growth and differentiation. K562 can be differentiated into erythroblasts and megakaryotic lineages by various chemical stimuli. However, the mechanisms underlying therein are not fully understood. In this study, we investigated a possible involvement of the melastatin family protein TRPM7, which is constitutively expressed in K562 cells, in cation-mediated proliferation and differentiation.Patch clamp and Ca^{2+} imaging experiments demonstrated a spontaneous cation activity and a concomitant continuous Ca^{2+} influx characteristic of TRPM7. Both the spontaneous current and influx were inhibited by application of a relatively selective TRPM7 channel blocker FTY720 or siRNA knockdown of TRPM7. Moreover, significant deceleration of K562 cell growth was observed by eliminating extracellular Ca^{2+} and by TRPM7-silencing. We also investigated a role of TRPM7 for the differentiation of K562 to red blood cells. Synthesis of hemoglobin elicited by sodium butyrate in K562 which represents its differentiation to an erythroid cell was significantly counteracted by FTY720 or TRPM7-silencing siRNA.The above results point to the pivotal significance of TRPM7-mediated cation influx in regulating both proliferative and differentiation potentials of K562. (COI: No)

1P-009

The outer pore region of the mouse PKD2L1 channel contributes to voltage-dependent inactivation

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Polycystic kidney disease 2-like 1 (PKD2L1) is a member of the transient receptor potential (TRP) superfamily. We have previously demonstrated that mouse PKD2L1 forms voltage-dependent nonselective cation channels, which are first activated but then immediately inactivated in response to membrane depolarization. However, the structural basis for the channel inactivation is poorly clarified. Since C-type inactivation of some voltage-dependent K^+ channels is known to be associated with structural changes of the outer pore region, in the present study, we focused on amino acid residues behind the putative selectivity filter of the PKD2L1 channel. The wild-type PKD2L1 channel exhibited small outward currents during depolarization and large tail currents upon the subsequent repolarization. On the other hand, the N531A/N533A mutation in the outer pore region of the PKD2L1 channel generated larger outward currents at depolarized potentials, suggesting that depolarization-triggered inactivation of the PKD2L1 channel was disrupted. Furthermore, we demonstrated that the N533 residue, but not the N531 residue, is essential for the voltage-dependent inactivation of the PKD2L1 channel. These results suggest that the voltage-dependent inactivation of the PKD2L1 channel is similar to C-type inactivation of some voltage-dependent K^+ channels. (COI:No)

1P-010

Characterization of the unique function and structure of voltage sensor domain-II of Two-pore Na^+ Channel 3 (TPC3)

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Two-pore Na^+ channels (TPCs) have 2 homologous repeats of 6 transmembrane helices, each of which is composed of a voltage sensor domain (VSD) and a pore domain (PD). These two domains are a functional unit of tetrameric type of voltage-gated cation channels, and therefore, TPCs function as a dimer. We investigated the role of two VSDs of TPC3 derived from *Xenopus tropicalis* (XtTPC3) by two-electrode voltage-clamp technique using *Xenopus* oocytes as an expression system. We focused on the differences between two 4th helices in VSDs (S4 of VSD-I and S10 of VSD-II). First, we analyzed the mutational effects of the voltage-sensing arginine residues. Whereas Arg167Gln and Arg170Gln in S4 showed no differences from wild-type, Arg517Gln and Arg523Gln in S10 positively shifted the conductance-voltage relationship. Second, we investigated a negatively charged residue (Asp511) which is evolutionally conserved only in S10. Previously, we have reported that Asp511 contributes to the voltage-dependent gating. By mutational analyses designed by utilizing the crystal structure of TPC1 as a template for XtTPC3, we found that Asp511 has electrostatic and inter-domain interaction with Arg225 in S5. Third, we further investigated the detailed movement of VSD-II by the voltage-clamp fluorometry technique. Taken together, we conclude that VSD-II, but not VSD-I, has major contribution to the whole channel gating by forming the inter-domain electrostatic interaction, which is unprecedented among voltage-dependent cation channels. (COI:No)

1P-011

External Ca^{2+} affects the efficacy and the desensitization of the FMRFamide-gated Na^+ channel by binding to the two negative rings at the bottom of outer vestibule of the channel pore

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FMRFamide-gated Na^+ channel (FaNaC) is a homo-trimeric peptide-gated sodium channel, and a subunit has two transmembrane domains (M1 and M2). Two negative rings by aspartate residues (D552, D556) were allocated at the bottom of the outer vestibule. To see the function of the negative rings, we made mutant channels at position 552 and 556 (D552N, D556N, D552ND556N) and analyzed their functions electrophysiologically in *Xenopus* oocytes. In a standard solution containing 1.8mM Ca^{2+}, EC50 of the wild type FaNaC (WT) was 4-5μM. In a nominally Ca^{2+}-free solution, EC50 was less than 2μM and the maximum current was more than doubled. By contrast, in a solution containing 10mM Ca^{2+}, the currents became smaller and EC50 was more than 20μM. The effects of Ca^{2+} were much less or absent in D552 and/or D556 mutants. The dose-inhibition curve of WT by Ca^{2+} contained three components. By contrast, the dose-inhibition curves of the mutants were approximated by a simple inhibition curve whose IC50 was close to the highest one in WT. The desensitization of FaNaC was approximated by single exponential function, and the onset but not offset rate constant was dependent on Ca^{2+} in WT. The Ca^{2+}-sensitivity was less in D552 and absent in D556 or D552ND556N. We conclude that the gating of FaNaC is modulated by ambient external Ca^{2+} which binds to D552 and D556. (COI:No)

1P-012

Antidepressants inhibit compound action potentials in a manner dependent on their chemical structures in the frog sciatic nerve

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Antidepressants have been used to alleviate chronic pain as adjuvant analgesics. Among cellular mechanisms for the antinociception produced by antidepressants, is there nerve conduction inhibition. This idea is supported by the fact that local anesthetics which inhibit nerve conduction are also used to relieve chronic pain. We have previously reported that several antidepressants inhibit nerve conduction, i.e., fast-conducting compound action potentials (CAPs) recorded from the frog sciatic nerve. Duloxetine (serotonin and norepinephrine reuptake inhibitor; SNRI), amitriptyline and desipramine (tricyclic ones; tertiary and secondary amines, respectively) reduced the peak amplitude of the CAP with the half-maximal inhibitory concentration (IC_{50}) values of 0.39, 0.16 and 1.4 mM, respectively. The present study further examined the effects of antidepressants on frog CAPs by using the air-gap method. Like duloxetine, a selective serotonin reuptake inhibitor (SSRI), fluoxetine, inhibited the CAP in a concentration dependent manner; IC_{50} value for this action was 1.5 mM. Similar inhibition was also seen by a tetracyclic antidepressant maprotiline with an IC_{50} value of 0.95 mM. The fluoxetine and maprotiline values were similar to those of local anesthetics (lidocaine and cocaine: 0.74 and 0.80 mM, respectively). It is concluded that antidepressants inhibit compound action potentials in a manner dependent on their chemical structures in the frog sciatic nerve. (COI:No)

1P-013

Phenylic acid-based NSAIDs inhibit compound action potentials recorded from the frog sciatic nerve

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Antinociception produced by analgesics has been partially attributed to nerve conduction inhibition. We have previously reported the inhibitory effects of a variety of drugs involved in antinociception on fast-conducting and voltage-gated Na^+-channel blocker tetrodotoxin-sensitive compound action potentials (CAPs) recorded from the frog sciatic nerve. The aim of the present study was to know how various types of NSAID affect frog sciatic nerve CAPs. The experiments were performed by applying the air-gap method to the frog sciatic nerve. Soaking the frog sciatic nerve for 20 min with a phenylic acid-based NSAID indomethacin (1 mM) reduced the peak amplitude of the CAP by about 40% in a partially reversible and concentration-dependent manner. A similar CAP inhibition was produced by other phenylic acid-based NSAIDs, diclofenac and etodolac; the extents of the inhibitions at 1 mM were some 50% and 15%, respectively. On the other hands, CAPs were almost unaffected by a salicylic acid-based NSAID aspirin (1 mM), propionic acid-based NSAIDs (ketoprofen, ibuprofen, naproxen and loxoprofen sodium; each 1 mM) and an oxicam-based NSAID meloxicam (0.5 mM). These results indicate that phenylic acid-based NSAIDs only exhibit an inhibitory effect on CAPs. At least a part of antinociception produced by phenylic acid-based NSAIDs used as a dematological drug to alleviate pain may be attributed to a nerve conduction inhibition produced by the drugs. (COI:No)

1P-014

Exogenous and endogenous nitric oxide regulate the persistent Na^+ current in Kenyon cells isolated from mushroom bodies in the brain of the cricket

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The nitric oxide (NO)/cyclic GMP signaling pathway has been implicated to be important in the formation of olfactory memory in insect. In this study, we investigated the effect of NO on single voltage-dependent Na^+ channels in intrinsic neurons, called Kenyon cells, in the mushroom bodies in the brain of the cricket. Step depolarization on cell-attached patch membrane induces the transient brief openings at the beginning of the voltage steps followed by a repeated brief openings all along the 150 ms pulses that may underlie the persistent component of Na^+ channels. Application of the NO donor S-nitrosoglutathione (GSNO) increased the number of openings of both types of single Na^+ channel currents. Adding a protein kinase G inhibitor, KT5823, diminished this excitatory effect of NO, indicating an involvement of PKG in the downstream pathway of NO. The membrane-permeable cGMP analog 8-Bro-cGMP increased the number of openings of both types of single Na^+ channels similar to the action of NO. Single application of Ca^{2+} ionophore, A23187, and NO synthase (NOS) inhibitor, L-NAME increased and decreased the activity of the persistent Na^+ channels, respectively. Taken together, the present results indicate that exogenous NO act as a critical modulator of both transient and persistent Na^+ channels but the latter channels are further found to be modulated endogenously by NO/cGMP/PKG signaling cascade. (COI:No)

1P-015

Aprotinin reduces the recycling rate of epithelial Na^+ channels (ENaC) to the apical membrane of an epithelial cell: a four-state model of intracellular ENaC trafficking

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Epithelial Na^+ channels (ENaC) play a crucial role in control of blood pressure by regulating renal Na^+ reabsorption. Intracellular trafficking of ENaC is one of the key regulators of ENaC function. We attempt here to provide a model for intracellular recycling applying hypotonic conditions. Our model consists of: 1) an intracellular site storing ENaCs translocated from the Golgi apparatus to the apical membrane, 2) an apical membrane site storing ENaCs that function as Na^+ conducting pathways across the apical membrane, and 3) an intracellular site storing ENaCs that are translocated from the plasma (apical) membrane site to an intracellular site or to degradation sites.We studied the effect of aprotinin (a protease inhibitor) blocking protease-induced cleavage of the extracellular loop of γ ENaC subunit on the rates of intracellular ENaC trafficking using the above-defined model of ENaC trafficking. We found that aprotinin significantly reduced the insertion rate, the recycling rate, the recycling ratio, the relocation number, the residency time of ENaC in the apical membrane and the whole life-time of ENaC to the apical membrane. (COI: No)

1P-016

Expression and function of amiloraide-blockable epithelial Na^+ channel in cement glands on hanging behavior in young Xenopus laevis tadpoles

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Young tadpoles in several species of frog, including Xenopus laevis and bullfrog, spend most of its time hanging from surface of water or solid object by its cement glands. Our previous studies showed epithelial sodium channel (ENaC) expressed specifically in embryonic cement glands in bullfrog. To examine function of ENaC in the hanging behavior, we studied behavioral change by addition of amiloraide, a blocker of ENaC, in tank water. Since Xenpus can be breed in laboratory all time of the year and its tadpole show the hanging behavior like bullfrog, we performed our experiment with Xenopus laevis as subjects. We could confirm that ENaC expressed in samples from head part of Xenopus tadpole using western blotting. Twenty Xenopus tadpoles in stage 37/38 were divided into two beakers with 200 ml water. Each beaker was stirred for 10 sec and number of hanging subjects were counted 15 min after the stirring. This procedure was repeated after addition of amiloraide (0.1mM) or same amount of water. Only in NaCl condition (100 mMN NaCl was used as tank water), amiloraide affected number of hanging subjects. From these results, we can conclude that under natural conditions, ENaC have no clear role in maintenance of hanging behavior. From the experiment in NaCl solution, however, it would appear that ENaC might be related with mechanisms of the hanging, since amiloride is specific blocker of ENaC. At the same time, we could confirm that ENaC in embryonic cement glands were functionally active in this stage. (COI:No)

1P-017

The mechanism for PKA-mediated facilitation of Cav1.2 channel: a new model

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It has been reported that PKA facilitates cardiac Cav1.2 channel via releasing the interaction between proximal and distal C-terminal regulatory domain (PCRD and DCRD), but the underlying mechanism is to be clarified. In this study, we found that the interaction between proximal C-terminal fragment (CT1) and distal C-terminal fragment (CT3) were inhibited by calmodulin (CaM). Furthermore, CT3D (a short CT3 without DCRD) still interacted with CT1B (a short CT1 without PCRD and EF hand) and the binding was also inhibited by CaM. These results suggested a CaM-competitive domain (CCD) in CT3D. The binding of CT3 to CT1SD (phosphorylation mimetic CT1 mutant) was smaller than that to CT1SA (dephosphorylation mimetic CT1 mutant), suggesting an inhibition of CT3-CT1 interaction by phosphorylaation. Our electrophysiological results showed that CT3D inhibited, while CT1B facilitated CaM-induced channel activity in inside-out patches and pre-incubation of patches with PKA abolished both effects of CT3D and CT1B. Based on these results, we brought up a mechanism underlying PKA-mediated facilitation of Cav1.2 that PKA-mediated phosphorylation facilitates Cav1.2 channel by releasing CCD from CaM binding sites through disruption of the interaction between distal and proximal C-terminus. (COI:No)

1P-018

Role of βsubunit of L-type Ca^{2+} channels in proliferation of vascular smooth muscle cells

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Enhanced cell proliferation of synthetic vascular smooth muscle cells (VSMC) are due to constantly activated cell cycle progression. Entries of Ca^{2+} are necessary both for the progression of G1 to S phase and for the M phase. We aimed to identify Ca^{2+} channels, which were involved in the regulation of cell cycle. After treatment with nifedipine (1, 10, and 50 μM) for up to 96 h, cell proliferation was measured by adding WST-1 reagent. The cell proliferation of VSMC was significantly inhibited by treatment with nifedipine in a dose-dependent manner. However, exposure of VSMC to high KCl (90 mM) also significantly inhibited the cell proliferation. When β subunit of L-type Ca^{2+} channel was constitutively expressed under the control by tetracycline in the Flp-In™T-REx™-293 cell line (derived from 293 human embryonic kidney cells), the β subunit protein was only localized in the nucleus. Microarray analysis demonstrated that 35 genes were significantly upregulated in the β subunit-expressing cells. These results suggest that the regulation of cell proliferation of VSMC might be determined by the specific pathway of Ca^{2+} entry and that the β subunit in nucleus plays an important role by regulating many genes that involves cytosolic Ca^{2+} homeostasis. (COI:No)

1P-019

Rectified proton permeation across a single-file water-chain in the channel pore

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Proton channels provide a narrow pore, in which water molecules are aligned in single-file, and proton jumps across a hydrogen-bonded water-chain. In bulk water, proton jumps across the hydrogen-bonded network of water (the three-dimensional Grotthuss mechanism), whereas the one-dimensional Grotthuss conduction still remains elusive. We exploited a narrow pore of the peptide channel, polytheonamide B (pTB), as a "test tube" to examine the proton conduction. pTB was incorporated into planar lipid bilayers, and single-channel currents were measured in various proton concentrations and membrane potentials. We found surprisingly that the *I-V* curves were asymmetric or inward rectified in symmetric proton concentrations, which has never been found for the proton channels. At lower proton concentrations, the degree of rectification was attenuated, but by adding high concentration buffer that supplies protons near the entrance, the rectification appeared. These results indicate that the rectification is yielded inside the pore rather than diffusion limitation at the pore entrance. The proton permeation process was modeled, having elementary processes of the proton jump across the hydrogen-bonded chain (hop) and the subsequent turnover of rearranged water molecules (turn). We found that the rectification is generated in the turn process such that the rate of turn is facilitated at negative potentials. This finding helps to understand proton permeation mechanism of proton channels. (COI:No)

1P-020

Subcellular localization and trafficking of voltage-gated proton channels in primary cultured microglia

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Voltage-sensor domain only protein, VSOP/Hv1, consists of voltage sensor domain lacking pore domain and functions as voltage-gated proton channels (Sasaki et al, Science. 2006). VSOP is mainly expressed in phagocytes and has a crucial role in the production of reactive oxygen species (ROS). In the previous experiments, we found numerous small VSOP-containing vesicles of primary cultured microglia by immunocytochemistry. In order to understand the machinery of vesicular trafficking of VSOP proteins in primary cultured microglia, we generated transgenic mice which expresses VSOP-EGFP fusion protein under CAG promoter activity. Time-lapse imaging experiments revealed that VSOP-EGFP vesicles show active movements inside the cell. While nocodazol which disrupts microtubules did not affect the vesicular motility, cytochalasin D, an actin polymerization inhibitor, completely inhibited it. Thus, it suggests that VSOP vesicular transport is dependent on actin dynamics. Furthermore, by using Structured Illumination Microscopy (SIM) we observed that VSOP-containing vesicles tightly associate with F-actin, supporting the idea that F-actin is required for the transport of VSOP-positive vesicles. In the present study, more detailed mechanism underlying the transport of VSOP-containing vesicles and its functional role is discussed. (COI:No)

1P-021

Voltage-gated proton channel in zebrafish (DrHv1): from culture dish to living animal

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Voltage-gated proton channel (Hv1) is a membrane protein that mediates the rapid movement of the proton across the cell membrane. The gene encoding Hv1, *hvcn1*, was widely identified throughout the animal kingdom, but their functions in the living organisms have remained indefinable. Here, we determine the functional roles of an Hv1 ortholog in zebrafish, *Danio rerio*, DrHv1. DrHv1 comprises 235 amino acids which share the similarity of molecular architecture among various species. Electrophysiological recording of DrHv1-expressed HEK293T cells shows the voltage-dependent proton conductance with the proton selectivity and pH-dependent gating. The activation kinetics of DrHv1 is similar to that of mouse Hv1; however, the current-voltage relationship indicates that DrHv1 requires lower membrane potential to be activated. Besides, while preserving the essential biophysical properties of Hv1, DrHv1 shows resistance to extracellular zinc ion (Zn^{2+}) than the mammalian orthologs due to the variation of zinc-coordinating residues at the linker between S3 and S4 transmembrane segments. We further identify the expression of DrHv1 in zebrafish neutrophils, suggesting the potential role of Hv1 in teleost phagocytes. CRISPR-Cas9-mediated genome editing of DrHv1 also shows successful results and the genetically modified DrHv1-deficient zebrafish can be used for the investigation of Hv1 *in vivo*. The study of DrHv1 spotlights the biological variation in different animal species and the potential of zebrafish as the animal model for studying the functional role of Hv1 in situ in the living organisms. (COI:No)

1P-022

Effects of gentamycin on postnatal closure of rat ductus arteriosus

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Background: The Ductus arteriosus (DA) is an essential artery that bypasses the main pulmonary artery and the descending aorta during a fetal period. DA closure sometimes delays in neonates with infection, although the DA immediately closes after birth. We reported that lipopolysaccharide-induced intrauterine infection delayed DA closure in neonatal rat. It was recently reported that the aminoglycoside antibiotic gentamycin caused patency of DA in neonatal rat with infections, although neonates with infections have to be treated with antibiotics. Aim: The aim of this study is to re-elucidate effects of gentamycin on postnatal closure of rat DA. Methods and Results: Neonates were delivered by caesarean section at term and were rapidly injected each regent into the peritoneal cavity. And then, the neonates were placed on the warm bed for 30 minutes and were rapidly frozen in liquid nitrogen. We measured the inner diameter of the DA, main pulmonary artery, and the aorta after we cut them in the frontal plane. Prostaglandin E_1, which is a potent vasodilator, significantly delayed rat DA closure compared to saline as previously reported. Gentamycin, which is frequently used in children, tended to delay rat DA closure in a dose-dependent manner, although there was no statistical significance. Conclusion: Gentamycin is not strongly associated with postnatal closure of rat DA. Further studies are needed in order to explore effects of antibiotics on postnatal closure of rat DA. (COI:No)

1P-023

Human gene analysis identified tissue plasminogen activator as a mediator of disrupting the internal elastic lamina in the ductus arteriosus

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Although intimal thickening (IT) is required to lead complete closure of the ductus arteriosus (DA), human gene profiling of IT has not been reported. We identified IT-specific genes using human DA and investigated the role in IT formation. Human DA tissues were obtained at surgery of congenital heart diseases and isolated to the tunica media and IT, which were subjected to DNA microarray analysis. 76 genes were expressed more than 3.0-fold in IT than in the tunica media (n=6, $p<0.05$). Among these genes, quantitative RT-PCR revealed that tissue plasminogen activator (PLAT) was highly expressed in endothelial cells (ECs) of the rat DA than aorta (2.5-fold, n=8, $p<0.05$). Immunohistochemistry showed that PLAT localized in IT of the human and rat DAs and up-regulated as IT development. PLAT converts plasminogen to plasmin, which induces matrix metalloproteinase (MMP) activation. When DA ECs were cultured with plasminogen, MMP-2 was markedly enhanced in DA ECs compared to aortic ECs (8.0-fold, n=4, $p<0.001$). In situ gelatin zymography showed that MMP activation was observed in the internal elastic lamina of the DA IT. By using an experimental 3D vascular models and organ culture, existence of plasminogen enhanced the MMP activity and disruption of the internal elastic lamina (n=4, $p<0.05$). These data suggest that PLAT may induce DA IT through disruption of the internal elastic lamina of the DA. Plasminogen administration may be a therapeutic strategy for patient DA by promoting IT formation. (COI:No)

1P-024

Determination of the elastic structures of connectin in vertebrate hearts with and without coronary circulation

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Vertebrate hearts are broadly classified into coronary circulation hearts and sinusoidal circulation hearts, and highly active and energy-consuming animals such as mammals and birds have coronary circulation hearts. Blood flow of coronary circulation occurs mostly during diastole because vascular compression limits flow during systole. Therefore, the coronary circulation hearts should have stiffer mechanical property to prevent excessive extensions of the heart during diastole resulting in a reduction of blood flow. Connectin is an elastic protein that regulates the extensibility of cardiac muscle by generating passive tension during diastole. To understand the extension restriction of coronary circulation hearts, we investigated the elastic region of connectin in chicken (bird), turtle (reptile), frog (amphibian) and zebrafish (fish) hearts and compared them to that in human (mammal) heart. We found the elastic regions of connectin were shorter in hearts with coronary circulation (human and chicken) than in hearts without coronary circulation (frog) or with coronary circulation that were only found outside of hearts (turtle and zebrafish). We also found that the elastic region of connectin in mammal hearts was shortened by different way from that of connectin in bird hearts. These results indicated that the shorter elastic regions of connectin may contribute to the extension restriction of coronary circulation hearts and the shortening of elastic region of connectin occurred independently in mammals and bird hearts. (COI:No)

1P-025

Novex-3 isoform of connectin/titin promotes proliferation of fetal cardiomyocytes in mice

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Mammalian cardiomyocytes lose their proliferative potential shortly after birth, and switch to hypertrophic growth afterward. We have previously shown that hypoxic fetal environments in utero are crucial for active cardiomyocyte division. In the present study, we surprisingly found that the short isoform of giant sarcomeric protein connectin/titin, known as novex-3, is localized in the nucleus of hypoxic fetal cardiomyocytes in mice. This nuclear localization appeared to be regulated by N-terminal nuclear localization signal of connectin gene which had been reported in earlier studies. Importantly, abundant nuclear expression of novex-3 in hypoxic fetal cardiomyocytes was sharply repressed both by oxygen exposure, and in postnatal cardiomyocytes when oxygen tension elevates by the onset of breathing. Moreover, novex-3 knockdown repressed cell cycle-promoting genes and proliferative activities of hypoxic fetal cardiomyocytes. Collectively, we propose that novex-3 isoform of connectin, the function of which has been ill-defined to date, is a novel cell cycle promoter localized in the nucleus of hypoxic fetal cardiomyocytes in mice. (COI:No)

1P-026

L-type Ca^{2+} channel in neonatal and infant stages exhibits a higher sensitivity to inhibition by verapamil compared with that in child and adult stages

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In the clinical settings, intravenous administration of verapamil for tachyarrhythmias is contraindicated in neonates and infants, due to the perceived risk of hypotension or bradycardia. In the present study, we investigated the postnatal developmental changes in the sensitivity of L-type Ca^{2+} current ($I_{Ca,L}$) to three structurally different blockers (verapamil, nifedipine, diltiazem) using mouse heart model. Ventricular myocytes were enzymatically dissociated from the heart of postnatal days 0, 7, 14, 21, 28 and adult (10-15 weeks) mice using Langendorff-perfusion methods. Whole-cell patch-clamp technique was applied to ventricular myocytes to record $I_{Ca,L}$ and to examine the sensitivity of $I_{Ca,L}$ to inhibition by three $I_{Ca,L}$ blockers. IC_{50} for verapamil was significantly smaller in day-0, 7, 14, 21, compared with day-28 and adult ventricular myocytes. On the other hand, there were no significant differences in IC_{50} for nifedipine or diltiazem in all postnatal ages. Next, we compared frequency-dependent blocking action of verapamil in day-7 with that in adult ventricular myocytes, using half-maximal concentration in each stage. There were no significant differences of $I_{Ca,L}$ obtained at the tenth pulse in day-7 (verapamil 50 nM) or adult (verapamil 150 nM) ventricular myocytes. $I_{Ca,L}$ in neonatal and infant stages exhibits a higher sensitivity to inhibition by verapamil compared with that in child and adult stages, which may contribute to the development of verapamil-induced hypotension in neonates and infants. (COI:No)

1P-027

Simultaneous nano-imaging of sarcomere dynamics and local calcium in rat neonatal cardiomyocytes via expression of yellow Cameleon-Nano140 fusion α-actinin in Z-disks

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In the present study, we developed a novel experimental system for simultaneous nano-imaging of the dynamics of the intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) and single sarcomeres in the subcellular partition, via expression of a FRET-based yellow Cameleon-Nano140 (YC-Nano140) fused into α-actinin for the localization at Z-disks in primary-cultured rat neonatal cardiomyocytes. The fusion protein enabled quantitative analyses of local Ca^{2+} transient (CaT) and the ensuing sarcomere dynamics at low and high temperatures during spontaneous beating and at electric stimulation at 5 Hz at 37°C. Although $[Ca^{2+}]_i$ changes were synchronized along a myofibril, the averaging of SL along the myofibrils caused marked underestimation of the magnitude of SL displacement due to superpositioning of shortening / lengthening of individual sarcomeres at different timings. Local Ca^{2+} waves were observed between CaT, and induced local sarcomeric contractions. There was a positive correlation between an increase in local $[Ca^{2+}]_i$ and the magnitude of sarcomere shortening. We conclude that the present experimental system has a broad range of application possibilities for unveiling molecular mechanisms of EC coupling in cardiomyocytes at the single sarcomere level in health and disease. (COI:No)

1P-028

Pathophysiological contribution of endothelial TRPM7 channel to pulmonary arterial hypertension

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Pulmonary arterial hypertension (PAH) comprises a multifactorial group of pulmonary vascular disorders that frequently lead to right heart failure and premature death. The pathogenesis of PAH involves complex remodeling processes in the endothelial cells. In this study, we explored a role of a mechanically-activated cation channel TRPM7 in these processes.Immunocytochemistry indicated that a TRPM7 antagonist FTY720 and cordyceps sinensis suppress TGF-β2-induced stress fiber formation. In immunoblot analyses using mesenchymal markers: N-cadherin and α-SMA and endothelial Markers: VE-cadherin and CD31, FTY-720 and siRNA knockdown of TRPM7 significantly suppressed TGF-β2-induced endothelial-mesenchymal transition (EndoMT). Treatment with cordyceps sinensis of monocrotarine-induced PAH rats ameliorated the development of pulmonary artery thickening, right ventricle hypertrophy and cardiac fibrosis. Moreover, activation of TRPM7-mediated signal transduction was detected in endothelial remodeling areas as well as plexiform lesions in the lung tissue from PAH patients.These results suggest that TRPM7 may contribute to an EndoMT process in vascular endothelial remodeling, and could thus be the novel targets of anti-remodeling therapy against some cardiovascular diseases. (COI:No)

1P-029

Additive/synergistic inhibitory effects of calcilytics with PDE5 inhibitor on excessive cell proliferation in idiopathic pulmonary arterial hypertension

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Pulmonary arterial hypertension is pathologically characterized by pulmonary vascular remodeling due to enhanced proliferation and reduced apoptosis of pulmonary arterial smooth muscle cells (PASMCs). Among PAH, idiopathic pulmonary arterial hypertension (IPAH) is a progressive and fatal disease of the pulmonary artery resulting from currently unidentified etiology. Phosphodiesterase type 5 (PDE5) inhibitors have been clinically used in the treatment of IPAH. Recently, we have shown that Ca^{2+}-sensing receptor (CaSR) antagonists (calcilytics) inhibit excessive cell proliferation of PASMCs from IPAH patients. In this study, the additive/synergistic effect of calcilytics on antiproliferation following PDE5 inhibition was examined in PASMCs from normal subjects and IPAH patients by MTT and BrdU incorporation assays. Sildenafil blocked the excessive cell proliferation of IPAH-PASMCs in a concentration-dependent manner. However, sildenafil did not affect the cell growth of normal-PASMCs. NPS2143 additively enhanced the antiproliferative effect induced by sildenafil in IPAH-PASMCs. Additionally, the inhibitory effect of NPS2143 or Calhex 231 on excessive cell proliferation of IPAH-PASMCs was synergistic increased in the presence of sildenafil. These findings reveal that calcilytics additively/synergistically enhance the antiproliferative activity mediated by PDE5 inhibition. In conclusion, a combination therapy of a PDE5 inhibitor with a calcilytic may be useful as a novel therapeutic approach for IPAH. (COI:No)

1P-030

Pulmonary hypertension due to left heart disease caused intrapulmonary venous arterialization in rats

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[Objective] A model of left atrial stenosis (LAS)-associated pulmonary hypertension due to left heart diseases (PH-LHD) was prepared and its mechanism was elucidated. [Methods] Five-week-old Sprague-Dawley rats were randomly divided into 2 groups (LAS and sham-operated control (SOC) groups). Echocardiography was performed at 2, 4, 6, and 10 weeks after surgery, and catheterization was performed and the organ was excised at 10 weeks after surgery. [Results] Left ventricular inflow velocity measured by echocardiography significantly increased in the LAS group compared with that in the SOC group, and the RV pressure/LV pressure ratio significantly rose in the LAS group compared with those in the SOC group. In addition, the right ventricular weight was significantly heavier in the LAS group than SOC group. On pathological examination, not only medial hypertrophy of the pulmonary artery, but also the arterialization of pulmonary vein, was noted. Increases in TGF-β signal and endothelin-1 were noted on microarray analysis, real-time PCR analyses, and Western blotting, suggesting involvement of endothelin-1 in arterialization of the pulmonary vein. [Conclusion] We succeeded in establishing a novel, reliable rat model of PH-LHD by generating LAS. Although PH was relatively moderate, the PH-LHD model rats demonstrated the characteristic intrapulmonary venous arterialization. (COI:No)

1P-031

Fluid shear stress enhances exocytosis of tissue-type plasminogen activator (t-PA) in vascular endothelial cells (VECs)

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Background: Fluid shear stress (FSS) has been demonstrated to alter the membrane polarity in VEC. Secretion of t-PA, an essential function of VECs for keeping fluidity of blood flow, is suggested to be influenced after exposure of FSS, though the precise mechanisms have not been elucidated. Purpose: We analyzed the effect of FSS on t-PA secretion and assessed underlying mechanism using cultured VECs. Methods and Results: 1) We introduced VECs in a collagen-coated microchip and applied FSS to the cells. The concentration of t-PA determined by ELISA transiently increased in the effluent solution after application of FSS. 2) GFP-fused t-PA (tPA-GFP) expressing cells were applied to FSS and exocytosis of tPA-GFP containing secretory granules was visualized by a total internal reflection fluorescence microscopy. The frequency of exocytotic events of tPA-GFP secretory granules (tPA-F) was significantly increased after application of FSS. 3) Treatment of cells with methyl-beta-cyclodextrin (MbCD), known to deplete cholesterol from plasma membrane, decreased the liquid-ordered phase of the plasma membrane as was demonstrated by fluorescence changes in membrane phase fluorescent probe, and significantly increased tPA-F, 4) The increases in tPA-F after either the addition of MbCD or the application of FSS were canceled by supplementation with cholesterol. Conclusion: FSS enhanced t-PA exocytosis, which appeared to depend on the cell membrane polarity, at least in part. (COI:No)

1P-032

Involvement of Rho-Rho kinase pathway in peripheral actin filament formation, an initial event during thrombin-induced endothelial barrier disruption

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We have reported that di-phosphorylation of myosin light chain (ppMLC) and actin filament formation at the cell periphery play initial events that proceed the stress fiber formation during the thrombin-induced endothelial barrier disruption (Sci Rep 6: 20989, 2016). Here we investigated the involvement of Rho-Rho kinase pathway in the peripheral actin filament formation induced by thrombin in porcine aortic endothelial cells. For this purpose, the effects of simvastatin and Y27632 on the actin filament formation were investigated. Both ppMLC and actin filament were scarcely observed before thrombin stimulation by immunofluorescence staining. Thrombin (1 unit/mL) induced a sustained decrease in transendothelial electrical resistance, which reached a peak at 3-5 min after stimulation, when ppMLC and actin filament formation were observed at the cell periphery. Pretreatment with 10μM simvastatin for 16-20 hr slightly decreased the actin filaments seen before thrombin stimulation, and significantly inhibited the thrombin-induced increase in ppMLC and formation of actin filaments at the cell periphery. Y27632 also abolished the thrombin-induced increase in ppMLC and the peripheral actin filament formation. These results suggest that the Rho-Rho kinase pathway is involved in the peripheral actin filament formation in the initial phase of the thrombin-induced endothelial barrier disruption. (COI:No)

1P-033

The role of adenosine triphosphate on VEGF mediated signaling

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Hypoxia induces angiogenesis through expression of hypoxia-inducible molecules, including vascular endothelial growth factor (VEGF). However, VEGF mediated signal under hypoxia is not clearly determined. We hypothesized that metabolic change under hypoxia influence signal formation of VEGF, and focused on a role of adenosine triphosphate (ATP) on VEGF-mediated signal alteration under hypoxia (1 % O_2).Exposure of cells to hypoxia decreased ATP concentration (65.93 ± 8.32 % of normoxia; mean ±SE; $p<0.01$) in HUVECs. VEGF-induced phosphorylation of VEGFR-2 and downstream molecules were significantly inhibited without change of VEGFR-2 expression (3h), which was associated with reduction of ATP level in cell. Inhibitor for ATP production, such as 2-deoxyglucose or antimycin-A, decreased ATP concentration in cells, and inhibited VEGF-induced phosphorylation of VEGFR-2 under normoxia. In contrast, hypoxia did not influence TGF-β1 meditated phosphorylation of SMAD2 and SMAD3. Interestingly, inhibition of VEGFR-2 phosphorylation in the prepared membrane from 3h hypoxia treated cells was fully recovered by supplementation of ATP. After 12h hypoxia, VEGF mediated phosphorylation of VEGFR-2 was inhibited with decreased of VEGFR-2 expression. Whereas, in the prepared membrane, inhibition of VEGFR-2 phosphorylation was not fully recovered by ATP, suggesting long-term hypoxia might modify receptors activation. This study demonstrates that ATP is an important regulator for VEGF mediated-signal under hypoxia. (COI:No)

1P-034

Activator of G-protein signaling 8 is involved in VEGF-induced choroidal angiogenesis

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Heterotrimeric G-proteins regulate multiple physiological and pathological events. Activator of G-protein signaling 8 (AGS8) was previously identified regulatory protein for heterotrimeric G-proteins from the heart of rat angina model. We recently reported that AGS8 was involved in VEGF mediated angiogenesis in cultured endothelial cells, however, role of AGS8 in vivo was not known. Here, we focus on a role of AGS8 on ocular angiogenesis. At first, tube formation assay was performed with cultured choroidal endothelial cells after AGS8 knockdown, which results in inhibition of VEGF-induced tube formation. Then, the laser coagulation induced choroidal neovascularization, known as a model of aged-related macular degeneration, was tested in the murine. Real-time PCR demonstrated that AGS8 mRNA was upregulated [238.3 ± 24.2 %] in choroidal lesion, and immunostaining indicated AGS8 expression was specific in newly formed vessels. Injection of AGS8 siRNA into murine vitreous successfully decreased AGS8 mRNA [54.0 ± 18.2 %] and inhibited choroidal angiogenesis. These results indicated that AGS8 play critical roles on ocular angiogenesis, specifically in choroidal angiogenesis in vivo as well as in vitro. (COI:No)

1P-035

Activator of G-protein signaling 8 regulates VEGFC induced lymphangiogenesis in human dermal lymphatic endothelial cells

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Cellular signal mediated by heterotrimeric G-protein is involved in the development of vascular diseases. Activator of G-protein signaling 8 (AGS8) was identified as a receptor-independent G-protein regulatory protein which bound G-protein βγ subunit. Recently, a role of AGS8 in angiogenesis has been characterized. Thus, AGS8-Gβ γ regulated the subcellular distribution of vascular endothelial growth factor receptor-2 (VEGFR-2) and influenced VEGF-induced signaling for angiogenesis. VEGFR-3, one of the VEGF receptor family, mediates VEGF signal in lymphatic endothelial cells, however a role of AGS8 in lymphangiogenesis is not identified. Here, we demonstrate that AGS8 is involved in VEGFC-induced signaling in human dermal lymphatic endothelial cells (HDLECs). Knockdown of AGS8 by siRNA inhibited VEGF-induced tube formation, cell proliferation, and cell migration. VEGFC stimulated the phosphorylation of the VEGFR-3, ERK1/2 and AKT, however, AGS8 knockdown inhibited these signaling events. Fluorescence-activated cell sorter analysis and immunofluorescence staining indicated that VEGFR-3 in the cell-surface was decreased following knockdown of AGS8, suggesting a role of AGS8 in trafficking of VEGFR-3 to the membrane. These data indicated the involvement of AGS8 in VEGFC-VEGFR-3 signaling, and in the lymphangiogenesis. (COI:No)

1P-036

Mechanisms of spontaneous Ca^{2+} transients in the mural cells of rat rectal arterioles

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Background: The mural cells of venules in hollow organs such as bladder, stomach or colon exhibit spontaneous Ca^{2+} transients resulting in spontaneous venular constrictions that may prevent blood stagnation during organ wall distension. On the other hand, the properties of spontaneous activity of fine arterioles are less understood. Methods: Cal-520 Ca^{2+} imaging was carried out to visualise spontaneous Ca^{2+} dynamics in the arteriolar mural cells of rat rectal submucosa. Results: The arteriolar mural cells (pericytes or vascular smooth muscle cells) had a round cell body extending several processes and developed rhythmic spontaneous Ca^{2+} transients arising from Ca^{2+} release from intracellular Ca^{2+} store. The synchrony of Ca^{2+} transients amongst mural cells was disrupted by carbenoxolone (3 μM), a gap junction blocker, DIDS (100 μM), a Ca^{2+}-activated Cl^- channel (CaCC) blocker, or lowering extracellular Cl^- concentration (from 134.4 mM to 12.4 mM). Blockers for T-type (1 μM mibefradil) or L-type (1 μM nifedipine) voltage-dependent Ca^{2+} channels slowed spontaneous Ca^{2+} transient or reduced their area under curve (AUC), respectively, without disrupting synchrony of Ca^{2+} transients. This is in contrast with rectal venules where nifedipine disrupted the synchrony of spontaneous Ca^{2+} transients. Conclusion: The spread of spontaneous depolarisation due to the opening of CaCCs via gap junctions appears to be sufficient to electrically couple arteriolar mural cells. Sequential activation of T-type and L-type Ca^{2+} channels accelerates and prolongs spontaneous Ca^{2+} transients, respectively. (COI:No)

1P-037

Paxillin as a novel signaling molecule regulates actin stress fiber formation and migration of vascular smooth muscle cells by its N-terminus binding to the active Fyn

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Rho-kinase (ROK)-mediated actin stress fiber formation and migration of vascular smooth muscle cells (VSMCs) plays important roles in progression of atherosclerosis and post-angioplasty restenosis. As an upstream molecule of ROK in such pathological pathway, we previously identified Fyn tyrosine kinase. However, the molecular mechanisms between the two kinases have not been clarified. Here, we identified paxillin as a novel downstream signaling molecule of the active Fyn by combined use of pulldown assay and tandem mass spectrometry. To clarify binding site of the active Fyn on paxillin, the direct interaction between recombinant Fyn and the full-length and fragments of paxillin were analyzed by surface plasmon resonance, and we determined the constitutively active Fyn (CA-Fyn) directly binds to N-terminus of paxillin. Colocalization of CA-Fyn and paxillin during the stress fiber formation in VSMCs further confirmed their binding. In addition, the knockdown of paxillin and the overexpression of N-terminus, but not C-terminus, of paxillin inhibited ROK activation, the actin stress fiber formation and migration of VSMCs. Re-expression of paxillin rescued the actin stress fiber formation and migration in paxillin knockdown VSMCs. These results demonstrate for the first time that paxillin is a novel downstream mediator of Fyn in migration of VSMCs and may be a new therapeutic target for atherosclerosis and post-angioplasty restenosis. (COI: No)

1P-038

A novel angiogenic agent COA-Cl promotes VEGF secretion from mouse C2C12 skeletal myocytes via the activation of cAMP/PKA/CREB/PGC-1α signaling axis

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We have recently reported that the angiogenic potency of an adenosine-like agent COA-Cl depends on the promotion of VEGF secretion that is mediated by the induction of a transcriptional coactivator PGC-1α in cultured human fibroblasts. In skeletal muscle, most of VEGF derives from skeletal myocytes rather than fibroblasts, and, a signaling cascade comprising cAMP/PKA/CREB regulates PGC-1α expression. Here, we explored whether or not and how COA-Cl promotes VEGF secretion from skeletal myocytes using mouse C2C12 cells as an in vitro model. Treatment with COA-Cl increased mRNA expression of both PGC-1α and VEGF, and the amount of VEGF protein secretion. COA-Cl increased the level of cAMP and phosphorylation of CREB at S133 (a PKA site). The COA-Cl-induced increases in PGC-1α and VEGF mRNA expression were attenuated by KT5720, a PKA inhibitor. Conversely, forskolin, an activator of adenylate cyclase, as well as 8-CPT-cAMP, a cell-permeable cAMP analogue, mimicked the effects of COA-Cl in inducing PGC-1α and VEGF mRNA. These data indicate that COA-Cl promotes VEGF secretion from mouse C2C12 skeletal myocytes via the activation of cAMP/PKA/CREB/PGC-1α signaling axis. (COI:No)

1P-039

Hyperplastic adipocytes in aortic wall can cause abdominal aortic aneurysm rupture

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Abdominal aortic aneurysm (AAA) is a common disease among the elderly. However, the mechanism of AAA rupture remain unknown. In this study, we used matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) to analyze the human AAA tissues and determine the mechanisms underlying human AAA. MALDI-IMS revealed abnormal accumulation of triglycerides (TG) in hyperplastic adipocytes, particularly in the adventitia. In histological aspect, the adipocytes were found to express pro-inflammatory cytokines and MMPs, suggesting that the adipocytes may be associated with the vulnerability of the aortic wall. Furthermore, we investigated the mechanism of AAA rupture using a hypoperfusion-induced animal model. We found that the administration of TG species increased the AAA rupture rate in the animal model and that hyperplastic adipocytes accumulated in ruptured aortic walls compared to non-ruptured walls. In the ruptured group, macrophage infiltration and the MMPs activity were increased in the areas around adipocytes. These results suggest that adventitial adipocytes were found to express pro-inflammatory cytokines and MMPs which suggested that hyperplastic adipocyte in the adventitia can be associated with AAA rupture subsequent to the vulnerability of the aortic wall. (COI:No)

1P-040

An EP4 antagonist has inhibitory and therapeutic effects on mouse models of abdominal aortic aneurysm

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Abdominal aortic aneurysm (AAA) is a chronic disease with inflammation and disruption of vascular smooth muscle cell layer. Our previous report demonstrated that the prostaglandin E receptor EP4 plays a major role in AAA progression and hence a potential target for treatment. In the present study, we investigated the effect of CJ-042794 (CJ), an EP4 receptor antagonist, on AAA using two different mouse models, i.e., angiotensin II- and CaCl2-induced AAA (AngII/AAA and CaCl2/AAA). To examine inhibitory effect on AAA, CJ (0.2 mg/kg/day) was orally administered together with AngII-infusion (1 μg/kg/min) for 4 weeks in ApoE-/- mice, and we found that CJ significantly inhibited AAA progression (aortic diameter: 0.7 ± 0.06-fold vs. saline control, n=6, $p<0.05$). We also investigated therapeutic effect of CJ on CaCl2/AAA in wild type mice. Oral administration of CJ started 2 weeks after periaortic CaCl2 application in which AAA was fully progressed, and continued for 4 weeks. Aortic diameter was significantly smaller in CJ group than in saline control group (0.8 ± 0.06-fold vs. control, n=6, $p<0.05$). In these two experimental AAA models, CJ significantly decreased MMP2 activity 0.2 ± 0.06- and 0.3 ± 0.02-fold (vs. saline control, n=5-6, $p<0.01$), respectively. Histological analysis using elastica van Gieson stain showed that disruption of elastic laminae was attenuated by CJ in the two AAA models. These results suggest that the EP4 antagonist CJ has protective and therapeutic effects in AAA. (COI: No)

1P-041

Role of TRPV4 in regulating spontaneous constriction of the mesenteric lymphatic vessels

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Collecting lymphatic vessels exhibit rhythmic spontaneous contractile activity. A previous study showed that the lymphatic vessel constrictions are facilitated upon a rise in the luminal pressure. In the present study, the involvement of TRPV4 channels, a stretch-activated cation channels, in the mechanical activation of lymphatic vessels - constrictions was investigated. Lymphatic vessels were isolated from the guinea pig and pinned in the organ bath to measure changes in their diameter by Diamtrak system and the membrane potential by the conventional microelectrode method. The lymphatic vessels exhibited spontaneous constriction and electrical activity. GSK1016790A, a TRPV4 channel agonist, increased the frequency of spontaneous contractions with a small reduction in the basal diameter. It also increased the frequency of spontaneous electrical oscillations of the membrane potential. HC067047, a TRPV4 channel antagonist, itself did not have significant effects on the frequency or the baseline, but inhibited the actions of GSK1016740A. The excitatory effects of GSK1016740A on the frequency were inhibited by ML218, a T-type voltage-dependent Ca^{2+} channel (TVDCC) blocker. These results suggested that the opening of TRPV4 channels may results in depolarization as well as intracellular Ca^{2+} rise to activate TVDCC. We conclude that TRPV4 activation may play a role in regulating lymphatic spontaneous constriction subsequent to an elevation of luminal pressure. (COI:No)

1P-042

Hypotensive effects of glucagon-like peptide-2 by the central administration: comparison of the effects in spontaneously hypertensive rats and Wistar-Kyoto rats

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The central administration of glucagon-like peptide-2 (GLP-2) decreases blood pressure in rats. In the present study, we compared the effects in SHRs and Wistar-Kyoto (WKY) rats.The central administration of GLP-2 (0.6 μg) decreased mean arterial pressure (MAP) in SHRs (-24.1 ± 4.5 %; $P < 0.05$), but not in WKY rats (-10.6 ± 7.4 %; $P > 0.05$), whereas GLP-2 (6 μg) decreased MAP in WKY rats (-23.5 ± 4.2 %; $P < 0.05$) and SHRs (-46.7 ± 11.6 %; $P < 0.01$) under anesthesia with urethane and α-chloralose. Histological analyses revealed that the central administration of GLP-2 (6 μg) induced Fos immunoreactivity (Fos-IR) in the hypothalamic and medullary areas in WKY rats and SHRs. However, the distribution of Fos-IR in catecholaminergic and GABAergic neurons differed between WKY rats and SHRs in the rostral ventrolateral medulla (RVLM). These results suggest that neuronal activity through the activation of GLP-2 receptors in the RVLM contributes to lowering blood pressure in SHRs. (COI:No)

1P-043

Resuscitation by prehospital transfusion with hemoglobin vesicles in trauma induced hemorrhagic shock / coagulopathy rabbits

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Background: We have developed Hemoglobin vesicle (HbV) as an artificial substitute for Red blood cells (RBC), and evaluated the efficacy of HbV treatment during prehospital period in traumatic hemorrhagic shock / coagulopathy rabbits.Methods: Hemorrhagic shock / thrombocytopenia were induced in rabbits by repeated blood withdrawal and isovolemic transfusion of RBC. Liver penetrating injury led lethal oozing hemorrhage. Prehospital phase: Uncontrolled hemorrhage was compensated by isovolemic transfusion of RBC transfusion (n=8), HbV (n=8) or Platelet poor plasma (PPP) (n=5) for initial 30 minutes. In-hospital phase: Platelet rich plasma (PRP) administration stopped bleeding. Subsequently, final RBC transfusion (n=16) or PPP (n=5) was administered. Acute prognosis was compared among them for 24 hr.Results: In thrombocytopenic rabbits (platelets < 50,000 / μL), liver penetrating injury caused uncontrolled bleeding, then PRP administration restored platelets and stopped bleeding within 40 min. At this point, dilutional anemia occurred as severe as class IV shock (Hb 6.0 g/dl, MAP approximately 50 mmHg). Prehospital administration of HbV as well as RBC transfusion regained MAP more than 55 mmHg, and they rescued more than 80 % animals, although rabbits receiving PPP showed 0% survival.Conclusions: HbV may be effective initial resuscitation fluids for acute hemorrhagic shock with trauma induced coagulopathy. (COI:No)

1P-044

Neonatal sevoflurane-induced impairment in the cognitive behavior of mice depends on the functional NKCC1 expression

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Sevoflurane, a widely used anesthetic, affects $GABA_A$ receptor-mediated tonic inhibition. Long-term impairment in cognitive behavior has been shown to emerge after the exposure to sevoflurane at an early age. However, the mechanisms underlying the sevoflurane-induced cognitive impairment are still unclear. In the immature brain, NKCC1 expression determines high $[Cl^-]_i$, promoting depolarizing GABA response. During the developmental maturation, NKCC1 expression decreases, resulting in hyperpolarizing GABA response. We observed the effects of the developmental maturation and bumetanide, a NKCC1 inhibitor, on the sevoflurane-induced cognitive impairment. Neonatal mice (3-5 and 19-21 days old) received an intraperitoneal injection of bumetanide or saline. Just after the injection, the mice were exposed to 2.1% sevoflurane in O_2 for 4 hrs. At 49 days of age, these mice were examined with behavioral tests, water maze, open field and prepulse inhibition. Unlike control mice, mice exposed to sevoflurane at P3-5 showed impaired water maze performance, traveling distance, and vertical movement in the open field arena as well as the inhibition of startle response by prepulse. Mice exposed to sevoflurane at P19-21 showed behaviors similar to those of the control mice. Mice exposed to sevoflurane at P3-5 with bumetanide pre-injection also showed behavior similar to those of the control mice. Our results suggest that the effects of neonatal sevoflurane exposure on cognitive behaviors depend on the functional NKCC1 expression. (COI:No)

1P-045

Enhancement of NMDA receptor-mediated synaptic transmission and nociceptive behaviour in serine rasemase knockout mice following spinal nerve injury

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D-serine has been reported to play an important role in the central nerves system (CNS). D-serine is converted from L-serine by serine racemase (SR). To clarify the contribution of SR to synaptic transmission in the CNS, we generated knockout (KO) mice lacking the gene for the SR. D-serine modulates N-methyl-D-aspartate (NMDA) receptor-mediated synaptic transmission as a coagonist of glycine binding site. The activation of NMDA receptors modulates synaptic transmission in the spinal superficial dorsal horn (SDH). To investigate the effect of D-serine in the neuropathic pain, NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) were recorded from neurons in the SDH in the spinal nerve ligated (SNL) mice. The ratio of NMDA/non-NMDA receptor-mediated EPSCs was smaller in SR-KO mice than in wild type (WT) mice. In contrast, the time decay course and the charge transfer of the NMDA component of EPSCs were slower and larger in SR-KO mice than in WT mice, respectively. Real-time RT-PCR analysis indicated that the expression level of the NR2B subunit of NMDA receptors was increased in SR-KO mice. SNL-induced nociceptive behavior, which is medicated by NMDA receptors in the spinal SDH, was enhanced in SR-KO mice. These results imply the possibility that the activity of SR modulates the neuropathic pain by changing the subunit composition of NMDA receptors. (COI:No)

1P-046

Melicope ptelefolia aqueous extract exerts analgesic effects in an animal model of neuropathic pain

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Neuropathic pain is caused by injury or diseases affecting the nervous system and characterised by symptoms such as allodynia and hyperalgesia which are difficult to treat. Hence, *Melicope ptelifolia* previously reported to have anti-inflammatory and antinociceptive effects, could be the plausible cure. Male ICR mice were divided into six groups: sham, vehicle, MPAE (50, 100 and 200 mg/kg) and amitriptyline. Neuropathic pain was induced by chronic constriction injury (CCI) on sciatic nerve. Cold plate and Randall-Sellitto test were used to measure the pain responses on days before and after treatment. Mice were sacrificed to collect brains for analysis of *c-fos* expression. Data were analysed using one-way ANOVA followed by Tukey's test with $p<0.05$ is significant. Single and repeated treatments of MPAE significantly alleviated cold allodynia and mechanical hyperalgesia. Meanwhile, analysis of *c-fos* expression in cingulate cortex region of the brain shows no significant differences among the groups. MPAE possess anti-allodynic and antihyperalgesic effects in neuropathic pain mice. (COI: No)

1P-047

Noradrenaline inhibits TRPV1 through α_2 adrenergic receptors in rat dorsal root ganglion neurons

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Adrenergic neurons are known to modulate the nociception. The most major neurotransmitter of the descending antinociceptive system is noradrenaline (NA). However, mechanisms of pain regulation by adrenergic neurons in peripheral tissues are ill studied. In this study, we have examined effects of NA on TRPV1 activated by capsaicin in rat dorsal root ganglion (DRG) neurons by the whole-cell voltage-clamp recordings. (1) DRG neurons were held at -60 mV and inward current responses to capsaicin (1 μM) were recorded. NA inhibited capsaicin-evoked currents dose-dependently. The maximal inhibition was 84% and observed by 10^{-13} M NA. (2) Inclusion of GDPβS, a non-hydrolysable GDP analog, in the intracellular solution or pretreatment of neurons with pertussis toxin, which inactivates $G_{i/o}$-protein, abolished the inhibition of the capsaicin response by NA (10^{-12} M). (3) Yohimbin, an α_2 receptor antagonist, or propranolol, a β receptor antagonist, reduced the inhibition. (4)Clonidine, an α_2 receptor agonist, also inhibited the capsaicin response and yohimbin abolished this effect of clonidine completely. These results suggest that the activation of TRPV1 is inhibited by NA and α_2 receptor is the strongest candidate involved in this effect. Further studies are required to clarify detailed intracellular mechanisms of inhibition of TRPV1 by adrenergic receptors. NA modulates the TRPV1 activity and this mechanism may contribute to the pain regulation on the peripheral sensory nerve under the skin. (COI:No)

1P-048

Resveratrol attenuates inflammation-induced hyperexcitability of rat trigeminal spinal nucleus caudalis neurons associated with hyperalgesia

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The aim of the present study was to determine whether daily systemic administration of resveratrol to rats attenuates the inflammation-induced hyperexcitability of trigeminal spinal nucleus caudalis (SpVc) wide-dynamic range (WDR) neurons associated with hyperalgesia. Inflammation was induced by injection of CFA into the whisker pad. The threshold of escape from mechanical stimulation applied to the orofacial area in inflamed rats was significantly lower than in control rats. The decreased mechanical threshold in inflamed rats was restored to control levels by daily systemic administration of resveratrol. The mean discharge frequency of SpVc WDR neurons to both non-noxious and noxious mechanical stimuli in inflamed rats was significantly decreased after resveratrol administration. In addition, the increased mean spontaneous discharge of SpVc WDR neurons in inflamed rats was significantly decreased after resveratrol administration.These results suggest that chronic administration of resveratrol attenuates inflammation-induced mechanical inflammatory hyperalgesia and that this effect is due primarily to the suppression of SpVc WDR neuron hyperexcitability via inhibition of both peripheral and central cyclooxygenase cascade signaling pathways. These findings support the idea of resveratrol as a potential complementary and alternative medicine for the treatment of trigeminal inflammatory hyperalgesia without side effects. (COI:Properly Declared)

1P-049

Spinal NO production visualized during hindpaw ischemia and NO-induced spinal potentiation in mice

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We have reported that bilateral spinal and cortical responses were potentiated during and after the ischemic treatment applied only to the left hindpaw in mice. In the present study, we investigated a possible role of nitric oxide (NO), one of the diffusible mediators that may induce neuropathic pain in a wide area of the spinal cord. We confirmed that NO donor applied to the intrathecal space alone potentiated the cortical responses. To investigate the effects of NO application at the spinal cord, we used von Frey test. Mechanical allodynia was induced after the spinal application of NO donor. The potentiation observed at the spinal cord and the cortex induced by hindpaw ischemia was clearly suppressed after spinal application of L-NAME, an inhibitor of NO synthase (NOS). Furthermore, no potentiation was observed in neural NOS knock out mice. To visualize NO formation during hindpaw ischemia in the spinal cord, we used DAF-FM, a fluorescent NO indicator. We found that an increase of fluorescence derived from the DAF-FM and NO complex was found at the ischemic side. A similar but slightly smaller increase was also observed at the contralateral side. These results clearly indicated the roles of NO production in the spinal and cortical potentiation. Similar mechanisms may also be important in the initial phase of neuropathic pain that is sometimes observed in a wider area beyond injured sites. (COI:No)

1P-050

Enhancement by orexin A and B of spontaneous excitatory transmission in adult rat spinal substantia gelatinosa neurons

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Orexin A and B, neuropeptides originating from the hypothalamus, inhibit nociceptive transmission in the spinal dorsal horn. We have previously reported that orexin B produces an inward current at -70 mV and/or increases the frequency of glutamatergic spontaneous excitatory postsynaptic current (sEPSC) in about 40% of the adult rat spinal dorsal horn lamina II (substantia gelatinosa, SG) neurons examined. The SG neurons play a pivotal role in the regulation of nociceptive transmission from the periphery. To more know a role of orexin in regulating nociceptive transmission in the dorsal horn, we examined a detail of the effects of orexin A and B on spontaneous excitatory transmission by applying the blind whole-cell patch-clamp technique to the SG neurons of adult rat spinal cord slices. The inward current and sEPSC frequency increase produced by orexin B enhanced in extent with an increase in concentration; their EC_{50} values were 0.020 and 0.051 μM, respectively. The orexin B activities were inhibited by orexin-2 but not -1 receptor antagonist (JNJ10397049 and SB334867, respectively). As with orexin B, orexin A repeatedly produced an inward current at -70 mV and increased sEPSC frequency in a manner resistant to voltage-gated Na^+-channel blocker tetrodotoxin. The orexin A activity was also concentration-dependent; this efficacy appeared not to be different from that of orexin B. These results suggest that orexin A and B inhibit nociceptive transmission with almost the same efficacy. (COI:No)

1P-051

Identification of substance P-mediated depolarization in the superficial dorsal horn evoked by electrical stimulation of primary afferents

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Substance P (SP) plays a critical role in the development of neuropathic pain via the neurokinin-1 (NK-1) receptor. To clarify the effects of SP on nociceptive synaptic plasticity, we investigated SP-mediated depolarization in the superficial dorsal horn (SDH) using a voltage-sensitive dye imaging. Three-week-old male ICR mice anaesthetized with halothane were subjected to partial sciatic nerve ligation. Transverse slices (500 μm thick) with dorsal root attached were obtained from the lumbar enlargement of 4-week-old mice. The spinal slices were stained with the voltage-sensitive dye Di-4-ANEPPS, and the fluorescence was measured using the MiCAM2 optical imaging system. We analyzed changes in fluorescence intensity of a slow C-fiber response by single-pulse electrical stimulation of the dorsal root. In the presence of Str(1 μM), Bic (10 μM), CNQX (10 μM), and AP-V(50 μM), electrical stimulation elicited a slight but distinct, long-lasting change in fluorescence. This response was abolished by the administration of the NK-1 receptor antagonist CP99994 (1 μM). These findings indicate that the long-lasting depolarization response is a slow C-fiber response. Additionally, the μ-selective opioid agonist DAMGO (30 μM) suppressed the SP-mediated depolarization response. These optical imaging experiments might provide a useful tool for investigating the synaptic effects of SP in the SDH. (COI:No)

1P-052

Muscarinic inhibition of GABAergic transmission onto striatal cholinergic interneurons involves M1 receptor activation

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Striatal cholinergic interneurons receive GABAergic input from medium spiny neurons (MSNs) in the striatum. Several studies have revealed that GABAergic transmission onto MSNs 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 of the GABAergic transmission onto striatal cholinergic interneurons. To selectively activate MSNs in the striatum, we used transgenic mice which show restricted expression of channelrhodopsin-2 in the striatal medium spiny neurons. Acute coronal slices (300 μm) were obtained from these transgenic mice of either sex. Striatal cholinergic interneurons were identified by their large somata. GABAA receptor-dependent inhibitory postsynaptic currents (IPSCs) were evoked by blue light stimulation (470 nm) 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 μM. The amplitude of optically evoked IPSCs was inhibited by carbachol (to 50.5 ± 7.8% of baseline, n = 5). However, the carbachol-induced inhibition of IPSCs was antagonized by prior application of a muscarinic 1 receptor antagonist, pirenzepine (1 μM, to 94.8 ± 5.1% of baseline, n = 7). These results suggest that cholinergic modulation of the GABAergic transmission from MSNs onto cholinergic interneurons involve M1 receptors. (COI:No)

1P-053

Targeting ATP sensitive potassium channel(K_{ATP}) to tackle the pathogenesis and levodopa-induced dyskinesia associated with Parkinson's disease

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Parkinson's disease (PD) is a common neurodegenerative movement disease caused by the loss of dopamine (DA) neurons in the substantia nigra pars compacta and their terminals in thecaudate/putamen. Levodopa-induced dyskinesia (LID) is a symptom occurred in more than half of PD patients after few years of levodopa treatment which lead to worsening motor performance then affect the quality of life. It has been known that ATP-sensitive potassium (K_{ATP}) channels express in the central nervous system and play an important role in dopaminergic degeneration. We investigated the role of K_{ATP} channels participated in the progression of PD and LID. We established hemiparkinsonian model by 6-hydroxydopamine (6-OHDA) and treated with K_{ATP} opener or blocker to know whether K_{ATP} channels activity affect the severity of PD by behavioral test. We showed the increasing K_{ATP} channels expression lead to a worsening motor function in PD model and so did the result in LID model. Administration of K_{ATP} channel opener increased the expression of K_{ATP} channels and decreased dopaminergic neurons number in striatum and substantia nigra but reversed by K_{ATP} blocker suggesting manipulate K_{ATP} channel activity can reduce the PD and LID progression. Some evidences showed that K_{ATP} channels also expressed in microglia which had been proved to participate in the pathology of PD. Our study will focus on the role of microglia and neurons in PD and LID patients, taking forward to find out a new therapeutic strategy via manipulating K_{ATP} channel activity. (COI:No)

1P-054

Dopamine-dependent synaptic plasticity at IPSC of Substantia Nigra pars reticulate (SNr) GABA neurons in the slices from an acute Pakinson's model rat brain

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I had already reported that a high frecuency electrical stimulation on subthalamic nucleus (STN-HFS), which imitated the deep brain stimulation (DBS) on STN for a progressed patient of Parkinson's disease, induced no synaptic plasticity at the IPSC of SNr GABA neurons evoked by an electirical stimulaiton onto a putative "direct pathway" in the slices from an acute model rat brain of Parkinson's disease (reserpinized rat) in the ACSF with 100 μM α-methyl-L-tyrosine (AMPT), a dopamine sythesis inhibitor. However, IPSC-LTP was induced by STN-HFS for 20 minutes in ACSF with a D_1-receptor agonist (5 μM SKF38393 hydrochloride) in almost neurons tested (7 out of 8 neurons). This LTP was accompanied with the significant increase in the frequency of spontaneous IPSC (sIPSC), indicating the presynaptic mechanism of LTP (n = 5, p = 0.02233). On the other hand, the similar STN-HFS in the ACFS with a D_2-dopamine receptor agonist (10 μM Quinpirole hydrochloride), induced IPSC-LTD in many neurons tested (7 out of 9 neurns). At 120 min after STN-HFS with Quinpirole, the normalized amplitude of evoked IPSC was 0.54574 ± 0.0919. This value was significantly different from the one before STN-HFS (n = 7, p = 0.000515). This decrease in IPSC amplitude was accompanied not with the amplitude of sIPSC but with the frequnecy of sIPSC. These synaptic plasticity due to the presynaptic mechanism (D_1-receptor activation dependent IPSC-LTP and D_2-receptor activation dependent IPSC-LTD) mentioned above might be one mechanism underling the side effect of DBS. (COI:No)

1P-055

Adrenergic alpha1 receptor agonist prolongs striatal firing via dopamine D1 receptor

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We have previously reported that phenylephrine, an alpha1 adrenergic receptor agonist, increases optogenetically induced firings of the medium spiny neuron in the channelrhodopsin-2 expressing rat striatum. Several hundred millisecond-long residual firings after 1-sec long photostimulation were observed and were enhanced by phenylephrine. However, mechanisms of the firing increase were not yet determined. We hypothesized that phenylephrine increases a release of dopamine from the dopaminergic terminal in the striatum, since the alpha1 receptor has been reported to exist in the dopaminergic fiber. Therefore, we investigated the effect of dopamine receptor agonists and an antagonist. Dopamine and 6-Br-APB, a dopamine D1 receptor agonist, significantly increased the post-photostimulation firings. And also, SCH23390, a D1 receptor antagonist, significantly decreased the phenylephrine induced firing increase. These observations suggest that phenylephrine increases the firing via the D1 receptor activation. To clarify whether the D1 receptor activation depends on the dopamine release, we measured the dopamine concentration after the application of phenylephrine. Our data supports the idea that adrenergic agents will be available for Parkinson's disease treatment in the future. (COI:No)

1P-056

Identification of substances affect MCH neurons and orexin neurons by calcium imaging screening

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Melanin concentrating hormone-containing (MCH) neurons and orexin-containing (ORX) neurons are distinct hypothalamic neurons. These neurons are implicated in the regulation of instinctive behaviors such as sleep/wakefulness and feeding. Recent study showed that these neurons have a functional relationship. However, substances regulate activity of these neurons are still elusive. Here we aimed to identify the substances affect these neurons and reveal physiological role of them. First, we made new transgenic mice strains express genetically encoded calcium indicator Yellow Cameleon-Nano50 in MCH neurons or ORX neurons. Next, an acute brain slice from those transgenic mice was subjected to calcium imaging. Artificial cerebrospinal fluid (aCSF) was perfused, and candidate substances were applied with aCSF perfusion. Forty candidates were examined. These were reported to be involved in the regulation of behaviors such as sleep/wakefulness and feeding. As a result, we identified 9 and 17 substances affect MCH neurons and ORX neurons, respectively. Among them, 6 substances affect both of neurons. Two substances on MCH neurons and 2 substances on ORX neurons were novel. In this study, we established new system to screen substances affect the activity of MCH neurons and ORX neurons, and detected novel substances affect these neurons. We will investigate physiological functions of these substances, and also search more candidate substances. (COI:No)

1P-057

Regulatory mechanism of serotonergic (5-HT) neurons in the sleep/wakefulness and stress responce

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In the modern stressful society, sleep disorder caused by stress is becoming more and more serious social problem. In previous study, sleep and stress related neural circuits are revealed including the hypothalamic-pituitary-adrenal (HPA) axis, which have a central role of stress response, and orexin neurons in sleep-awake systems. In association with these circuits the neurotransmitters and modulators are becoming revealed, for example corticotropin releasing factor (CRF) and orexin. Serotonin (5-HT) is also important to stress response and associated with variety of physiological activities such as sleep rhythm and memory. However, the regulatory mechanism of 5-HT neurons is not revealed in detail. Since the activity of 5-HT neurons is predicted to be controlled by various neurotransmitters and modulators, we focused on the bioactive substances which affect the activity of 5-HT neurons. To identify the factors, we performed Ca^{2+} imaging in acute mouse brain slices. We made transgenic mice which express Ca^{2+} indicator Yellow Cameleon (YC)-Nano50 in 5-HT neurons. We screened various bioactive substances such as hormone, prostaglandin, interleukin and cytokine, particularly related to sleep and stress response. To study in detail, we performed electrophysiological slice patch clamp recording. We will study the relationship between these factors and stress response using behavior experiments. (COI:No)

1P-058

Enhancement of 5-HT4 receptor-mediated serotonergic synaptic modulation by electroconvulsive stimulation

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Electroconvulsive therapy (ECT) is a rapidly acting and highly effective treatment for depression. However, its mechanism of action is not well understood. We have recently shown that ECT-like stimulation in mice enhances dopamine D1-like receptor-mediated synaptic potentiation at the synapse between the hippocampal mossy fibers and CA3 pyramidal cells. The mossy fiber synaptic transmission is potentiated by serotonin via activation of the 5-HT4 receptor as well. In the present study, we examined whether electroconvulsive stimulation (ECS), an animal model of ECT, can affect the serotonergic modulation at the mossy fiber synapse in mouse hippocampal slices. We found that the potentiating effects of serotonin at the mossy fiber synapse were greatly enhanced in ECS-treated mice. The significant effect of ECS was detectable after a few times of treatments. To assess synaptic modulation mediated by endogenous monoamines including serotonin, we examined the effect of the monoamine releaser methamphetamine on the mossy fiber synaptic transmission. Bath application of methamphetamine induced slowly developing synaptic potentiation. In mice lacking the 5-HT4 receptor, methamphetamine failed to induce significant synaptic potentiation. Repeated ECS strongly enhanced this 5-HT4 receptor-dependent synaptic potentiation induced by methamphetamine, indicating that ECS can enhance synaptic modulation mediated by endogenous serotonin. Our findings suggest potential importance of 5-HT4 receptor-mediated synaptic modulation in the mechanism of action of ECT. (COI:No)

1P-059

A double knockout zebrafish revealed distinctive regulations of nicotinic acetylcholine receptors (nAChRs) in slow and fast muscles

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Nicotinic acetylcholine receptors (nAChRs) expressed in the neuromuscular junction are pentamers, composed of α, β, δ, and ε (or γ) subunits. Recent electrophysiological studies suggested that the subunit composition of nAChRs in slow muscles are different from that of fast muscles. In slow muscles, nAChRs lack ε /γ subunit and are composed of αβδ subunits. To investigate the distinctive compositions and functions of nAChRs in slow and fast muscles further in vivo, we generated a double knockout zebrafish line that lacked both ε and γ subunits (ε /γ-DKO zebrafish), which has never been studied in any other animal model. In zebrafish slow and fast muscle cells are spatially segregated and can easily be distinguished by their location. We found that the ε /γ-DKO zebrafish have the ability to swim In addition, histological analysis of the epsilon; /γ-DKO zebrafish showed that the nAChR expression was limited to slow muscles. These results strongly support the hypothesis that nAChRs lacking ε /γ subunit function in vivo and are specifically expressed in slow muscles, which underlie the swimming of the ε /γ-DKO zebrafish. Interestingly, nAChRs lacking ε /γ subunit cannot be expressed in fast muscle. Thus, distinctive molecular mechanisms regulate nAChR subunit compositions in slow and fast muscles. (COI:No)

1P-060

NMDA receptor-dependent presynaptic inhibition at the juvenile rat calyx of Held synapse

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N-methyl-D-aspartate receptors (NMDARs) play diverse roles in synaptic transmission, synaptic plasticity, neuronal development, and neurological diseases. In addition to their postsynaptic expression, NMDARs are also expressed in presynaptic terminals at some central synapses, and their activation modulates transmitter release. Here we demonstrate that activation of NMDARs in nerve terminals at a central glutamatergic synapse inhibits presynaptic Ca^{2+} currents (I_{Ca}) by means of GluN2C/2D subunit-dependent manner, thereby suppressing nerve-evoked transmitter release. Although neither presynaptically-loaded fast Ca^{2+} chelator BAPTA nor non-hydrolyzable GTP analogue GTPgammaS affected the NMDAR-mediated I_{Ca} inhibition, elimination of Na^+ from extracellular or intracellular solution altered the strength of this presynaptic inhibition, suggesting that Na^+ is the key mediator for the process. Repetitive activation of I_{Ca} in the presence of a glutamate uptake blocker attenuated the decline of I_{ca} amplitude, suggesting that endogenous glutamate has a potential to activate presynaptic NMDARs. We conclude that presynaptic NMDARs can attenuate glutamate release by inhibiting voltage-gated Ca^{2+} channels at a model synapse in the immature rat brainstem. (COI:No)

1P-061

Restorative effect of dark rearing on the visual cortical response of amblyopic cat

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Function and neural circuit of brain are modified by experience during the critical period of postnatal development. For example, monocular deprivation during early postnatal life induces amblyopia, and most neurons in the primary visual cortex lose their responses to the deprived eye and respond exclusively to the eye that has remained open. Although it has been difficult to restore visual acuity after maturation, recent studies in rodents showed that a period of exposure to complete darkness could promote recovery from amblyopia induced by prior monocular deprivation. Dark rearing is noninvasive and potentially suitable for clinical application. In cats, which share many similarities to human with respect to the organization of central visual pathways, dark rearing was reported to improve visual acuity of the deprived eye. The reports on cats, however, examined the effect of darkness only by behavioral assessment and it is not known which brain region contributes to the behavioral restoration. Therefore, we reared cats in darkness after brief monocular deprivation and examined restorative effect of darkness on visual responsiveness of neurons in the primary visual cortex electrophysiologically. We demonstrate that dark rearing can restore the response of visual cortical neurons to the deprived eye. This study was supported by KAKENHI(15H01440). (A.K., T.G.: equal contribution to this study) (COI:No)

1P-062

Single-unit response by prosthetic retinal stimulation: comparison between ON cells and OFF cells in cat LGN

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We have been developed the novel retinal prosthetic system, Suprachoroidal Transretinal Stimulation (STS), in which stimulating current is provided from scleral electrodes. One of its advantages is safety because the stimulating electrodes do not contact the retinal tissue directly to avoid physical damage of the retina. Previously we reported that the retinal area activated by STS is limited near the electrode by single-unit recording study from relay cells in cat lateral geniculate nucleus (LGN)(93rd PSJ meeting). However, we did not show the difference of unitary responses among the cell types by prosthetic stimulation. Here we investigated the response properties of ON cells and OFF cells in LGN. The electrode array was implanted into the scleral pocket made on the posterior of eyeball (n=5). The size of single electrode is 0.5 mm in diameter and 0.3 mm in height, same as for clinical trials. More than 10 days after surgery, the cat was anesthetized again and single unit responses of LGN relay neurons to biphasic retinal stimulation (100 - 1000 μA and 0.5 ms/phase) were recorded. Both ON cells and OFF cells showed the burstic activities by STS. The first responses of OFF cells were less burstic than those of ON cells, and the following bursts of each cell type appeared alternatively. In the response probability by STS, no apparent difference between ON cells and OFF cells was observed. (COI:Properly Declared)

1P-063

The effect of histamine for the voltage-gated inward current on amacrine cells in the mouse retina

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Mammalian retinae express histamine receptors. Immunohistochemical studies showed that histamine H1 receptor (H1R) was expressed in the mammalian amacrine cells. At the last annual meeting, we reported that histamine affects voltage-gated outward currents in amacrine cells in the mouse retina. In this research, we found that some types of amacrine cells exhibit voltage-gated inward currents (n = 18 / 37). Here we investigated the effect of histamine for the inward current in mouse amacrine cells, using the whole-cell version of the patch-clamp technique. Mouse retinae were sliced at 200 μm in thickness. The slice patch-clamp recording was performed at the inner nuclear layer of the retina. The amacrine cells were identified by the locations of somata in the retinal layer and by the shapes of the fluorescence with injected Lucifer yellow. Under voltage-clamp conditions, amplitudes of the voltage-gated inward currents were enhanced by the application of 100 μM histamine in thirteen amacrine cells (46.9 ± 10.3%, mean ± SEM). However, these responses were reduced by pyliramine or triprolidine, specific H1R antagonists. These results indicate that histamine contributes to the modulation of the membrane potential in some amacrine cells. Furthermore, histamine can affect inward currents via the H1R. The amacrine cells participate in the lateral modulation in the retina. Therefore, histamine may be one of the important neuromodulators in the visual processing. (COI:No)

1P-064

Electrical synapses between gap-junctionally connected excitatory visual neurons will enhance chemical output synapses from these neurons onto postsynaptic neurons

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Electrical synapses are present in many types of visual neurons expressing channel subunit, connexins (Hidaka et al., J. Neurosci., 2004; J. Integra. Neurosci., 2005, 2008; Brain Res., 2012). Electrical current spread through gap junctions between presynaptic neurons is expected to modulate chemical output synapses from these neurons onto postsynaptic neurons. In our recent studies, physiological properties of electrical synapses between α retinal ganglion cells have been characterized (J. Neurosci., 2004; J. Integra. Neurosci., 2016). In the present study, we examined electrical synapses of pyramidal cells in visual cortex of developing rats and primate common marmosets. First, we investigated the localization of gap junctions between these excitatory visual cells by immunocytochemical studies of connexin proteins. Second, we analyzed physiological properties of electrical synapses between these cells under dual whole-cell patch clamp recordings. We then investigated relationship between electrical synapses and chemical output synapses of these cells. Connexin36-gap junctions were localized in these cells. Electrical synapses occur between them, where depolarizing output responses of the cells increased through cells' electrical synapses. These results suggest that visual cells' excitatory synapses onto postsynaptic cells appear to increase through electrical synapses between gap-junctionally connected excitatory visual cells. (COI:No)

1P-065

The expressions of tyrosine hydroxylase immunoreactive amacrine cells in the gerbil ritina

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The gerbil has specific characteristics in retaining the central retinal artery. We have examined shape localization and change in number of the TH-IR cells in the retina from postnatal day (P) 1 to adult gerbils using immunocytochemical method. The two types were clearly different in the shape of somata and stratification of TH-IR dendrites in the inner plexiform layer (IPL) at P7. Type-A TH-IR cells had monostratified dendrites extending in the outermost layer of the IPL, while type-B cells had dendrites extending in the middle of IPL. At P7, type-A somata were observed in the inner part of the inner nuclear layer (INL). its dendrites extended into the outer part of the IPL. Type-B somata were located in the inner part of the newly formed INL. In adult type A, densely stained, round-shaped and large somata were located in the innermost part of the INL. Adult type-B somata, on the other hand, were stained weakly, with their thin dendrites penetrating the outer part of the IPL. These results suggest that two kinds of dopaminergic amacrine cells have different developmental properties in the developing gerbil retina. (COI:No)

1P-066

Frequency organization of the secondary auditory fields reflecting tonotopically-arranged afferents from the primary auditory thalamus

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Frequency organization of the auditory cortex reflects cochleotopy that is kept through the course of the auditory pathway. In contrast to classical views, the secondary auditory field (A2) is tonotopically arranged in mice, although A2 has been thought to receive thalamic inputs from the dorsal division of MGB (MGd) with no tonotopy. To reconcile the discrepancy, we injected tracers to reveal distribution of thalamic neurons projecting to A2, and found that A2 received dense projections from the caudal part of the ventral division of MGB (MGv), in addition to some projections from MGd. Injection of tracers along the frequency gradient of A2 revealed topological organization in the caudal part that run ventrodorsally. We noticed that MGv neurons labeled by injection to a particular site in A2 were distributed wider than MGv neurons labeled by similar injection to A1, suggesting that an A2 neuron receives a wider range of tonal information from MGv compared with an A1 neuron. To validate this, we used two-photon calcium imaging and observed tonal responses of neurons in cortical input layers stained with Cal520. As expected, band width of neurons in layer 4 was broader in A2 than that in A1, and tonotopic arrangement of layer 4 in A2 was less ordered than that in A1. Moreover, we removed other auditory parts except A2 by aspiration, and found that tonotopy in A2 remained without inputs from other parts. These findings suggest that functional properties of A2 with the tonotopy are largely determined by the thalamocortical projections from MGv. (COI:No)

1P-067

Response patterns of higher auditory cortex neurons during ramped and damped sounds

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Temporal features of sounds influence sound perception and identification. Psychophysical experiments have shown that sounds whose amplitude is ramping up with time (ramped sounds) are perceived louder in strength and longer in subjective duration than their time-reversed sound (damped sounds) even if they are equal in intensity and physical duration. In the previous study we examined neural responses of the primary auditory cortex (A1) during the asymmetric sound stimuli in awake animals to elucidate the neural bases related to this perceptual asymmetry. We found that the edge cells in A1 sensitive to the abrupt change of stimulus envelope show the persistence of excitation after ramped sounds which is longer than that after damped sounds. Because the majority of A1 neurons are tuned to the velocity (slow or quick) but not to the direction (increase or decrease) of the amplitude change, the coding mechanism for asymmetric perception may not yet be fully executed at the level of A1. In the present study we recorded single unit activities from the secondary auditory cortex and posterior auditory fields of awake animals and examined response patterns during ramped and damped sounds. We found specific cells sensitive to direction of the amplitude change in those higher auditory cortices. (COI: No)

1P-068

The sound response properties of the optogenetically identified GABAergic neurons in the mouse inferior colliculus

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The inferior colliculus (IC) is an obligatory auditory center where all the auditory information from the brainstem is integrated and sent to the forebrain. In the 20% of neurons are GABAergic and virtually all the IC neurons receive inhibitory inputs that strongly affect the responses to sound. However, despite of its importance, the GABAergic neurons in the IC have never been identified in electrophysiological recordings in vivo. To distinguish GABAergic neurons from glutamatergic neurons in vivo, we used VGAT-ChR2 mice in which inhibitory neurons specifically express channelrhodopsin-2. In this animal, the light stimuli from the brain surface evoked excitatory or inhibitory responses in GABAergic or glutamatergic neurons in the IC, respectively. Using this method, we recorded from the identified GABAergic and glutamatergic neurons in the IC. We found that both cell types were heterogeneous with diverse response properties to sound. In the responses to tones, both cell types showed no clear difference in the population data. Further, we found that the response properties of both were affected by their location in the IC: The nearby neurons shared similar frequency response areas (FRAs), regardless of the cell types. In addition, the maximum firing rate and the band width of FRA of both cell types varied in sub-regions in the IC. These results suggested that the patterns of afferent input might predominantly affect the response property of both GABAergic and glutamatergic neurons in the local circuit of the IC. (COI:No)

1P-069

The new surgical method for vestibular lesion

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Vestibular lesion (VL) is required in order to examine the physiological function via the vestibular system using animals. The peripheral VL has been conducted chemically or electrically, however there are two problems in these methods using mice; 1) chemical lesion does not work in mice, and 2) there is a lack of lesion specificity in the method of electrical lesion because electric current is uncontrollable. To solve these problems, we developed the new method of VL using sonicator. There are 4 groups in this experiment; 1) only tympanic membranes are destructed (Sham) 2) unilateral VL using sonicator (Uni) 3) bilateral VL using sonicator (Bi) 4) bilateral electrical VL (Ele). In order to evaluate the effect of VL, we conducted following three tests; 1) Swimming test 2) Balance test 3) Righting reflex test. We performed these three tests after 1 day, 1 week, 2 weeks, and 3 weeks after VL. The performance of above 3 tests was significantly suppressed in Bi and Ele groups compared with Sham group even at the 3rd week (0.16 ± 0.01 s in Sham, 0.31 ± 0.01 s in Bi, and 0.29 ± 0.01 s in Ele). There was no difference in their values between Bi and Ele mice. In the Uni mice, although the attenuation of the performance was observed after the surgery, vestibular function was recovered. Accordingly, although the effect of the VL using sonicator is same as electrical lesion, sonicator would be better from the aspect of the specificity. (COI:No)

1P-070

Higher cortical functions required for sound-shape associative learning in mice

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Physiological functions of higher sensory areas are largely unknown in mice. One of such higher functions is associative learning between multimodal sensory inputs. For example, we can recall a particular sound from the shape stimulus intimately associated with the sound, and vice versa. We confirmed that mice could produce sound-shape association memory by using an M-shaped maze. Passive exposure to sounds plus shapes for a few days was sufficient to produce similar sound-shape association memory in mice. Cortical responses to the associated shape stimuli were investigated using flavoprotein fluorescence imaging. The associated responses to the shape stimuli appeared diffusely in the auditory cortex. These responses in the auditory cortex may be the basis for sound-shape associative memory. Clustered protocadherins (cPcdhs) are neuron-specific cell adhesion molecules with multiple gene clusters. We found that mice cPcdhα of reduced gene clusters had impaired sound-shape association memory in the M-shaped maze test. The responses in the auditory cortex were not activated by the associated shape stimuli. We investigated whether mice produce the associated sound-shape memory using not only the complicated stimuli but also the pure tonal stimuli.The associative auditory responses were not observed after the association between shape stimuli and pure tonal stimuli. (COI:No)

1P-071

Modulation of neuronal activity in primate somatosensory cortex during tactile self-stimulation task

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During a voluntary action, the replicated motor command (efference copy) is sent back to the sensory system. This enables the brain to predict the subsequent sensory input that is compared with the actual sensory feedback. Such a predictive mechanism is thought to be essential in precise sensorimotor control. This mechanism also explains attenuation of perceptual sensory feedback during voluntary action as proposed in the comparator model by Blakemore et al (1999). However, the neurophysiological underpinnings of the comparator model are still unclear. In the present study, we investigated the activity of neurons in the primate somatosensory cortex during a tactile self-stimulation task. In this task, the monkey applied tactile stimulations to his own hand with a brush controlled by a lever manipulated with the other hand. The brush followed the lever movement synchronously (no-delay condition) or with temporal delays (delayed conditions). We recorded single cell activities from somatosensory cortex contralateral to the tactile stimulation. As a result, certain proportion of the neurons showed smaller tactile response in no-delay condition than in delayed conditions, although the physical stimulation was almost identical. This is consistent with the sensory attenuation for expected feedback during voluntary action. Our result suggests that the interaction between the predicted and actual sensory feedbacks is taken place as a modulation of neuronal activity in relatively early stages of cortical somatosensory processing. (COI:No)

1P-072

Using wearable cameras, optical imaging of brain activity in rat sensory cortex

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Imaging of brain activity in freely moving animals seems technically challenging. However, this imaging technology is important to elucidate neural mechanisms underlying behavior under spontaneous fluctuations in arousal level. To image and monitor the brain activity, we have gotten an idea that small-sized cameras with fiberscope connected to cranial window and narrow-band LED light source are utilized and mounted on laboratory animal jackets. In this study we investigated whether it is possible to apply commercially-available wearable cameras (e.g. GoPro) in functional brain imaging. While delivering electrical pulses to the rat hind paw for 5 s and at 8 Hz, we imaged activity in the contralateral parietal cortex using reflected light at wavelengths of 586 and 620 nm. In the imaging of optical signals at 586 nm (an equal absorption point of oxy- and deoxy-hemoglobin) that represents changes in total Hb concentration, we observed a monophasic signal change, peaking at 4-5 s after the stimulation onset. In the imaging at 620 nm (deoxHb-dominant absorption) that reflects the balance of oxygen metabolism and blood flow/volume, we observed biphasic (early negative, late positive) changes. These time courses are similar to those of optical intrinsic signals that have been reported. In conclusion, the expensive CCD image sensors are not indispensable, and it is possible to detect and image brain activity using wearable cameras. The results suggest the possibility of functional brain imaging in small animals without constraint using wearable cameras. (COI:No)

1P-073

Two-photon Ca^{2+} imaging using GCaMP6f in awake marmoset neocortex

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Two-photon calcium imaging with genetically-encoded calcium indicators has been employed for the investigation of neural circuit organization especially in mice studies. However, there were limitations in the application of this technique to primate brains, even though the investigation of primate brains is crucial for the understanding of the human brain. Recently, we developed a novel system to chronically image the activity of cortical neurons of the adult marmoset (Callithrix jacchus), a small New World monkey. We used a tetracycline-inducible system to robustly amplify the expression of GCaMP6f. In the previous report, we conducted the experiments under anesthetized condition. Here, we applied our system to the early visual cortex of awake marmosets. Visual stimuli, including natural scene images and artificial bar stimuli, were presented onto the display in front of the animal. The animal was allowed to view the visual stimuli freely, and the movement of their gaze was continuously monitored using a video-based eye tracking system. Using this system, we succeeded in monitoring visual responses of cortical neurons at the frame rate of 30 Hz. In conclusion, we succeeded in extending our system to monitor neuronal population activities from primate neocortex in awake condition. This method will be applicable to functional analysis of neuronal population activity in behaving marmosets. (COI:No)

1P-074

Acute effect of the median or ulnar nerve crush on the propagating excitation wave pattern of the somatosensory cortex in the rat

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Using our original optical recording system, we have reported that neural excitation induced by a somatic stimulation appears in the somatotopically corresponding site and spreads over the somatosensory cortex as a wave. In this study, we compared the effect of the median (MC) or ulnar nerve crush (UC) on the spatiotemporal pattern of this wave. Rats were separated into two groups (MC or UC). The forepaw response was elicited by an electrical stimulation at the right hypothenar pad. The optical recordings were performed before (PRE), soon after (t0) and 30 min after (t30) the nerve crush for each group. We measured three parameters in the optical signals as follows; the latency and the amplitude of the fastest response, and the propagation velocity around the initiation site. There were no effects on the three parameters in MC group. On the other hand, in UC group, the latency was significantly longer at both t0 and t30 and the amplitude was larger at t0 than those at PRE. The velocity in UC group was significantly smaller at t0 and t30 than that at PRE, only in the medial-posterior direction. Survival of the cortical response after MC or UC is probably ascribed to the overlapping innervation of the skin by ulnar and median nerves. These two nerves, however, appear to contribute differently to the cortical response since the effect of the crush was not identical. Thus, these alterations of the propagating excitation wave pattern of the somatosensory cortex might be attributable to the lower central nervous system. (COI:No)

1P-075

Influence of motivation on neuronal activities of V4 in a visual search task

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Neuronal activities selecting a target during visual search (VS) behavior have been investigated in several brain areas. However, influences of motivation, which must be a large impact on goal directed behaviors, remain unclear. To investigate these influences, we recorded neuronal activities of V4 when monkeys performed a delayed VS tasks with 3 grades of reward (RWD). In the task the animal memorized location of a singleton and made a saccade to the location after a delay period to get a RWD. If the animal performed correctly in succeeding two trials, the grade of the RWD was increased. Wrong performance made the grade decreased. Reaction times (RTs), which were influenced by animal's motivation because of the delay period, changed depending on the grade of the RWD, implying the animal's prediction of the grade of the forthcoming RWD. Activities of V4 from 110 to 155 ms after onset of stimulus (early period) responded strongly in trials with shorter RT. This effect was irrelevant with whether the stimulus within neuron's receptive field (RF) was a target or a distractor. On the other hand, activities from 155 to 280 ms (late period), which distinguished whether the stimulus within the RF was a target or a distractor, were not different depending on RT. These results suggested that higher motivation evoked general enhancement in neuronal activities of V4 in the early period. Such modulation would be overridden by attentional modulation in the late period. (COI:No)

1P-076

Analysis of prediction error responses in the mouse posterior parietal cortex

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Previously, we have reported that prediction errors between whisker and visual inputs were detected in the posterior parietal cortex (PPC) of mice. In flavoprotein fluorescence imaging, visual or whisker stimulation alone hardly activated PPC. However, anti-phase but not in-phase combination of moving grating patterns and whisker stimulation, the former of which is very unlikely in natural environment for mice, produced prediction error responses in PPC. We have reported that cortical depression and map shifts were induced in the primary visual cortex (V1) of young mice that had worn a monocular prism goggle, suggesting that the prediction errors detected in PPC produced cortical depression in V1 level. Clustered protocadherins (cPcdhs) are neuron-specific cell adhesion molecules with multiple gene clusters. Both of the prism-induced depression in V1 and the prediction error responses in PPC were impaired in mice with reduced cPcdh-α clusters. These results strongly suggest that the diversity of cPcdh-α is important for the PPC function to detect prediction errors between visual and whisker inputs. We investigated the prediction error responses and found that they were dependent on the exposure to normal environment in which mice likely experienced only in-phase combination of visual-whisker stimulation. We further confirmed that the directional differences between whisker and visual stimulation were proportional to the magnitude of the prediction error responses. (COI:No)

1P-077

Action of hinokitiol on compound action potentials in the frog sciatic nerve

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A tropolone derivative (β-thujaplicin; hinokitiol, a natural component contained in Chamaecyparis taiwanensis), has various actions including antitumor, antibacterial and antiinflammatory activities. We have previously reported that various plant-derived chemicals inhibit voltage-gated Na^+-channel blocker tetrodotoxin-sensitive compound action potentials (CAPs) recorded from the frog sciatic nerve by using the air-gap method; this inhibition is dependent on their chemical structures. The aim of the present study was to know whether like other plant-derived chemicals hinokitiol inhibits CAPs and if so what chemical structure of hinokitiol is important in this inhibition. Hinokitiol concentration-dependently reduced the peak amplitude of the CAP with a half-maximal inhibitory concentration (IC_{50}) value of 0.54 mM. A threshold to elicit CAPs was increased by hinokitiol. A stereoisomer of hinokitiol, γ-thujaplicin, also inhibited CAPs with the IC_{50} value of 0.54 mM, a value similar to that of hinokitiol. On the other hand, tropolone, which lacks the isopropyl group of hinokitiol, at 0.5 mM had no effects on CAPs. Moreover, CAPs were unaffected by hinokitiol-related chemicals, kojic acid and guaiazulene (each 0.5 mM). It is concluded that hinokitiol has an ability to inhibit CAPs and an interaction among the substituted groups (isopropyl, carbonyl and hydroxyl groups) of 1,3,5-cycloheptatriene plays a role in this inhibition. Nerve conduction inhibition by hinokitiol could contribute to at least a part of its pharmacological actions. (COI:No)

1P-078

Induction of astroglial Ca^{2+} signaling by IP_3R-SOC coordinated system

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Astrocytes, one type of glial cells, not only play supporting roles of neurons but also regulate local blood flow and synaptic transmission using intracellular calcium (Ca^{2+}) signaling. Therefore, understanding of astroglial Ca^{2+} signaling system contributes to elucidate the mechanism underlying astrocytic physiological functions. However, the Ca^{2+} pathway to induce astrocytic Ca^{2+} signaling is still unclear. One of the well-characterized pathways is Ca^{2+} release from the endoplasmic reticulum (ER) via inositol 1, 4, 5-trisphosphate receptor (IP_3R). Some studies indicated Ca^{2+} channels on the plasma membrane are also required although the importance of them remains unknown. We report here that Store Operated Calcium entry channels (SOCs), which are constituted by Ca^{2+} sensing STIM proteins and Ca^{2+}-selective Orai channels, are crucially involved in astrocytic spontaneous Ca^{2+} transients. Using Ca^{2+} imaging of rat hippocampal primary cultures, we showed that astroglial Ca^{2+} transients were blocked by SOC inhibition and the removal of extracellular Ca^{2+}. The inhibition of SOC resulted in the depletion of Ca^{2+} store and impairment of Ca^{2+} release through IP_3R. Our findings suggest that astrocytic Ca^{2+} transients were generated by the co-operation of Ca^{2+} influx through SOC and Ca^{2+} release from ER through IP_3R. (COI:No)

1P-079

The contribution of 5-HT3 receptors to responses of dopamine release in the nucleus accumbens to tactile stimulation in rats

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We have shown that tactile stimulation increases dopamine (DA) release in the nucleus accumbens (NAc) in rats (Maruyama et al., 2012). On the other hand, it has been reported that infusion of serotonin (5-HT) into the NAc increases DA release in the NAc and the increase is mediated via 5-HT3 receptors. However, the contribution of 5-HT3 receptors in the NAc to the actual physiological responses had not yet been shown. Present study aimed to clarify the involvement of 5-HT3 receptors in the responses of DA release in the NAc to tactile stimulation in the anesthetized rats. For this purpose ondansetron, a selective 5-HT3 antagonist, was administered into the NAc with use of reverse microdialysis method. A coaxial microdialysis probe was stereotaxically implanted in the NAc and perfused with modified Ringer's solution at a speed of 2 µl/min. The amount of DA in the dialysate was measured by a high-performance liquid chromatograph with an electrochemical detector. Tactile stimulation was applied to the back for 5 min. Treatment with ondansetron significantly reduced the increase in DA release induced by the tactile stimulation. Present results suggest that the increases in DA release in the NAc are partly mediated via the 5-HT3 receptors in the NAc. (COI:No)

1P-080

Effects of sex steroids on the sexual differentiation of the sexually dimorphic nucleus of the dorsal hypothalamus (SDN-DH) in mice

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We identified a sexually dimorphic nucleus of the dorsal hypothalamus (SDN-DH) in mice. The SDN-DH was located between two known male-biased sexually dimorphic nuclei, the principal nucleus of the bed nucleus of the stria terminalis (BNSTp) and the calbindin-sexually dimorphic nucleus (CALB-SDN), and exhibited female-biased sex difference in volume and neuron number. Next, we examined the effects of testicular testosterone during the postnatal period on the sexual differentiation of the SDN-DH in mice. The volume and neuron number of the SDN-DH was increased in males by neonatal orchiectomy and decreased in females by treatment with testosterone, dihydrotestosterone, or estradiol within 5 days after birth. Additionally, we examined the SDN-DH of prepubertal mice and adult mice, some of which were gonadectomized before puberty. The female SDN-DH enlarged during puberty, although the SDN-DH of prepubertally ovariectomized females did not change in volume but decreased in neuron number. The number of neurons in the male SDN-DH decreased during puberty, and this was not affected by prepubertal orchidectomy. Testicular testosterone during the postnatal period may act to defeminize the SDN-DH via binding to androgen receptor and estrogen receptor after aromatization, although defeminization may proceed independently of testicular hormones during puberty. Ovarian hormones during puberty may act to feminize the SDN-DH. (COI:No)

1P-081

Sexual behavior-associated changes in neuronal activity of the sagittalis nucleus of the hypothalamus in male rats

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The medial preoptic nucleus is a center for male sexual behavior and contains a male-biased sexually dimorphic nucleus composed of calbindin neurons. Testicular testosterone affects the medial preoptic nucleus to facilitate male sexual behavior via binding to estrogen receptor after aromatization. The sagittalis nucleus of the hypothalamus (SGN) has been identified as a sexual dimorphic nucleus that exhibits male-biased sex differences in volume, number of calbindin neurons, and expression of estrogen receptor, although the physiological roles of the SGN have not yet been determined. In this study, we measured the number of activated neurons in the SGN during sexual behavior to determine whether the SGN of male rats is involved in the regulation of sexual behavior. The brains were sampled from male rats displayed sniffing, mounting, intromission, and ejaculation. Brain sections were immunostained for c-Fos, a neuronal activity marker, and calbindin, and we counted the number of c-Fos-immunoreactive (ir) cells in the SGN. As the results, the number of c-Fos-ir cells gradually increased with displaying sniffing and mounting. The number of c-Fos-ir cells reached a peak level when males displayed mounting, because there was no significant difference in the number among males displayed mounting, intromission, and ejaculation. These findings suggest that the SGN is related to the control of sexual motivation and/or appetitive sexual behavior in male rats. (COI:No)

1P-082

Increased expression of Iba1 after focal infarction of the macaque internal capsule

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We recently established a macaque model of focal internal capsular infarcts, and found that. a decrease of large neurons in layer V of the primary motor cortex (M1) was associated with long-lasting impairment of hand movements. As the first step to investigate cellular and molecular processes underlying the selective neuronal loss in preserved M1 tissue, we focus on the expression change of Iba1, a marker of microglia. Immunohistochemistry was performed in brain sections obtained from macaques several hours to 3 months after infarctionIba1-immunoreactivity in M1 layer V significantly increased during 4 days to 3 weeks after infarction, compared with that of intact macaques. The immunoreactivity decreased to the level of intact macaques at 1 month and later after infarction, when the number of large neurons in layer V decreased. The transiently increased iba1-positive microglia in M1 may participate in loss of large neurons. We also investigated Iba1 expression around the core of infarcts in the internal capsule, and found that Iba1-immunoreactivity continued to increase at 3 months after infarction. The result is in contrast to that reported in rat, in which Iba1 expression around the infarct core increased during several days but decreased by 2 weeks after infarction. Therefore, the temporal dynamics of microglial activation may differ between primates and rodents. The present macaque model will contribute to understand microglial activation, which may inhibit or promote functional recovery, and effect of therapeutic interventions on it. (COI:No)

1P-083

Ameliorative effects of subcutaneous administration of a mixture containing IL-3 and GM-CSF on a rat experimental stroke model

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Bone marrow-derived macrophages are densely accumulated in the ischemic core in the brain of a rat stroke model. Since the macrophages express NG2 proteoglycan and Iba1, they are called BINCs (brain Iba1+/NG2+ cells). We have considered that BINCs are protective cells for the ischemic brain, because they abundantly express neuroprotective factors such IGF-1 or HGF and also because transplantation of BINCs in to the ischemic core ameliorate the outcomes. Human stroke brains contained the similar cells in the core lesions; however, the density of the cells was much lower than that in the rat ischemic core lesions. The difference may be attributable to the age; aged (over than 65years old) people and young (2-month-old) rats, the latter of which outcome is normally much better than that of the formers. We suspected the responsibility of the slow and declined activity of the bone marrow of aged people. To activate their bone marrow and BINCs, we thought the utilization of hematopoietic cytokines. Because BINCs expressed receptors for IL-3 and GM-CSF, a mixture containing the both cytokines, was subcutaneously administered to the rat stroke model once per day for 5 days. The mixture resulted in much ameliorative outcomes of the rats in terms of the stroke volume and behavioral functions. (COI:No)

1P-084

Alternative splicing for activation of coagulation factor XIII-A in the fish retina and optic nerve after optic nerve injury

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Unlike mammalian retinal ganglion cells (RGCs), fish RGCs have the capacity to repair their axons, even after optic nerve transection. Factor XIII-A (FXIII-A), which has become known as cellular transglutaminase, plays important roles in mediating cross-linking reactions in various tissues. FXIII-A acts as one of the regeneration molecules in the fish retina and optic nerve after optic nerve injury, and becomes activated at the site of injury within a few hours. Previous research has shown that activated FXIII-A induces neurite outgrowth from injured retinal ganglion cells, and supports elongation of the regenerating optic nerve. However, the activation mechanism of FXIII-A remains unknown. We found that expression of the region encoding the activation peptide was markedly suppressed compared with the region encoding the active site. An overexpression study with a short-type FXIII-A cDNA lacking the activation peptide revealed induction of long neurite outgrowth in fish retinal explant cultures compared with full-length FXIII-A cDNA. The present findings suggest that alternative splicing may occur in the FXIII-A gene, resulting in deletion of the region encoding the activation peptide and thus allowing direct production of activated FXIII-A protein in the fish retina and optic nerve after optic nerve injury. (COI:No)

1P-085

Cell-permeable p38 MAP kinase promotes migration of adult neural stem/progenitor cells

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In the adult damaged brain, endogenous neural stem/progenitor cells (NPCs) can migrate toward the sites of injury at some extent, but the migration activity of NPCs is insufficient to restore damaged brain tissue. In this study, we have focused on p38 MAP kinase (p38), because p38 expression has been found in embryonic NPC that migrate extensively throughout the embryonic brain. We showed that p38 is expressed in doublecortin-positive adult NPCs, and Inhibitor experiments using the compound SB203580 revealed that endogenous p38 participates in NPC migration. To enhance NPC migration, we prepared a cell-permeable p38 wild-type protein (PTD-p38WT) consisting of the HIV protein transduction domain (PTD) fused to the N-terminus of p38. Treatment with PTD-p38WT protein significantly promoted the random migration of adult NPCs without disturbing cell survival or differentiation; this effect depended on the cell permeability and kinase activity of the fusion protein. These findings indicate that PTD-p38WT is a novel and useful tool for unraveling the roles of p38, and that PTD-p38WT provides a reasonable approach for regenerating injured brain by enhancing NPC migration. (COI:No)

1P-086

The role of PlexinA1 receptor in the pioneer axonal extension and the guidepost formation during the early phase of corpus callosum development

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The corpus callosum is the large bundle of axons that mainly link similar regions of the left and right hemisphere. Both semaphorins and the receptor critically contribute to the corpus callosum development. Our study disclosed the high incidence of agenesis of corpus callosum in BALB/c mice lacking PlexinA1, a receptor for semaphorins. To know the expression pattern of semaphorins and the receptors in the corpus callosum development of BALB/c mice, immunohistochemistry of Neuropillin-1 (Npn-1) expressed in the pioneer axons and Sema3C expressed on the midline guidepost. The analysis confirmed the localization of Npn-1 positive pioneer axons and the expression of Sema3C in the calretinin-positive cells on the midline of the embryonic day 15.5 (E15.5) brain.To analyze the direction of pioneer axonal extension, immunohistochemistry of Npn-1 was performed on both embryonic brains from wild-type (WT) and PlexinA1-deficient mice. The incidence in which Npn-1 positive pioneer axons crossed the midline was significantly lower in E17.5 PlexinA1-deficient brain as compared with WT. Thus, PlexinA1-deficient pioneer axons may not respond to the chemoattractive guidance molecule such as Sema3C. To trace the pioneer axonal pathway in more detailed manner, we are analyzing the projection pattern of pioneer axons by microinjecting diI into E17.5 mouse cingulate cortex. (COI:No)

1P-087

Mood stabilizing drugs activate adult neural stem cell-neurogenesis system

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Neural stem cells (NSCs) not only produce all neurons and glia in the developing brain but also reside in the adult brain and provide new neurons in the olfactory bulb and hippocampus, which play significant roles in the olfaction and some types of memory. NSCs attract much attention as a resource of cell replacement therapy for impaired central nervous system. However, efficient and clinically feasible strategy to activate endogenous NSCs is not currently available. We have previously demonstrated that mood stabilizing drugs, which are used to treat patients with bipolar disorder, enhance the self-renewal capability of mouse NSCs in vitro and that this enhancement is achieved at therapeutically relevant concentrations in the cerebrospinal fluid. The pharmacological effects of classical mood stabiliazers are mediated by the activation of Notch signaling in the NSC. In this study, we examined the effect of a novel type of mood stabilizer, lamotrigine, on the the self-renewal of NSCs in vitro and neurogenesis in the olfactory bulb and dentate gyrus in vivo. Our data suggest that lamotrigine possesses similar pharmacological function to classical mood stabiliazers, such as valproate and carbamazepine. (COI:No)

1P-088

Activation of Serotonin 2A receptor modulates NMDA receptor-mediated glutamate responses via Src in dendrites of rat jaw-closing motoneurons

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Masseter motoneurons (MMNs) have the well-developed dendrites where MMNs receive a variety of inputs. MMNs receive both glutamatergic and serotonergic inputs in the dendrites; however it is unclear how the two inputs interact with each other. In this study we examined effects of serotonin (5-HT) on the glutamatergic responses in the dendrites of the MMNs in brainstem slice preparations obtained from postnatal day 2-5 neonatal rats using whole-cell recordings. Focal photolysis of MNI-caged glutamate was accomplished using a 365 nm nitrogen-pulsed laser or 730 nm two photon laser systems. Laser photolysis of caged glutamate in the dendrites induced somatic depolarization. Bath application of 5-HT increased the amplitude of the laser-evoked responses in the dendrites, in addition to induction of membrane potential depolarization. 5-HT-induced enhancement of the laser-evoked responses was mimicked by application of the 5-HT_{2A} receptor agonist. Application of NMDA receptor antagonist blocked the 5-HT-induced enhancement, whereas application of the AMPA receptor antagonist did not affect the 5-HT-induced enhancement. Pretreatment with a Src inhibitor reduced the 5-HT-induced enhancement. These results suggest that activation of 5-HT_{2A} receptors enhanced the NMDA receptor-mediated glutamate responses via Src in the dendrites of the MMNs. 5-HT may enhance the glutamatergic motor command onto MMNs. (COI:No)

1P-089

Analysis of rhythmic jaw movements induced by stimulation of the amygdaloid nucleus of the rat

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The present study determined which part of the central amygdaloid nucleus (Ce) is effective in inducing rhythmic jaw movements; made an analysis of the rhythmic jaw movements; and investigated whether rhythmic jaw movements are induced by repetitive electrical stimulation of the lateral amygdaloid nucleus (La) in the rat. Experiments were performed on anesthetized rats. Repetitive electrical stimulation was applied to the Ce or La. The electromyograms were recorded from the anterior belly of digastric and masseter muscles. Jaw movements were recorded in the vertical and horizontal directions. Rhythmic jaw movements were induced by repetitive electrical stimulation of the medial division of the Ce (CeM) and the lateral division of the Ce (CeL). These movements always began with opening of the jaw, and were followed by rhythmic movements consisting of simple opening-closing movements. The frequency of rhythmic jaw movements was 3-5 Hz. In fact the electromyographic activity of the anterior belly of the digastric muscle induced by stimulation of the CeL did not correspond to the rhythmic jaw movements, whereas the electromyographic activity induced by stimulation of the CeM did. Rhythmic jaw movements were not induced by stimulation of the capsular part of the Ce, dorsolateral part of the La, ventromedial part of the La, anterior part of the basolateral amygdaloid nucleus (BL), or posterior part of the BL. These results show that the CeM is the most effective part in inducing rhythmic jaw movements. (COI:No)

1P-090

Involvement of PLD in β-agonist-induced amylase release via MARCKS phosphorylation in rat parotid acinar cells

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In parotid acinar cells, stimulation of β-adrenergic receptors provokes amylase release via activation of cAMP-dependent protein kinase. Myristoylated alanine-rich C kinase substrate (MARCKS) is known as a major cellular substrate for protein kinase C (PKC). MARCKS has been implicated in membrane trafficking in several cell types. We have previously demonstrated that MARCKS phosphorylation via the activation of PKC δ, a PKC isotype, is involved in β-agonist-induced amylase release in rat parotid acinar cells. On the other hand, it has also been thought that phospholipase D (PLD) is involved in amylase release. Here, we investigated the involvement of PLD in β-agonist-induced amylase release in rat parotid acinar cells. Rat parotid acinar cells were prepared using trypsin and collagenase. MARCKS and phosphorylated-MARCKS were detected by Western blotting. Amylase activity was measured by Bernfeld's method. The β-agonist isoproterenol (IPR) induced amylase release. FIPI, a PLD inhibitor, inhibited IPR-induced amylase release. IPR also induced MARCKS phosphorylation. FIPI inhibited IPR-induced MARCKS phosphorylation. Dioctanoylglycol, a diacylglycerol kinase inhibitor, has no significant effect on IPR-induced amylase release. PLD catalyzes the hydrolysis of phosphatidylcholine to form phosphatidic acid. Therefore, our observations suggest that PLD is a regulating factor for IPR-induced amylase release via MARCKS phosphorylation by PKC δ activation in rat parotid acinar cells. (COI:No)

1P-091

Paracellular fluid secretion and microcirculation in the perfused submandibular gland

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Upon muscarinic stimulation the hydrostatic pressure could increase according to increase of the microcirculation of the perfused submandibular gland of rat. The raised pressure could drive paracellular fluid secretion and fluorescent dye (Lucifer Yellow, 500 Da) secreted in proportion to the applied pressure. In the present study, we perfused with fluorescent dye (DyLight488) sized ca. 1000 Da and the secreted dye concentration (Cs/Cp) was measured and plotted against the salivary flow rate during different perfusion rate. The Cs/Cp was constant through various flow rate suggesting that the pattern of dye transfer from circulation to saliva indicates paracellular transport. Whereas the perfusion pressure (AV pressure difference) decreased upon cholinergic stimulation in the gland perfused at the constant rate, suggesting a dilatation of the microcirculation. However the glandular capillaries diameter did not dilatated but uneven part to part. In addition, we also found that Danshen, Chinese herb, decreased the arterio-venous pressure immediately but the paracellular secretion was delayed around 5 min after start of stimulation. This finding was concluded that the onset of paracellular transport requires not only the increase in microcirculation but also the increase in the permeability across the tight junction. This work was supported by JSPS KAKENHI (26460308). (COI:No)

1P-092

Influence of type 2 diabetes on parasympathetic vasodilation in the salivary glands of rats

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We examined the hemodynamics in the major salivary glands of urethane-anesthetized, spontaneously-developed type 2 diabetic rats and nondiabetic control rats during electrical stimulation of the central cut end of the lingual nerve (LN), using a laser speckle imaging flow meter. The blood glucose level was significantly higher in diabetic rats than in the nondiabetic rats, indicating the pathogenesis of diabetes. No significant differences in body and salivary gland weights were noted between the two types of rats. LN stimulation induced intensity- and frequency- dependent blood flow increases in the salivary glands in both diabetic and nondiabetic rats; however, the magnitude of increase in the parotid gland was significantly lower in the diabetic rats, although this is not case in early developmental stage of diabetes. Blood flow increase in the parotid gland was markedly inhibited by intravenous administration of the autonomic ganglion blocking drugs, hexamethonium or antimuscarinic drugs, atropine. Although intravenous administration of acetylcholine increased the parotid gland blood flow in a dose-dependent manner, the response of diabetic rats was significantly lower than that of the nondiabetic rats. Our results indicate that type 2 diabetes selectively impairs parasympathetic vasodilation in the parotid gland, suggesting that a disturbance in the cholinergic vasodilator pathway may contribute to impairment of parasympathetic vasodilation in the parotid gland of diabetic rats. (COI: No)

1P-093

Peripheral mechanisms underlying tongue hypersensitivity associated with dry tongue

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Dry mouth syndrome is a common disease in dentistry. It is well known to cause persistent oral pain that usually starts from the tongue. Underlying mechanisms of this dry mouth pain are not fully understood. Transient receptor potential (TRP) channels are Ca^{2+}-permeable cation channels that play important roles in sensory function. TRPV4 is a member of the vanilloid subfamily of TRP channels and plays a significant role in pain signaling. It can be activated by osmotic changes, mechanical and thermal stimuli. p38 is involved in inflammation-induced pain and phosphorylated by a variety of stimuli. The phosphorylation of p38 enlarges the pain signal from the peripheral nociceptors. Local injection of p38 inhibitors significantly suppresses thermal and mechanical hyperalgesia. Activation of TRPV4 channel leads to an increase in the intracellular calcium. This increase in intracellular calcium enhances phosphorylation of p38, mediating mechanical allodynia and inflammation-induced hyperalgesia. To find out whether TRPV4 participates in mechanical hyperalgesia of tongue, we use dry-tongue models. Immunohistochemistry of trigeminal ganglion was conducted using TRPV4 and pp38 antibodies and found a significant increase in the number of TRPV4 and pp38-IR cells in the TG in dry-tongue rats compared with sham rats. The present findings suggest that activation of TRPV4 leads to phosphorylation of p38 in TG neurons, resulting in tongue hypersensitivity of dry mouth patients. (COI:No)

1P-094

Involvement of endothelin signaling in modulation of tongue pain in the early stage of tongue carcinogenesis

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Clinically, tongue cancer patients do not occasionally complain of obvious tongue pain in the early stage of tongue carcinogenesis. However, the exact mechanism of nociceptive modulation in cancerous tongue is not still known. In this study, we examined the involvement of endothelin signaling in modulation of tongue pain in the early stage of tongue carcinogenesis in rats. Cancer cells (SCC-158) were inoculated into the tongue under the deep anesthesia. Tongue mechanical sensitivity did not change in the early stage of tongue carcinogenesis, was significantly reduced from day 11 onward. Endothelin-A receptor was expressed in cancer cells. A mount of Endothelin-1 was significantly increased, and Endothelin-A receptor antagonist administration into cancerous tongue induced the mechanical hypersensitivity in the early stage of tongue carcinogenesis. μ-opioid receptor was expressed in trigeminal ganglion neurons innervating to the cancerous tongue, μ-opioid receptor antagonist administration to the tongue significantly enhanced the mechanical hypersensitivity, and amount of β-endorphin was significantly increased in the early stage of tongue carcinogenesis. These findings suggest that β-endorphin released from the cancer cells via endothelin-1 signaling is involved in modulation of tongue pain in the early stage of tongue carcinogenesis. (COI:No)

1P-095

Characteristics of the elicited responses of rat somatosensory and insular cortices by electrical stimulation to periodontal ligament

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An orofacial nociception is transmitted to the primary somatosensory cortex (S1) via the ventral posteromedial nucleus, and then to the secondary somatosensory cortex (S2). It has reported that electrical stimulation of the rat periodontal ligament (PDL) simultaneously elicited neural excitation in S1 and the specific region composed of S2 and the insular oral region (IOR). However, the physiological relationship between S1 and S2/IOR has been little understood. To address this issue, we investigated the intracortical interaction between two response areas in S1 and S2/IOR by an *in vivo* optical imaging using a voltage sensitive dye. The responses in S2/IOR were observed by the electrical stimulation within the response area in S1 without no latency, and vice versa. After confirming those reciprocal interactions, we blocked the intracortical communication between S1 and S2/IOR by injecting tetrodotoxin and then stimulated each region in turn. Such operation resulted in attenuation of the evoked response in non-stimulated cortical partner site. In addition, morphological study using a retrograde neurotracer, FluoroGold (FG), showed the presence of bi-directional intracortical connection between the initial response areas in the S1 and S2/IOR. These results suggest the presence of a mutual connection between S1 and S2/IOR as an intracortical signal processing network. (COI:No)

1P-096

Anandamide induces network oscillations between the gustatory and gastrointestinalinsular cortices

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Anandamide (AEA) and N-oleoylethanolamine (OEA) are produced in the intestine and brain during fasting and satiety, respectively. Subsequently, AEA facilitates food intake via activation of cannabinoid type-1 receptors (CB1Rs) while OEA decreases food intake via activation of peroxisome proliferator-activated receptor-α (PPARα) and/or G-protein-coupled receptor 119 (GPR119). Neuronal activity in the gastrointestinal region of the autonomic insula (GI-Au-I) that rostrally adjoins the gustatory insula (Gu-I) increases during fasting, enhancing appetite while umami and sweet taste sensations in Gu-I enhances appetite in GI-Au-I, strongly suggesting the presence of a neural interaction between the Gu-I and GI-Au-I which changes depending on the concentrations of AEA and OEA. However, this possibility has never been investigated. In rat slice preparations, we demonstrate with voltage-sensitive dye imaging that activation of CB1Rs by AEA induces θ-rhythm oscillatory synchronization in the Gu-I which propagates into the GI-Au-I but stops at its caudal end, displaying an oscillatory coordination. The AEA-induced oscillation was abolished by a CB1R antagonist or OEA through activation of GPR119. Our results demonstrate that the neural coordination between the Gu-I and GI-Au-I is generated or suppressed by the opposing activities between CB1R and GPR119. This mechanism may be involved in the feeding behavior based on taste recognition. (COI:No)

1P-097

Effects of hyperglycemia on brainstem neurons in fasted rats

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The present study was performed to demonstrate the relation between blood glucose level and neuronal excitability in the nucleus tractus solitaries (NTS) and the area postrema (AP). Sprague-Dawley rats (250-500 g) fasted for 24 h were used. We firstly measured blood glucose concentration at 15, 30, 60, 90 120 min after i.p. injection of 1.1 M glucose (10 ml/kg). Immunoreactivity of c-Fos protein was detected in the NTS and the AP at 2 h after i.p. injection (10 ml/kg) of 1.1 M glucose,1.1 M mannitol or saline (control). The blood glucose concentration peaked at 15 min after injection of glucose and then gradually decreased for 90-120 minutes toward the normal level. Fos-immunoreactive (Fos-ir) neurons in the NTS were markedly detected in glucose and mannitol groups as compared with control group. Slight changes in the number of Fos-ir AP neurons were hardly compatible with glucose- and osmolarity-sensitive AP neurons reported in the previous studies. The present study suggested the strong relation between hyperglycemia and the excitability of NTS neurons. (COI:No)

1P-099

Study of S-PRG filler eluate optimum concentration in human gingival fibroblasts experiment

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The S-PRG filler is known to have a high cariostatic effect material and to release 6 ions (Na^+, F^-, Al^{3+}, BO_3^{3-}, Sr^{2+} and SiO_3^{2-}). In this study, we examined the effect of S-PRG filler eluate to human gingival fibroblasts (HGF). HGF were incubated for 3 days in the S-PRG filler eluate containing various concentrations of FBS (0, 2, 5, 10, 20%). The control group was MEM-α containing 10% FBS. As a result, cell proliferation was not observed by the S-PRG filler eluate. In the same way, HGF were cultured for 3 days and then stained with 0.4% trypan blue to test cell viability. We confirmed cell death in various concentrations of S-PRG filler eluate. Next, we dilute the S-PRG filler eluate to 11 steps with MEM-α (1, 1/2, 1/5, 1/10, 1/100, 1/200, 1/500, 1/1000, 1/2000, 1/5000 and 1/10000) containing 10% FBS. As a result, the solution diluted to 1/100 or more showed a cell proliferation similar to the control group. These data suggest that it is preferable to use the S-PRG filler eluate diluted to 1/100 or more in the experiments with S-PRG filler eluate to HGF. (COI:No)

1P-100

Alkali- and ADP-sensitive store-operated Ca^{2+} entry (SOCE) mediated by Ca^{2+} release-activated Ca^{2+} (CRAC) channels in rat odontoblasts

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In a previous study, we reported that depletion of Ca^{2+} stores activated store-operated Ca^{2+} entry (SOCE) in odontoblasts. However, the detailed properties of SOCE remain unclear. In this study, we examined the pharmacological properties of SOCE in rat odontoblasts. From these cells we measured intracellular free calcium concentration ($[Ca^{2+}]_i$) by fura-2 fluorescence. In the absence of extracellular Ca^{2+}, thapsigargin (TG), a sarcoendoplasmic reticulum Ca^{2+}-ATPase inhibitor, evoked transient $[Ca^{2+}]_i$ increases. After $[Ca^{2+}]_i$ returned to the near-resting levels, subsequent application of 2.5 mM extracellular Ca^{2+} increased $[Ca^{2+}]_i$ as SOCE. The SOCE was inhibited by Ca^{2+} release-activated Ca^{2+} (CRAC) channel inhibitors. After pretreatment of TG, alkaline solution containing 2.5 mM Ca^{2+} induced $[Ca^{2+}]_i$ increases which were inhibited by a TRPA1 channel antagonist. In the absence of extracellular Ca^{2+}, a $P2Y_{1,12,13}$ receptor agonist evoked transient $[Ca^{2+}]_i$ increases. After pretreatment of the $P2Y_{1,12,13}$ receptor agonist, SOCE was elicited by subsequent application of 2.5 mM extracellular Ca^{2+}. These results suggested that activation of CRAC channels and SOCE were augmented by alkaline solution and P2Y receptor agonists in odontoblasts. (COI:No)

1P-101

Effect of gum chewing training on the oral functions in healthy adults

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The aim of this study was to investigate the effect of the gum chewing training on the oral functions. Subjects were 16 healthy women with normal occlusion. We instructed the subjects to perform the gum chewing training (chewing by ten times with the molar tooth of the right and left side in turn with the mouth closed) three times a day for 3 months every day. Maximum tongue pressure and cheek pressure was measured using JMS tongue pressure measurement device. The masticatory efficiency were evaluated using gummy-jelly, the glucose extraction were measured after chewed. The measurement were performed at the point before training and at the points of 2 weeks and 1, 2, and 3 months after the beginning of training, and at the points of 2 weeks and 1 month after discontinuation of training. Changes in the oral functions according to an exemplary period of the gum chewing training were analyzed by One-way repeated measures ANOVA. The maximum tongue pressure, cheek pressure and masticatory efficiency were increased markedly at 2 weeks after the beginning of the training. These effects were appeared for 3 months. At 2 weeks after discontinuation training, the maximum tongue pressure and cheek pressure tended to decrease than 3 months after beginning training, but these values were significantly higher than before training and approximated to them of the 2 months after beginning of the training. The results showed that the continuation of gum chewing training was increased the perioral muscle pressure and masticatory efficiency might be the effective training methods to improve oral function. (COI:No)

1P-102

Association of oral fat sensitivity with mental state in healthy young adults

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Sensitivity for prototypical tastes may be related to mental state (MS). In addition, fatty acid taste has been suggested to be a novel prototypical taste, while body mass index (BMI) has been related to oral fat sensitivity (OFS). However, the relationship between OFS and MS is still unknown, even in healthy young adults. Thus, we investigated the association of OFS and MS among this population. We measured OFS, sensitivity for three prototypical tastes (sweet, salty, and sour), and BMI in the subjects (n = 41, mean age: 27.0 ± 5.7 years). They also completed a self-reported questionnaire on their health condition including MS. Then, we analysed the relations between the values for taste sensitivities and the MS or BMI scores. We confirmed the inverse correlation between BMI scores and OFS among the subjects. In addition, we found that the MS scores for (i) the frequency of relaxation and (ii) number of mentors during personal distress were inversely associated with OFS. The frequency of relaxation was also associated with oral acetic acid sensitivity, but there were no other associations between the three prototypical taste sensitivities and the two aspects of MS. These results suggest that OFS may have a unique relationships with MS regarding mental stress accumulation in healthy young adults. (COI:No)

1P-103

Development of a new sensor array for noninvasive measurement of laryngeal movement during swallowing

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During swallowing, the larynx rises adequately and closes trachea tube with epiglottis. Measurement of the laryngeal movement would be useful to evaluate the swallowing function. Therefore, we tried to develop a new noninvasive equipment to do this. We made small piezo electric sensors (length 1.5 mm, width 7.0 mm, thickness 3.6 mm). Then these five sensors were lined with 3.0 mm interval accurately and embedded in the middle of polyurethane gel sheet (80 × 100 × 8 mm). The sensor sheet was lightly attached to the ventral surface of the neck near the laryngeal prominence. Then the subject was instructed to swallow 3 ml water ten times. The sensors that positioned between the resting and highest position of the larynx during swallowing showed two positive peaks. The first and second peak corresponded to the period when the larynx passing to upper and lower position during swallowing. Mean rising velocity for men (n=6) and women (n=6) was 79 ± 23 mm/s and 106 ± 21 mm/s, respectively. Similarly, mean lowering velocity for men and women was 94 ± 29 mm/s and 110 ± 57 mm/s. The latency of the command swallow for men and women was 0.487 ± 0.111 s and 0.531 ± 0.180 s. All three parameters have no significant sex difference. In conclusion, we succeeded to develop a new sensor array to detect the laryngeal movement noninvasively, and this array will be useful to evaluate the swallowing function. (COI:No)

1P-104

fMRI study of brain activation during teeth tapping in the aged people

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The cortical activities during jaw movements have been investigated with various noninvasive brain imaging methods (e.g., PET, MRI, MEG), yet how orofacial sensory inputs contribute to jaw movement remains unclear. Here, we examined brain activities during a simple teeth tapping by functional magnetic resonance imaging (fMRI) in three groups, an elderly dentulous (ED) group, an elderly edentulous group with (EDW) and without denture wearing (EE). A general linear model analysis revealed that teeth tapping induced activation in sensorimotor cortex, supplementary motor area, cerebellum, thalamus, basal ganglia and insula in all groups (conjunction analysis FWE, p=0.05). Comparisons between groups indicated that activity in sensorimotor cortex and supplementary motor area significantly greater in ED group (p=0.005). The dorsolateral prefrontal cortex (DLPFC) was activated in ED and EDW, but not EE. A functional connectivity analysis, psychophysiological interaction (PPI) analysis, showed that tapping with denture wearing enhanced effective connectivity from sensorimotor cortex to thalamus, basal ganglia, cerebellum, DLPFC and supplementary motor area (p=0.005). These results suggest that sensory inputs from oral region are significantly involved in the fine control coordination and modulation of teeth tapping that in the past have been attributed largely to brain stem regulatory mechanisms. (COI:No)

1P-105

A novel method for assessment of reaching and grasping movements in head-fixed mice

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Reaching and grasping is a common forelimb movement in mammals and essential for animals life. To understand the mechanisms of reaching and grasping at the level of neural circuits, it is required to observe activities of individual neurons during the behavior. Head-fixation is necessary for minimizing motion artifacts to establish stable electrical and/or optical recordings of neural activities in behaving animals. Therefore, we have developed a new method for investigating reaching and grasping behavior of head-fixed mice. Male and female C57BL/6J mice (>4 weeks of age) were used. After recovery from the surgery for attaching a metal plate on mice head, a series of procedures were started. The procedures consist of habituation, water restriction, and behavioral training. A piece of 2 % agar (a 5-milimeter cube) was provided in front of the mouse as reward by using an automated dispenser. We monitored the reaching and grasping movements with the right forelimb by using two cameras at the maximum sampling rate of 200 frames per second. Mice could successfully learn to get the agar cube around seven days. By combining this behavioral task with two-photon calcium imaging and optogenetics, neural activities during reaching and grasping movements can be observed and manipulated, respectively, for the investigation of motor control and its underlying circuit mechanisms. (COI:No)

1P-106

Effects of speed on kinematics and muscle activity during treadmill locomotion in unrestrained monkeys

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To investigate how Japanese monkeys, which are a quadruped by nature, adapt to speed when walking bipedally, animals were asked to perform quadrupedal (QL) and bipedal locomotion (BL) on a treadmill at different constant speeds. We recorded side and back views of the monkeys using high-speed video cameras and EMG activity from limb and back muscles. Kinematics and EMGs were compared between QL and BL. We found that, during both QL and BL, the stride length, stepping frequency and EMG amplitudes of recorded muscles increased with increases in speed. However, three critical differences were found in the EMG activity. First, recruited activity with increasing speed was larger for BL than QL. Second, although majority of hind-limb extensors peaked around touchdown for both QL and BL, another peak progressively emerged in the late stance phase during BL at higher speeds. Third, back muscles during QL showed discrete brief bursts twice a step at the timing of touchdowns of the left and right hindlimbs. However, during BL, they showed broad biphasic modulation, troughs of which located around the touchdowns of two hindlimbs. Such activity patterns were far more pronounced at higher speeds during BL than QL. The results suggest that, when walking bipedally, the monkeys adjust the speed by accommodating acceleration and deceleration forces generated by one limb and the other, and they secure the stability by integrating rhythmic limb movements and dynamic truncal posture, as observed in human gait. Partially supported by JSPS KAKENHI Grant No. 26120004. (COI:No)

1P-107

Alterations in movement representations in the motor cortex of type 1 diabetic rats

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We recently reported a decrease in the number of corticospinal tract (CST) neurons that descend their axons to lumbosacral segment in type 1 diabetic rats. While CST injury induces alteration in movement representations in the sensorimotor cortex, there is no such information available in diabetic animals. Here, we examined movement representations in the sensorimotor cortex of type 1 diabetic rats using electrophysiological technique. Twelve Wistar male rats, aged 13 weeks, were used in this study (n = 6, diabetic group, by streptozotocin (STZ) injection at 13 weeks of age; n = 6, control group). At 13 weeks after STZ injection, the animals were anesthetized, and movement representations in the sensorimotor cortex were assessed by intra-cortical microstimulation. Experimental diabetes significantly decreased the net area of the motor cortex representing the hind limb and trunk ($P < 0.01$). On the other hand, the forelimb area remained nearly unchanged. Additionally, motor conduction velocity was decreased in diabetic rats ($P < 0.01$). This study showed that diabetes elicits a marked attenuation of cortical-evoked motor function, i.e., predominant decrease in the hind limb and trunk area. These alterations seem to coincide with the decrease in number of CST neurons that descend their axons to lumbar and sacral segments and might account for muscle weakness around the knee and ankle described in diabetic patients. (COI:No)

1P-108

Cardiovascular response to up-ramp load in cycling exercise

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Aim: We analysed cardiovascular responses to up-ramp load during cycling exercise. Methods: Eight healthy young adults executed cycling exercise with three minutes up-ramp of various ramp slope inclinations. Cardiovascular functions were continuously monitored with a finger blood pressure monitoring system. Responses were normalized and delays of the responses to the up-ramp load increase were analysed. Results: Responses of cardiovascular parameters showed considerable delays to the increase of the ramp load intensity, though the amounts of delay were smaller than those of respiratory parameters. Significant differences were also observed in the amounts of the delay among the measured cardiovascular parameters: while cardiac output showed only a negligible amount of delay to the up-ramp load, heart rate increase showed more than 10 second-delay. This discrepancy was explained by the initial rapid decrease in total peripheral vascular resistance. Discussion: The increase in cardiac output follows the increase in load intensity almost faithfully without significant delay. This may indicate that cardiovascular system acts to an initial small increase in the load rather exaggeratedly prospecting further increase of the load. (COI:No)

1P-109

Cardiovascular responses to standing-up and sitting-down in healthy young adults

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Aim: Cardiovascular responses to standing-up from sitting position were observed and compared with the opposite sitting-down responses. Method: Cardiovascular functions in healthy young adults were continuously monitored with a finger blood pressure monitoring system during standing-up and sitting-down with one minute intervals. Systolic and diastolic blood pressure, cardiac output, heart rate, stroke volume and total peripheral vascular resistance (TPR) were analysed. Results: there existed two distinguishable components in cardiovascular response to the postural change. One is a phasic response lasting for the initial 15 seconds, which was most clearly visible as a decrease in TPR, and was partially compensated by a transient increase in cardiac output, leaving only a small fluctuation of the blood pressure. The other is a static component, that had opposite direction in the standing-up and in the sitting-down response, lasting till the next postural change, though gradual decrease of response amplitude could be observed. Discussion: The phasic decrease in TPR occurred both to the standing-up and to the sitting down. These phasic responses seem to be due to primarily an autonomic nervous response to postural change itself regardless of the direction of the change. (COI:No)

1P-110

Cardiovascular response to breath-holding during cycling exercise

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Background: Diving reflex is a mechanism enabling the body to manage and tolerate a lower level of oxygen when faces are cooled or we hold our breath. Dependency of this reflex to exercise intensity has been not exactly investigated yet. Aim: To clarify load dependency of cardiovascular response to breath-holding (BH), we performed short-term BH during cycling exercise at various levels of intensity. Method: Seven healthy young adult males were participated in the experiment. Subjects performed 15 second BH during cycling exercise and cardiovascular parameters were continuously monitored by a finger-blood pressure system with 5 minutes interval of definite exercise intensity. Results: By BH, cardiac output as well as heart rate showed decreasing response. Total peripheral vascular resistance, on the other hand, remarkably increased during BH. The responses tended to increase with the exercise intensity. Conclusion: Hemodynamic response is supposed to be mainly caused by abrupt change of vascular resistance on even short-term breath-hold during exercise. (COI: No)

1P-111

Effect to the lactate disappearance of the cool down method decreasing load

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The cool down is effective in recovery of muscle fatigue. Previous research reported the time of the cool down to put it into effect by the fixation strength, but the study about the way to load volume decreasing method wasn't accomplished so much. This time, we performed the cool down which reduces the loading volume method for 10 people for each of adult men and women. One cool down way was the strength of 50%VO_2max, the other cool down way was making the loading volume decrease with 50%, 40% and 30%VO_2max .They were performed on different day. We made the wattage increased from the rest position to the loading volume which becomes 80%VO_2max. The cool down was done for 15 minutes after the movement. The blood lactate was measured in the time of rest, after the movement end, 5 minutes later of cool down starting, 10 minutes later of cool down starting and 15 minutes later of cool down. The rate of lactate disappearance each time was calculated based on blood lactate of the time of rest. The statistical significance of the results was assessed using one-way analysis of variance and Bonferroni test. The significant level was set to 5%. The rate of lactate recovery of cool down starting 5 minutes later, 10 minutes later and 15 minutes later were 22.6%, 87.2% and 105% by fixed method. They were 41.9%, 93.5% and 122.9% by decreasing method. The significant difference was obtained by decreasing method between 10 minutes later and 15 minutes later ($p<0.05$). The decreasing method was useful for lactate disappearance. (COI:No)

1P-112

The effects of downhill running training on skeletal muscle and hippocampal BDNF expression in type II diabetic rat

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Type II diabetes (T2D) induces muscle fragility and impairs cognitive function. Downhill (DH) running may prevent skeletal muscle fragility and improve cognitive function in the T2D. The purpose of this study was to examine the effects of DH running training on skeletal muscle and hippocampal brain-derived neurotrophic factor (BDNF) expression in T2D rat. Wistar (NM) and Goto-Kakizaki (T2D) rats were divided into training and control groups: NM control, NM with DH training, T2D control (T2D-CONT), and T2D with DH-TR (T2D-DH). Training groups performed intermittent DH running (10 degree decline) five days a week for six weeks. After the training, mechanical stress was applied to the tibialis anterior muscle with surface electrical stimulation. Muscle damage was evaluated from hematoxylin-eosin stained cross-sections as relative number of damaged fibers in relation to intact fibers. BDNF concentration was measured using enzyme-linked immunosorbent assay. Muscle-to-body weight ratio was higher in the T2D-DH than in the T2D-CONT ($p < 0.05$). Muscle damage reduced in the T2D-DH ($3.2 \pm 2.5\%$) relative to the T2D-CONT ($38.1 \pm 10.5\%$, $p < 0.001$). BDNF concentration was higher in the T2D-DH than in the T2D-CONT ($p < 0.05$). The present results suggest that DH running training may improve resistance to mechanical stress in skeletal muscle and cognitive function in T2D. (COI:No)

1P-113

The effect of force movement on motor function recovery in a rat model of intracerebral hemorrhage

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A stroke is induced by sudden loss of blood supply to an area of the brain because of hemorrhage or thrombosis of a blood vessel. It causes a severe problem because of its secondary disease that impairment of motor and sensory functions. Furthermore these dysfunctions reduce the activities of daily living and the quality of life. It is well known that the exercise is effective method for functional recovery after intracerebral hemorrhage (ICH). However, the effective types of exercise have not been well known. This study aimed at examining the effect of force exercise on recovery of motor function using treadmill running in ICH rat. Male SD rats were injected with collagenase (200U/mL) into striatum to induce ICH. They were divided into the ICH group and the ICH + treadmill group. The treadmill group was trained from 4-14 days after surgery. Motor functions were assessed by motor deficit score (MDS) at 0-15 day in both groups. The ICH + treadmill group showed significantly motor function recovery compared with ICH group. We next evaluated subdivision of MDS in the ICH + treadmill group. The effect of treadmill exercise on the recovery of motor function significantly increased in the hindlimb and coordination, but not in the forelimb and trunk function. It was revealed that the treadmill exercise is more effective for the recovery of hindlimb than forelimb and trunk. These data suggest that the treadmill exercise promotes the limbs coordination recovery. (COI:No)

1P-114

Downregulation of KCC2 accelerates motor function recovery after axonal injury

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Neuronal K^+-Cl^- co-transporter 2 (KCC2) through its Cl^- extrusion activity ensures hyperpolarizing GABA/glycine responses. Increased excitability of neurons coincident with KCC2 downregulation in several pathological conditions likely affects circuit remodeling and ultimately functional recovery. However, whether KCC2 downregulation benefits or impedes functional recovery is still unknown. We investigated the relationship between KCC2 downregulation and functional recovery after sciatic nerve injury. We first checked the time course of KCC2 expression after injury and found it was significantly reduced in the ventral horn of the spinal cord ipsilateral to the injury at 3 days after injury. We rescued KCC2 downregulation using CaMKII-tTA/KCC2-tetO mice and found that this impaired motor recovery as measured by a rotarod test. To test whether the impact of KCC2 involved GABA signaling, we injected Bicuculline into the ventral horn at 3 and 5 days after injury. Functional recovery of mice injected with Bicuculline was reduced compared with ACSF injected mice. Finally, we found significantly reduced GAD67 expression at 42 days after injury in wild type but not in KCC2 mice. These results suggest that motor function recovery depends on acute downregulation of KCC2 and potentially involves chronically reduced GAD expression. (COI:No)

1P-115

Development of sympathetic and parasympathetic regulations in muscle-like cellular organizations derived from mouse ES cells

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Gut-like cellular organizations are derived from embryonic stem (ES) cells and induced-pluripotent (iPS) cells, and the degree of differentiation is estimated by morphological and histological features. However, gut-like organoids have not been sufficiently assessed in functional aspect. We thus developed organoids following the methods previously used for gut-like organoids, and observed spontaneous contractions, intracellular Ca^{2+} oscillations and responses to sympathetic and parasympathetic agonists. We induced embryonic bodies (EB) from mouse ES cells by a procedure of hanging drop culture for 6-7 days, and subsequently cultured EB on gelatin-coated dishes for 2-3 weeks to obtain small sheet-like organoids showing spontaneous contraction. We distinguished two types of organoids by the frequency of contraction: gut muscle-like and cardiac muscle-like sheets. In the former with c-Kit immunoreactivity the frequency was significantly lower. In addition, the distance and duration of contraction quantified using an image tracking analysis were significantly larger in the former. Intracellular Ca^{2+} waves measured in the former samples using Fluo-4 mimicked those previously observed in gut musculatures of mice. Applications of sympathetic and parasympathetic agonists prolonged and shortened the interval of Ca^{2+} activity in gut muscle-like samples, respectively. Even in the same culture dish, gut muscle-like and cardiac muscle-like organoids were differently conferred reciprocal regulations of the autonomic nervous system, depending on their physiological functions. (COI:No)

1P-116

Transcription factor ATF5 is involved in the maturation of goblet cells in mouse colon

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The endoplasmic reticulum (ER) stress is caused by the accumulation of unfolding proteins in the lumen of the ER. The ER stress activates a network of signaling pathways termed the unfolded protein response (UPR) to maintain the cellular homeostasis. Recently, genetic deletion studies have revealed that the UPR is closely associated with the colitis. Activating transcription factor 5 (ATF5) is a bZIP type transcription factor, which is activated by post-transcriptional regulation via the phosphorylation of eIF2α in response to UPR. In this study, we investigated the role of ATF5 on the differentiation of intestinal mucosa using ATF5-deficient mice. ATF5 mRNA was expressed in small and large intestines, and the gross morphology of villi and crypts appeared normal by the disruption of ATF5. We found that the expression of Muc2, a mature goblet cell marker, was decreased in ATF5-deficient colon compared with the wild type by qPCR. We confirmed that the number of mature goblet cells was significantly decreased in ATF5-deficient colon by PAS staining and Muc2 immunohistochemistry. However, the expression of Tff3, a progenitor goblet marker was not affected in ATF5-deficient colon. Interestingly, we observed the increase of inflammatory cytokines such as IL-1β in ATF5-deficent colon compared with the wild type. These results indicated that ATF5 is involved in the maturation of goblet cells in the colon, suggesting its protective role against the colitis by promoting mucus production. (COI:No)

1P-117

Functional assessment of intestinal morphological changes during metamorphosis in frog

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Introduction: The abrupt morphological changes of intestine during metamorphosis has been well documented. The features of intestinal metamorphosis were shortening of the intestine and remodeling of the intestinal epithelium. It is believed that the purpose of the morphological changes of intestine is adaptation from aquatic herbivorous to terrestrial carnivorous life. However, little is known about the physiological functional importance of these morphological changes. To elucidate the functional changes during metamorphosis, we measured luminal Na concentrations and Na-dependent glucose uptake in tadpoles and adult frogs. Methods: The small intestine was isolated and divided into four segments, the luminal contents collected for analysis of ion concentration for determining by ion chromatography. Phlorizin-sensitive glucose-induced short-circuit current (δIsc) was measured in intestinal preparations mounted in Ussing chambers. Results: Although dietary sodium intake was extremely low in tadpoles, luminal Na concentration gradually increased along proximal to middle part of intestine (>70mM). And this Na concentration was comparable to carnivorous adult frog. The increment of δIsc was observed in tadpole intestine. These results suggesting that luminal Na homeostasis is important and is kept at a high concentration for Na-dependent nutrient mechanisms. (COI:No)

1P-118

Na-dependent glucose absorption and Na recirculation via paracellular pathways in mouse small intestine

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Many nutrients are absorbed by Na-dependent transport mechanisms in the small intestine. However, little is known about how the intestine meet the needs of Na for nutrient absorption. It is thought that Na diffuses back into the lumen via paracellular pathways to support nutrient absorption. However, direct experimental evidence in support this idea has not been shown. In this study, to investigate whether paracellular pathways are involved in this Na recycling, we measured glucose-induced short-circuit currents (δIsc) under open and short-circuit conditions in Ussing chambers. And we simultaneously measured changes in unidirectional ^{22}Na fluxes (δJ) in a wild-type mice mucosal sheets and compared them with those of claudin 15 deficient mice. The results showed that, under the short-circuit condition of the wild-type, luminal application of glucose resulted in an increase in δJ which corresponded to the amplitude of δIsc. However, under the open-circuit condition, δIsc was observed but δJ was strongly inhibited. In claudin 15 KO, a robust increase in δJ was observed under the open-circuit condition. These observations raise the possibility that the Na which is absorbed into enterocytes is rapidly recycled back into the lumen via paracellular pathways which are driven by increased luminal negativity. (COI:No)

1P-119

Characterization of the biological activity of xenin fragments on spontaneous circular muscle contraction in rat distal colon

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Xenin25 is a 25-amino acid neurotensin-related peptide produced by GIP-producing K cells in the small intestine. In the upper intestine, xenin25 induces an increase in insulin release at physiological glucose concentrations. Xenin25 affects smooth muscle activity in the rat colon. Native xenin25 is efficiently degraded by plasma enzymes. However, little is known about the physiological importance of these degradation fragments on colonic intestinal motility. The present study examined the effect of xenin fragments on smooth muscle activity in the rat distal colon. Xenin25 and its fragments were synthesized by the Fmoc-strategy and were purified by reverse-phase HPLC. For the mechanical experiments, full-thickness circular muscle strips were attached to isometric transducer under a constant load of 10~20 mN in 15 mL oxygenated Krebs solution maintained at 37 OC. An amplifier (Quad Bridge Amp.) and a Power Lab system (ML846) were used to record circular muscle activity. The results showed that Xenin5-25, 9-25, 11-25 and 14-25 had essentially similar biological actions as native xenin25. Although further study is needed to identify the bioactive domain(s) with the xenin25 molecule, these results indicate that action of xenin25 in the colon is different from that of upper intestine. (COI:No)

1P-120

Contribution of the apical Na^+/H^+ exchanger 3 to intestinal calcium absorption in hemizygous beta-globin knockout thalassemic mice

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In the apical membrane of the intestinal epithelial cells, Na^+/H^+ exchanger (NHE)-3 is an essential transporter that provides a driving force for nutrient absorption and also helps maintain intracellular pH. A previous in vitro study has shown that intracellular pH imbalance could negatively affect cytoplasmic calcium translocation during calcium absorption. We, therefore, hypothesized that NHE3 inhibitor tenapanor might diminish calcium absorption in hemizygous beta-globin knockout thalassemic (BKO) mice, in which calcium absorption had already impaired. Herein, iron and calcium absorption rates were determined by radioactive Fe-59 and Ca-45 in Ussing chamber, respectively. Our results showed that BKO mice had low hemoglobin levels and increased duodenal iron transport, the latter of which might be a compensatory response to chronic anemia. The duodenal calcium transport was markedly lower in BKO mice as compared to wild-type littermate. However, this impaired calcium transport was rescued by hepcidin, an inhibitor of iron absorption. Finally, as expected, NHE3 inhibitor tenapanor completely abolished the hepcidin-induced calcium transport in BKO mice. In conclusion, we have provided evidence that NHE3 contributes to the intestinal calcium absorption particularly in BKO mice. Supported by grants from Thailand Research Fund (RTA5780001 and PHD/0219/2553) and Mahidol University. (COI:No)

1P-121

Timing of post-exercise nutrient ingestion: effects on gastric emptying and glycemic response in humans

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After resistance exercise, the immediate protein ingestion is recommended to promote the muscle protein synthesis during recovery. In other hand, during the short duration immediately after the resistance exercise, the gastrointestinal tissues are often impaired by hypo-perfusion, possibly resulting to the acute dysfunction of digestion and absorption, such as gastric emptying (GE). From the latter viewpoint, the aim of this study was to examine the effect of timing of post-exercise protein ingestion on GE and blood glucose (BG). Eleven healthy subjects randomly ingested 400 mL of protein drink containing 12 g carbohydrate and 20 g protein either at rest (Con), the timing of either 5 min (PE5), or 30 min (PE30) after a single bout of resistance exercises which were consisted of 6 sets of 10 repetitions on leg press, lat pull-down and chest press machines. Each 1st and 2nd sets were performed at 50% of the subject's one repetition maximum (1RM). The subsequent sets were performed at 75% of 1RM until the exhaustion. The GE were assessed by the ^{13}C sodium acetate breath test. The times when the $[^{13}CO_2]$ recovery per hour reaches a maximum ($T_{max\text{-}calc}$) and when the total cumulative dose of the $[^{13}CO_2]$ reaches one-half ($T_{1/2}$) were calculated according to a standard analytical method. $T_{max\text{-}calc}$, $T_{1/2}$ and BG response were slower for PE5 than that for PE30 and Con. Protein ingestion immediately after strenuous resistance exercise transiently impaired digestion and absorption, and consequentially affects glycemic response. (COI:No)

1P-122

Anti-stress effects of indirect moxibustion via central oxytocin on gastric emptying in rats

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Japanese-style acupuncture often involves the use of moxibustion to achieve optimal results for gastric disorders. Previous studies have shown that both electro-acupuncture (EA) and indirect moxibustion (iMOX) at ST36 of the tibialis anterior muscle, improved stress-induced delayed gastric emptying (GE) by stimulating the parasympathetic nerve. In addition, the central oxytocin elicited by EA attenuates the response of delayed-GE to stress. The purpose of this study was to investigate whether iMOX at ST-36 improves restraint stress-induced delayed GE via the central oxytocin. Rats were given of solid food after 24 hours of fasting. Immediately after the ingestion, rats were loaded to restraint stress. Ninety minutes after the feeding, rats were euthanized and their gastric content was removed to calculate GE. Six iMOXs were performed at both of the ST-36 locations during the stress loading. To investigate whether central oxytocin was involved in mediating the stress-induced alterations of GE by iMOX, oxytocin antagonist was administered (i.c.v.) just after the start of restraint stress. GE in the 90-minute study period was significantly delayed by restraint stress. This delayed GE was significantly accelerated by iMOX, whose effects were blocked by the oxytocin antagonist. In conclusion, endogenous central oxytocin is involved in mediating the stimulatory effects of iMOX on restraint stress-induced delayed GE. (COI:No)

1P-123

Measurement of geometric center in colonic transit by the radiopaque marker under the X-ray

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We had shown that the new method for the mesurement of the colonic transit (CT), was able to evaluate repeatly in a rat that had been using the radiopaque marker under the X-ray. Furthermore, we had already evaluated CT changes by pharmacological technique. However, geometric center (GC), that is widely using for the indicator for the CT, have been not calculated in our studies that is using just maximum migration length of the marker. Therefore, aim of the present study is to assess CT of normal conscious rats calculating the GC. Thirteen male Sprague-Dawley rats were used. Under isoflurane anesthesia, an in-dwelling silastic cannula was inserted into the caecum and positioned to enter the proximal colon. Five days after the surgery, 20 metal radiopaque markers, whose diameter is 1.5mm, were administrated into the proximal colon with saline (1.0 ml). It was visible throughout the GI tract via soft X-ray when the first maker output with fecal pellet. Just after imaging in vivo, the entire colon was surgically removed and imaged. CT was calculated by the GC on the images of those. It is possible calculating the GC at the time of the first marker output using the method. GC is no difference between in vivo and the removed entire colon. These results have shown that CT is able to be measured in vivo chronologically using the radiopaque marker by the GC. (COI:No)

1P-124

Effects of cabbage vinegar on isolated intestinal functions of mice

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Gunma Agriculture Technology Center developed a new vinegar using cabbage because Gunma prefecture is the major production area of cabbage in Japan. Various functions of vinegars including inhibition of postprandial blood glucose level are reported. The present study examined effects of cabbage vinegar (CV) on isolated intestinal functions of mice for evaluating characteristics of CV by comparing with those of grain vinegar (GV) and acetic acid solution (AA). In order to investigate effects of each sample (CV,GV,AA; vinegar sample) on starch digestion and glucose absorption, everted sacs of isolated small intestine of mice were dipped in Ringer solution including 0.5% soluble starch or 10mM glucose with neutralized vinegar samples (acidity: 0.0225%-1%). Intestinal motility was measured by using Magnus method and effects of vinegar samples were examined by changing Krebs solution including neutralized vinegar samples (acidity: 0.25%-1.0%). All vinegar samples inhibited each intestinal function acidity-dependently, but their effects were different from intestinal positions. The results showed that inhibitory effects by vinegar samples occurred by action of AA. When results obtained from applications of each vinegar sample were compared, CV showed larger inhibition than others. Not yet known, but some components in CV might be also related with inhibitory effects. Experiments for clarifying mechanisms of these inhibitory effects by CV are in progress. (COI:No)

1P-125

High-salt diet and intestinal Na^+/K^+-ATPase in hypertensive Dahl salt-sensitive rats

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We have previously demonstrated that high-salt diet increases intestinal secretion of Na^+, Cl^-, and water in Sprague-Dawley (SD) rats but not in hypertensive Dahl salt-sensitive (DSS) rats. In the proximal renal tubules (PRT) of SD rats, high-salt diet enhances the endocytosis of basolateral Na^+/K^+-ATPase (NKA) and luminal Na^+/H^+ exchanger 3 to stimulate natriuresis, which is suppressed in hypertensive DSS rats. Since the mechanism of an intestinal ion transport has many similarities with PRT, the aim of this study is to elucidate whether high-salt diet changes intestinal NKA activity and its endocytosis in DSS rats. Male DSS and SD rats were divided into two groups; one fed on a high-salt diet (DSSH, SDH), and the other fed on a regular diet (DSSR, SDR) for 4 weeks. The intestinal mucosa-submucosal preparations from each groups were mounted on the Ussing chamber to measure short-circuit current (I_{sc}) induced by nystatin to permeabilize mucosal side in the presence or absence of mucosal Na^+. Nystatin-induced I_{sc} in the presence of mucosal Na^+ represents basolateral Na^+ outward current driven by NKA, which almost all varnishes in the absence of mucosal Na^+. High-salt diet significantly decreased nystatin-induced I_{sc} in SD (129.4±6.9 for SDH versus 179.7±7.3μA/cm^2 for SDR) but not in Dahl (208.7±10.8 for DSSH versus 198.4±9.6μA/cm^2 for DSSR) rats indicating that high-salt diet decreases NKA activity in SDH but not in DSSH. Effects on its endocytosis is under study by comparing the NKA distribution ratio (plasma membrane versus cytosol) among 4 rat groups. (COI:No)

1P-126

Butyrate activates XE991 sensitive potassium secretion in rat rectal colon

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Short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate are synthesized from dietary carbohydrate by colonic bacteria fermentation. These SCFAs are considered to contribute not only to an energy source or prevention of cancer but also regulation of ion transport. Previously, we have shown that the effects of 30 mM butyrate application on rat colon with short-circuit current (I_{SC}) measurements. In this study, we revealed that butyrate induced I_{SC} shift after amiloride application (amiloride-resistant I_{SC} shift, referred as $I_{r\text{-}amil}$ shift) toward negative direction which was inhibited by 100 μM bumetanide, a Na^+-K^+-$2Cl^-$ cotransporter inhibitor. This $I_{r\text{-}amil}$ shift was also inhibited by XE991, a KCNQ type potassium channel inhibitor, in dose-dependent manner. RT-PCR and immunofluorescence analyses demonstrated that KCNQ2 and KCNE2 localized in luminal membrane of surface cells of rat rectal colon. These results indicate that butyrate might activate electrogenic transport through KCNQ type potassium channels in the luminal membrane of rat rectal colon. (COI:No)

1P-127

Molecular evidence for the involvement of free-fatty acid receptor 3 (FFA3, GPR41) in the short-chain fatty acid-evoked intestinal anion secretion

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Short-chain fatty acids (SCFAs), which are 2-6 carbon monocarboxylates, are the predominant fermented products by intestinal microbiota. SCFAs not only are known to be absorbed as nutrients, but also induce an epithelial anion (fluid) secretion and an intestinal smooth muscle contractions. These effects of SCFAs are reported to be mediated via an epithelial stimulation from luminal (apical) side of the intestine. However, there had been no molecular evidence of the mechanism of receptors in the secretory effects. We therefore investigated the involvement of free-fatty acid receptors (FFAs) in the SCFA-evoked intestinal secretion. FFAs including FFA2 (GPR43) and FFA3 (GPR41) have previously been identified as receptors for SCFAs from orphan GPCRs in 2003. Mucosa-submucosa preparations of the cecum from wild type, FFA2-KO and FFA3-KO mice were mounted on Ussing chambers, and effects of SCFAs on short-circuit current (Isc) as an index of the electrogenic transepithelial ion transport. As results, propionate (C3 SCFA) evoked an transient increase in Isc in wild type and FFA2-KO, but evoked no response in FFA3-KO. Consequently, present study indicated a molecular evidence for the first time that the intestinal secretory effects of SCFAs were mediated via FFA3 (GPR41) receptors. (COI:No)

1P-128

Functional expression of adenosine A_{2B} receptor in pancreatic duct cells

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Introduction: Adenosine regulates transepithelial anion secretion in duct cells and is considered to play a role in acini-to-duct signaling in the pancreas. Pancreatic duct cells were found to express adenosine receptors at the messenger RNA level. However, the molecular basis of functional adenosine receptors and intracellular mechanism of ductal secretion via adenosine remain inconclusive. Objectives: The present study aimed to identify functional adenosine receptors in pancreatic duct cells. Methods: We measured whole-cell currents in guinea pig duct cells and in human adenocarcinoma cell line (Capan-1) single cells using gramicidin-perforated patch techniques. We measured transepithelial potential difference (V_{te}) in Capan-1 monolayer. The immunolocalization of adenosine receptors was performed using Capan-1 monolayer. Results: The application of adenosine induced sustained inward Cl^- currents at −83 mV with the half-maximal effective concentration (EC_{50}) values of 21 μM and 9 μM in guinea pig duct and Capan-1 cells, respectively. The luminal addition of adenosine increased negative V_{te} with the EC_{50} value of 12 μM in Capan-1 monolayer. These values approximately corresponded to that previously reported on adenosine A_{2B} receptors. Adenosine A_{2B} receptors localized in the luminal membrane of Capan-1 monolayer. Conclusion: These results indicated that adenosine regulates anion secretion via adenosine A_{2B} receptors on the luminal membranes of pancreatic duct cells. (COI:No)

1P-129

Computational model of bicarbonate transport by pancreatic duct epithelium with basement membrane

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Pancreatic duct cell produces isotonic HCO_3^- -rich fluid secretion. We constructed a mathematical model cell using MATLAB/Simulink. The model was composed of 4 compartments (bath, cell, intercellular space, and lumen) with basement membrane, basolateral and apical membranes, and tight junction. Solute permeability of basement membrane was set at 10^{-3} cm s^{-1}. The initial interspace volume was set at 1% of the cell volume. The negative hydrostatic pressure in the interspace required to draw water through basement membrane was calculated using an approximation of the pressure-volume characteristics of a thin-walled elastic tube (Spring & Hope, 1978). Cell stimulation was mimicked by increasing the activities of basolateral Na^+- HCO_3^- cotransporter and apical SLC26A6 Cl^-/HCO_3^- exchanger, increasing the permeability of basolateral K^+ channel and apical CFTR Cl^-/HCO_3^- channel, and decreasing the activity of basolateral AE2 Cl^-/HCO_3^- exchanger. The model produces fluid secretion containing ~140 mM HCO_3^- at the rate of ~3 nl min^{-1} mm^{-2} epithelium. The osmolarity of the bath, interspace, cell, and secreted/luminal fluid was 306.0, 306.0, 306.5, 314.2 mOsm, respectively. The hydrostatic pressure difference between bath and intercellular space was ~2 x 10^{-5} cm H_2O, and the decrease of the interspace volume required to achieve the value was infinitesimally small. (COI:No)

1P-130

Bicarbonate transport in interlobular pancreatic ducts from cystic fibrosis mice

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Cystic fibrosis (CF) is an autosomal recessive disorder caused by mutations of *CFTR* gene. HCO_3^--rich fluid secretion in pancreatic juice is derived from ductal epithelium and depends on CFTR. Pancreatic juice from CF patients is sometimes acidic. In the present study, we investigated the pathophysiology of H^+/HCO_3^- transport in CF exocrine pancreas by using interlobular ducts (diameter: ~100 μm) isolated from ΔF mice, a CF model in which the ΔF508 mutation was introduced. The isolated ducts were superfused with the standard HCO_3^--CO_2-buffered solution and the rate of fluid secretion was calculated from the increment of luminal volume and expressed as rate per unit area of ductal epithelium. In ducts from wild-type mice (wt/wt ducts), stimulation with forskolin (1 μM) increased the secretory rate to 0.41 ± 0.10 nl min^{-1} mm^{-2} (n=4). In ΔF/ΔF ducts, forskolin simulation caused fluid absorption (shrinkage of the lumen) at the rate of -0.30 ± 0.14 nl min^{-1} mm^{-2} (n=4). Intracellular pH (pH_i) was measured using BCECF in luminally microperfused ducts. Duct cells were acid-loaded by NH_4^+ (20 mM) pulse followed by Na^+ removal under forskolin stimulation. The rate of pH_i recovery upon restoration of luminal Na^+ was 0.17 ± 0.03 pH unit min^{-1} (n=10) in ΔF/ΔF ducts, which was significantly ($p<0.01$) faster compared to wt/wt ducts (0.09 ± 0.02, n=15). Thus, HCO_3^- influx/H^+ efflux across the apical membrane was up-regulated in ΔF/ΔF ducts. Our data suggest that CF pancreatic duct absorbs and acidifies luminal fluid. (COI: No)

1P-131

Investigation of ion channel role in trans- chorioallantonic-membrane potential

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Chick embryo chorioallantonic membrane (CAM) is an extraembryonic membrane which is formed by fusion of chorion from the ectoderm and allantosis from the endoderm. CAM is the vascular rich membrane mediating gas and nutrient exchanges. Chick embryo CAM is commonly used in the study of angiogenesis and tumor metastasis. This technique is known as CAM assay, considered as a classical method. Recently CAM assay has been refocused as an alternative to animal testing. However only few molecular study of trans-CAM potential were reported. In this study, Reverse-transcriptase PCR was performed to evaluate channel expression of CAM. Expression of ClC2, ClC5, ClC7, CFTR, CLCA, NKCC and ENaC was ditected. And we evaluated ion channel role in trans-CAM potential by in vitro Ussing's chamber method. Immunofluorescence was performed on some channels. These results add new insights into CAM assay. (COI:No)

1P-132

Transport of β-hydroxybutyrate by Monocarboxylate Transporter 9 (MCT9)

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Monocarboxylate transporter 9 (MCT9), an orphan transporter member of the 16th solute carrier family (SLC16), has amino acid sequence only 30-35% identity to its family members and distinguishes from MCT1 to 4. Less information about MCT9 function has been reported. We tried to characterize its physiological roles. The quantitative RT-PCR (qRT-PCR) against human tissue cDNAs revealed that the mRNA expression of MCT9 is ubiquitous expression in many tissues, highest in kidney. *Xenopus* oocytes expressing MCT9 demonstrated the [^{14}C]β-hydroxybutyrate transport in Na^+-and Cl^- dependent manner. The transport was also reduced in acidic condition. The concentration-dependent β-hydroxybutyrate transport of MCT9 was fitted to the two-component kinetics suggesting for the multiple binding sites of the substrate. MCT9-mediated β-hydroxybutyrate transport was inhibited by various organic anionic compounds such as probenecid, estrone sulfate, acetoacetate and lactate. This study indicates that MCT9 may function as a transporter for monocarboxylates such as ketone bodies and contribute for its renal excretion. (COI:No)

1P-133

Epithelial ion secretion of human nasal ciliary epithelium

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Airway epithelia play key roles in maintaining the volume and composition of airway surface liquid by regulating transepithelial ion transport. However, little information is available on ion transport in upper airway epithelia. We studied transepithelial ion transport of airway epithelia by measuring the short circuit current (Isc) in human nasal ciliary cells. In nasal ciliary epithelia, the amount of anion secretion was much larger than that of Na^+ absorption. To focus on the anion transport, experiments were carried out under a condition blocking Na^+ absorption by benzamil. Under a Cl^-/HCO_3^--containing condition, the basolateral addition of DIDS, a blocker of AE, NBC and Cl^- channels, transiently increased Isc followed by decrease of Isc. The results suggest that the transient increase in Isc would be caused by inhibition of Cl^- release via Cl^- channels to the basolateral side, and the decrease in Isc would be due to $[Cl^-]_i$ diminishment induced by inhibition of Cl^- uptake from the basolateral side. Under a Cl^--free HCO_3^--containing condition, Isc was much smaller than that under a Cl^-/HCO_3^--containing condition and the basolateral addition of DIDS slightly decreased the Isc without any transient increase. These results suggest that human nasal ciliary cells secrete mainly Cl^- but also HCO_3^-, contributing to control of the volume and composition of airway surface liquid. (COI:No)

1P-134

Mitochondrial substrate dependent changes of mitochondrial function

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Mitochondria are essential organelles in the energy metabolism of cells. The production of ATP from the mitochondria is based on their metabolites, however, their functional changes depending on mitochondrial substrates has not been clearly described. In this study, NADH, mitochondrial membrane potential(ψm) and oxygen consumption were monitored in different set of mitochondrial metabolites such as malate(M), pyruvate(P), glutamate(G) and succinate(S) in the presence or absence of inorganic phosphate. Any single metabolite could not maintain mitochondrial function, except S, which could consume the oxygen, however, ψm was not effectively formed. In combinations of two metabolites, only P/S and P/M combination could maintain mitochondrial functions. When the combinations of three metabolites were tested, generally mitochondrial functions were maintained except M/G/S combinations. When all metabolites were included, the mitochondrial functions were maintained. However, there were clear differences in NADH production, ψm formation, and oxygen consumption. The highest value was achieved in conditions of P/G/S. In general, the addition of inorganic phosphate increased the generation of ψm up to threefold and the oxygen consumption, however, the maximal NADH was decreased. In conclusion, mitochondrial function was not maintained with only P or other single metabolite. This study suggests a suitable metabolite combination for production of mitochondrial energy and indicates an effective energy metabolism through its combination. Support: 2014M3A9D7034366 & 2015M3A9B6028310 from NRF. (COI:No)

1P-135

Metabolic change of neuromedin U overexpression mice by using hydrodynamic gene delivery method

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Neuromedin U (NMU) is a neuropeptide isolated from porcine spinal cord and has multiple functions of regulating food intake, energy expenditure and circadian rhythmicity. NMU binds to two specific receptors, NMUR1 and NMUR2. Central or peripheral administration of NMU reduces food intake and increases energy expenditure. It is reported that NMU and NMS, which is another ligand for NMUR1 and NMUR2, are highly expressed at suprachiasmatic nucleus (SCN) of the hypothalamus. SCN is a main part of regulating circadian rhythms, and NMU system plays important roles for circadian rhythmicity. Not only in SCN but also in peripheral organs including liver and adipose tissues, circadian oscillator genes, such as *Per2* and *Bmal1*, are expressed, and peripheral circadian regulating genes play a role for metabolic homeostasis. Here, we established NMU overexpression mice by using hydrodynamic tail vein injection procedure (SB-NMU mice) and analyzed of their metabolic changes. Then we found SB-NMU mice reduced their body weight without change of food intake. On the other hand, locomotor activity, oxygen consumption (VO_2) and carbon dioxide emission (VCO_2) of SB-NMU mice were increased in the light phase. In addition, *Per2* and *Bmal1* genes in the liver of SB-NMU mice were changed. Based on these evidences, NMU system may have some roles for metabolic homeostasis by regulating peripheral circadian rhythmicity. (COI:No)

1P-136

Role of myokines in metabolic abnormalities of streptozotocin-induced diabetes

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We found that inhibition of AMPK activity in skeletal muscle by preferentially expressing dominant-negative AMPK (DN-AMPK) significantly improved STZ (streptozotocin)-induced metabolic abnormalities. To clarify the mechanism, I investigated effects of parabiotic mice between wild type (WT) and DN-AMPK STZ-treated mice. Parabiotic WT as well as DN-AMPK mice improved the metabolic abnormalities including hyperglycemia and high levels of ketone bodies and fatty acids, suggesting that humoral factors released from skeletal muscles regulate metabolic abnormalities in STZ-induced diabetes. We investigated a role of IL-6 (Interleukin-6), myokine released from skeletal muscle in STZ-induced diabetes. STZ-induced diabetes increased IL-6 protein expression in skeletal muscle and plasma IL-6 level, and those were returned to the control levels in DN-AMPK mice. Infusion of neutral antibody for IL-6 into STZ-induced diabetic mice improved the metabolic abnormalities, similar to those in STZ-treated DN-AMPK mice. Conversely, infusion of recombinant protein of IL-6 aggravated the metabolic parameters in STZ DN-AMPK mice. On the other hand, myonectin, a novel myokine which promotes fatty acid uptake in adipose tissue and liver, showed a lower concentration in STZ-induced diabetic mice, and plasma myonectin level was returned to the control level in STZ DN-AMPK mice. Infusion of neutral antibody for myonectin aggravated the metabolic parameters in STZ DN-AMPK mice. These results suggest that myokines, such as IL-6 and myonectin, play important roles in STZ-induced diabetes. (COI:No)

1P-137

Activation of nesfatin-1-containing neurons in the hypothalamus and brainstem by peripheral administration of oxytocin and oxytocin analog and central administration of kisspeptin in rats

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Newly identified anorectic neuropeptide, nesfatin-1 synthesizes not only in the peripheral tissue but also in the central nervous system. In the present study, we examined the effects of intraperitoneal (ip) administration of oxytocin and oxytocin analog and central administration of kisspeptin on nesfatin-1-immunoreactive (ir) neurons in the hypothalamus and the brainstem of rats, using immunohistochemistry for Fos. Ip administration of oxytocin and oxytocin analog caused marked increases of nesfatin-1-ir neurons expressing Fos-ir in the paraventricular nucleus (PVN), arcuate nucleus (Arc) and nucleus tractus solitarius (NTS). Intracerebroventricular administration of kisspeptin caused marked increases of nesfatin-1-ir neurons expressing Fos-ir in the supraoptic nucleus (SON), the PVN and the Arc. These results suggest that neurfatin-1 neurons in the hypothalamus and brainstem may be an important role to sense fine levels of peripheral oxytocin and central kisspeptin in rat. (COI:No)

1P-138

A possible feedforward mechanism for human selective brain cooling through gustatory sweating induced by TRPV1 activation

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The ingestion of capsaicin induces gustatory sweating predominantly in the face in humans. We studied its significance by investigating a 25-year-old man with a hemifacial gustatory sweating deficit with no thermoregulatory sweat impairment on either side of his face. We examined his sudomotor function using Minor's method (the iodine-starch test) and skin temperature distributions using infrared thermography simultaneously. Tastants were applied on the lingual apex at an ambient temperature of 25°C. Tabasco™ (capsaicin) and ginger (gingerol) (both activate transient receptor potential vanilloid 1: TRPV1) application induced hemifacial sweating, flushing, and decrease of skin temperature only on the unimpaired side of his face. Application of horseradish, oriental mustard (allyl isothiocyanate; activates TRPA [Ankyrin] 1), gum syrup (sweet), salt (salty), ume (sour), and instant coffee (bitter) (the last four tastants activate taste receptor cells) did not produce laterality of facial skin temperature. These results suggest that gustatory sweating may be induced by an activation of TRPV1, the receptor stimulated by hyperthermia. This sweating might induce selective brain cooling at higher temperatures because its distribution is predominantly facial. Since this sweating occurred immediately after tastant ingestion and increased rapidly, we hypothesize that this sweating may be a form of feedforward regulation. In conclusion, the significance of gustatory sweating may be cerebral protection from heat. (COI:No)

1P-139

Activated brown adipose tissue shows nuclear accumulation of phosho-nuclear factor kappa B-like immunoreactive protein

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Brown adipose tissue (BAT) generates heat in response to sympathetic activation, and is involved in body temperature regulation and body weight control. Our previous immunohistochemical studies showed that a protein (or proteins) detected by an antibody against phosphorylated nuclear factor kappa B p65 (pNFkB) accumulated in the nuclei of activated BAT cells. In this study, to examine if the pNFkB-like protein accumulated in the nuclei of activated BAT is real NFkB, we studied its molecular weight by Western blotting (WB). ***In vivo* study:** Mice were maintained at 30°C for 18 hours, and exposed to cold (4°C) for 15, 30 or 60 min. Mice kept at 30°C were used as the control. Their BAT was sampled under anesthesia, and the nuclear fraction was processed for WB. Exposing mice to 4°C significantly augmented a protein band with molecular weight 120,000. This response was evident after 15 min cold exposure and remained at similar levels after 30 and 60 min cold exposure. ***In vitro* study:** Primary culture of rat BAT cells were stimulated with a beta adrenergic agonist, isoproterenol (ISO: 1-10 μM), for 10, 30 or 60 min. The nuclear fraction was analyzed by WB. A protein band with molecular weight 45,000 was significantly augmented by ISO (1μM, 10 min). In both the *in vivo* and *in vitro* studies, we could not detect a protein band with molecular weight around 65,000 corresponding to that of NFkB p65. These results indicate that the protein that is accumulated in the nuclei of activated BAT is not NFkB p65. (COI:No)

1P-140

Role of microglial gap junction in the high-fat diet-induced feeding rhythm disturbance

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Background: High-fat diet (HFD) causes diet-induced obesity (DIO) and disturbs feeding rhythm, and correcting feeding rhythm is sufficient to prevent DIO. Yet, the exact mechanisms for HFD-induced feeding rhythm disturbance remain elusive. Saturated fatty acids (SFA) activate microglia in the arcuate nucleus of the hypothalamus and cause hypothalamic inflammation, leading to DIO. Activation of microglia triggers two different pathways, inflammatory cytokine signaling, and the detachment of gap junction. The former process is known to cause leptin resistance and contributes to DIO, but the role of the latter process was unaddressed. We used central-acting gap junction inhibitor INI-0602 (INI) to address the question. Method: We asked following questions in B6 male mice: Does INI prevent DIO? Is SFA responsible for feeding rhythm disturbance? Does INI prevent the feeding rhythm disturbance? Results: INI prevented DIO by mainly suppressing the initial hyperphagia induced by HFD feeding. SFA caused feeding rhythm disturbance by promoting feeding specifically in the light phase. INI prevented, but did not improve, the feeding rhythm disturbance by HFD-feeding. Conclusions: Signal propagation through detached microglial gap junction is important for the early phase of feeding rhythm disturbance caused by HFD, while the canonical cytokine signals plays more prominent role in the propagation and the maintenance of hypothalamic inflammation caused by HFD. (COI:No)

1P-141

EID1 inhibits adipogenesis through downregulation of GPDH

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Adipocytes (also called fat cells) are derived from mesenchymal/mesodermal stem cells. There are two types of adipocyte: white adipocyte contains large fat droplets; and brown adipocyte contains smaller droplets and numerous mitochondria for thermoregulation. A recent study has shown that EP300-interacting inhibitor of differentiation 1 (EID1) reduces the accumulation of triglycerides in mouse pre-adipocyte 3T3-L1 cells. However, little is known about the mechanism of inhibitory effect on fat accumulation of EID1. Here we report that over-expressed EID1 suppresses fat accumulation of 3T3-L1 cells through downregulation of glycerol-3-phosphate dehydrogenase (GPDH; EC.1.1.1.8), a marker of adipocyte differentiation. EID1 expression vector was transfected to cultured 3T3-L1. Then, transfected cells were stimulated with IBMX, dexamethasone, insulin, and rosiglitazone for induction to differentiated adipocytes. After 9 days of stimulation, almost all 3T3-L1 cells were observed the accumulation of lipid droplets. In contrast, 70% of 3T3-L1 cells transfected with EID1 did not differentiate and the activity of GPDH was significantly reduced. These findings indicate an important function of EID1 in the regulation of adipocyte differentiation through downregulation of GPDH. (COI:No)

1P-142

Hypothalamic neuropeptide Y inhibits brown adipose tissue thermogenesis via activation of medullary reticular GABAergic neurons

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Hunger stimulates release of neuropeptide Y (NPY) in the paraventricular hypothalamus (PVH) from projections from the arcuate nucleus. The NPY action in the PVH inhibits brown adipose tissue (BAT) thermogenesis, leading to reduced energy expenditure for the survival of starvation. However, the neural circuit mechanism by which the NPY action leads to inhibition of BAT thermogenesis has been unknown. In this study, we found that hypothalamic action of NPY activates GABAegic neurons in the intermediate and parvicellular reticular nuclei (IRt/PCRt) of the medulla oblongata that project to the rostral medullary raphe, which contains sympathetic premotor neurons controlling BAT thermogenesis. Pharmacological stimulation of IRt/PCRt neurons inhibited BAT thermogenesis as well as elicited mastication and increased food intake. Furthermore, selective stimulation of GABAergic neurons in the IRt/PCRt with pharmacogenetic technology inhibited cooling-induced BAT thermogenesis. Finally, inactivation of IRt/PCRt neurons reversed NPY-induced inhibition of BAT thermogenesis. These results demonstrate a hunger response circuit in which NPY signaling from the hypothalamus activates GABAergic IRt/PCRt neurons, which then inhibit thermogenic sympathetic outflow to BAT to reduce energy expenditure. (COI:No)

1P-143

Molecular basis underlying white adipose tissue remodeling that precedes hibernation in Syrian hamster

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Hibernation is an adaptive strategy for winter survival with little or no food by remarkably suppressing the metabolism and core body temperature. Mammalian hibernators undergo body remodeling in fall to survive a cold winter; they store fat extensively in white adipose tissue (WAT) from late summer to fall and utilize it as an energy source during winter hibernation. However, molecular mechanisms underlying WAT remodeling for hibernation have not been fully elucidated. To address this, we conducted morphological and transcriptome analyses of inguinal WAT (iWAT) in Syrian hamsters among non-, pre-, and hibernation period. We found that the animals exposed to prolonged short photoperiod and cold (SD-Cold) stimuli reduced the size of adipocytes and became to have beige-like cells in iWAT during the transition from non-hibernation state to hibernation state. The prolonged SD-Cold stimuli induced genes involved in lipid catabolism and anabolism prior to the onset of hibernation. These genes known as targets of peroxisome proliferator-activated receptor (PPAR) were indeed up-regulated by pharmacological treatment of PPAR agonists in iWAT explants from Syrian hamster. These results suggest that seasonal activation of PPAR signaling induces iWAT remodeling during pre-hibernation period in Syrian hamster to adjust their metabolism for hibernation. (COI:No)

1P-144

Hypometabolism during daily torpor in mice is dominated by reduction in the sensitivity of the thermoregulatory system

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Some mammals enter a hypometabolic state either daily torpor (minutes to hours in length) or hibernation (days to weeks), when reducing metabolism would benefit survival. Hibernators demonstrate deep torpor by reducing both the sensitivity (H) and the theoretical set-point temperature (T_R) of the thermogenesis system, resulting in extreme hypothermia close to ambient temperature. However, these properties during daily torpor remain poorly understood due to the very short steady state of the hypometabolism and the large variation among species and individuals. To overcome these difficulties in observing and evaluating daily torpor, we developed a novel torpor-detection algorithm based on Bayesian estimation of the basal metabolism of individual mice. Applying this robust method, we evaluated fasting induced torpor in various ambient temperatures (T_As) and found that H decreased 88% during daily torpor while T_R only decreased 3.79 °C in mice. These results indicate that thermogenesis during daily torpor shares a common property of sensitivity reduction with hibernation while it is distinct from hibernation by not lowering T_R. Moreover, our findings support that mice are suitable model animals to investigate the regulation of the heat production during active hypometabolism, thus suggesting further study of mice may provide clues to regulating hypometabolism in mammals. (COI:No)

1P-145

Establishment of methods for inducing hibernation-like hypothermia in rats

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Although hypothermia would be a potential therapeutic target for treating ischemic diseases, hypothermia itself also causes circulatory disturbance. It is thus desired to establish safe methods inducing hypothermia. Hibernators decrease body temperature during hibernation without adverse cardiovascular events. This prompted us to consider that it is possible to induce deep hypothermia in non-hibernators by adopting the strategy used in hibernators. In this study, we aimed to establish methods for inducing hibernation-like hypothermia in rats. Since activation of central adenosine A1 receptors has been shown to induce hypothermia in hibernators, the adenosine A1 agonist N6-cyclohexyladenosine (CHA) were intracerebroventricularly injected in rats. They were placed in a cold room maintained at 4°C, and rectal temperature and electrocardiogram (ECG) were recorded. In comparison, rats anesthetized with pentobarbital were also kept in the cold room. Body temperature of the rat injected with CHA decreased to 15°C During inducing hypothermia, the rat showed no abnormal ECG. By contrast, the rat induced hypothermia by pentobarbital showed abnormal ECG, before body temperature reached 15°C. These results indicate that it is possible to induce hibernation-like hypothermia in non-hibernator, the rat. Activation of central adenosine A1 would be effective to inducing hypothermia without cardiac arrest. (COI:No)

1P-146

Regulation of alternative splicing of cold-inducible RNA-binding protein in hypothermic animals

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Background Although low temperature exhibits protective effects on cell functions, it paradoxically damages organ functions. Interestingly, hibernators can avoid hypothermic damages during hibernation. We hypothesized that cold-inducible RNA-binding proteins (CIRP) play a role in resistance to hypothermia. Recently, we revealed that splicing variants of CIRP in euthermic hamsters converge to one specific variant during hibernation. Thus, we aimed to examine factors of the splicing regulation. Methods We induced hypothermia in hamsters and mice by cooling after isoflurane inhalation or intracerebroventricular injection of an adenosine A1 receptor agonist. We performed RT-PCR to analyze the expressions of CIRP. Results In hamsters, when body temperature was rapidly decreased, the hibernation-specific splicing was not reproduced. In contrast, slow decreasing of body temperature caused unification of CIRPmRNA variants. Three splicing variants of mRNA constitutively expressed in euthermic mice, but single PCR product encoding functional CIRP was detected after decreasing body temperature with a slow rate. Discussion Decrease in body temperature, which is comparable with that observed in hibernators, reproduce the hibernation-specific splicing pattern. The moderate low temperature (around 30 degrees) would be suitable for inducing the alternative splicing of CIRPmRNA. Furthermore, this study suggested that non-hibernating animal also retains the regulation. (COI:No)

1P-147

Sympathetic responses to ozone exposure

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Ozone is an oxidative air pollutant, and epidemiological studies have shown that exposure to ozone is associated with respiratory and cardiovascular morbidity. In rodents, inhalation of ozone induces dose-dependent decreases in heart rate and body temperature, but their mechanisms are unclear. The activity of sympathetic nerves innervating thermoregulatory effectors (brown adipose tissues (BAT) and plantar cutaneous vessels), and the heart, were recorded in urethane-anesthetized, artificially ventilated rats. I tested whether ozone inhalation (1, 2, 5 ppm, 90 min) inhibited their activity. High concentrations of ozone (5ppm) significantly inhibited sympathetic nerve activity to BAT (BAT SNA) and to plantar cutaneous vasoconstrictor (CVC) nerves, as well as causing a delayed fall in body temperature; but although heart rate fell, cardiac sympathetic nerve activity was unaffected. Spectral analysis of heart rate variability showed a large increase in high frequency (1.0~3.0 Hz) power in response to ozone, indicating increased cardiac parasympathetic nerve activity. These results suggest that ozone lowers body temperature by inhibition of BAT SNA and CVC activity, while it decreases heart rate by increasing cardiac parasympathetic nerve activity rather than decreasing cardiac sympathetic activity. (COI:No)

1P-148

Orexin-A diminishes the suppressive effect of GLP-1 on the reflex swallowing

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We examined the relationship between orexin-A and GLP-1 on the regulation of reflex swallowing in anaesthetized rats. Swallowing was elicited by repeated electrical stimulation of the superior laryngeal nerve (SLN) and was identified by the electromyogram of the mylohyoide muscle. The injection of GLP-1 (20 pmol) into the medial nucleus of the solitary tract (mNTS) decreased the frequency of swallowing during the SLN-stimulation. The latency of the first swallowing tended to be extended after the administration of GLP-1. Fourth ventricular administration of orexin-A prior to the injection of GLP-1 attenuated the degree of suppression of the swallowing response induced by GLP-1-administration. The injection of small dose of GLP-1 (6 pmol) into the mNTS did not affect the reflex swallowing. The injection of small dose of GLP-1 induced the suppressive response of the reflex swallowing after the pre-administration of orexin-1 receptor antagonist into the fourth ventricle. After the microinjection of SB334867 into the commissural part of NTS (cNTS), the injection of small dose of GLP-1 also induced the suppressive response. These results revealed that the orexin-A suppresses GLP-1-response of the reflex swallowing by way of orexin-1 receptors situated in the cNTS. This work was supported by JSPS KAKENHI Gant Number 15K00818. (COI:No)

1P-149

The evaluation of swallowing function by analyzing the smoothing wave form of surface EMG on the neck

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[Purpose] One of the reason of poor nutritional status in the elderly seems to be the food intake shortage due to the disturbance of chewing and swallowing. We evaluated the swallowing function by analyzing the surface EMG on the neck. [Method] Subjects were 10 healthy youth (21.5±0.5 year old) and 13 healthy elderly (74.7±5.5 year old). We recorded surface EMG by the bipolar lead between both side of thyrohyoid venter in sampling time 0.1m/s. EMG was recorded during swallowing 10 ml of liquid type of sport drinks and 10ml of jelly type of sport drinks by Neuropack μ (Nihon Koden). The movement average were taken from 500 points of EMG wave form and they were smoothed (EMG). The duration, amplitude and number of notch were taken from EMG. [Result] In elderly, there was significant difference. The duration of EMG was significantly extended (youth; 1.21±0.44, elderly; 6.15±2.95, p=0.0001) and its amplitude was significantly reduced (199.5±115.3, 51.0±3.8, p=0.005) than youth. The amplitude of EMG during swallowing jerry materials was significantly reduced compared with liquid materials (liquid; 51.0±3.8, jerry; 49.4±5.5, p=0.031). There are significant correlation between the duration of EMG and the number of notch on EMG (R=0.778, p=0.001). [Conclusion] Using surface EMG on the neck seems to be convenient and noninvasive technique for the evaluation of swallowing, especially as useful for the subclinical swallowing disturbance. It is suggested that prolonged duration and low amplitude of EMG will be the indication of swallowing disorders. (COI:No)

1P-150

Rice and the polishing degree of rice influence the antithrombotic activity of vascular endothelial cells

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It is reported that western diet promotes thrombotic diseases such as myocardial infarction and cerebral infarction, which increase in Japanese. In Japan, the western dietary patterns have caused the decrease in intake of rice. It is thought that the reduction of rice consumption may be one of the factors for thrombotic disorders. Rice can be classified into white rice, whole rice, and brown rice according to polishing degree. Therefore, the effects of rice and its polishing degree on antithrombotic activity of vascular endothelial cells (ECs) were investigated. Furthermore, we analyzed the effects of these kinds of raw and cooked rice. Each rice was extracted with methanol. The established ECs (TKD-2) were cultured with each extracted sample. Each sample did not influence the proliferation of ECs. In comparison with vehicle control, the extracts of raw white, whole and brown rice significantly increased t-PA activity in both conditioned medium and cell extract of ECs. Moreover, the extract of raw brown rice augmented t-PA activity more than that of raw white rice. However, these samples did not alter the expression of t-PA, u-PA and PAI-1 mRNAs, respectively. These findings suggest that the extracts of raw rice enhance stability of t-PA mRNA or translation of t-PA mRNA to t-PA protein. On the other hand, in comparison with control, the extracts of cooked whole and brown rice significantly increased t-PA activity in cell extract of ECs. Thus, even if whole and brown rice are cooked, their effects demonstrated in raw materials are maintained. (COI:No)

1P-151

Mitochondrial fission in liver physiology

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Recently, mitochondrial research has led to a dramatic expansion of the existing paradigms that define mitochondria behavior and function. It is well established that mitochondria are highly dynamic organelles that frequently fuse and divide in response to cellular physiological and pathological conditions. It is also well known that its interaction with other organelles such as endoplasmic reticulum (ER) is important for the overall cellular function. In vertebrates, mitofusin-1 and -2 are involved in mitochondrial fusion while dynamin-related protein 1 (DRP1) and mitochondrial fission factor control mitochondrial fission. In this study, the role of mitochondrial fission on liver physiological and pathological signaling was explored. By using liver specific DRP1 knockout (*Drp1*LiKO) mice, we showed that a defect in mitochondrial fission and subsequent ER stress promoted the expression of fibroblast growth factor 21 (FGF21) and that the increased FGF21 in turn functions as a metabolic regulator, exhibiting anti-obesity and anti-diabetes effects. On the other hand, histological analysis revealed that *Drp1*LiKO liver exhibited severe inflammation, fibrosis and apoptosis, possibly associated with the decreased mitophagy formation and the increased reactive oxygen species generation. In addition, by using mouse primary hepatocytes, we also investigate these important roles that mitochondrial fission on liver physiology *in vitro*. Our results present new insights into the functional importance of mitochondrial fission in liver metabolic function and immunology. (COI:No)

1P-152

Wx/ae brown rice changed the expression of hepatic gene related to lipid methabolism to improve dyslipidemia

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Wx/ae rice is a consequence of double mutant of amylose-free waxy (wx) mutant and amylose-extender (ae) genes in Kinmaze rice (wild type :WT). wx/ae brown rice contains abundant resistant starch and ganma-oryzanol which reduces the blood lipid compared to Koshihikari. In the previous study, we reported that wx/ae brown rice has an effect on preventing fatty liver. In this study, to reveal the mechanisms of this effect we examined the expression of various molecules involved in metabolisms of lipid in mice by RT-PCR method. Male C57BL/6J mice were fed of chow diet (CD), high-fat diet (HFD), HFD + WT brown rice, HFD+ wx/ae brown rice, HFD + resistant starch derived wx/ae for 12 weeks. The FAS, and HMG-CoA synthetase genes were significantly downregulated in HFD+ wx/ae brown rice group compared to HFD group. In addition, after feeding HFD for 8 weeks, these mice switched to CD, HFD+ WT brown rice, HFD+ wx/ae brown rice, HFD+ resistant starch for 4 weeks. The FAS, and HMG-CoA synthetase genes were significantly reduced in the HFD+ wx/ae brown rice and HFD+ resistant starch groups. The concentration of triacylglyceride in blood was significantly decreased after exchange to HFD+ wx/ae brown rice and HFD+ resistant starch. These results suggest that wx/ae brown rice improves dyslipemia and fatty liver by changing hepatic gene expressions related to lipid metabolism. (COI:No)

1P-153

Cynanchum wilfordii extract attenuates non-alcoholic fatty liver disease

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In this study we investigated whether Cynanchum wilfordii extract (CWE) supplementation would decrease fat accumulation and damage in the liver. The beneficial effect of CWE was evaluated in a murine model of nonalcoholic fatty liver disease. Mice were fed either a normal diet or an atherogenic diet with fructose (ATHFR) in the presence or absence of CWE (50, 100, or 200 mg/kg). Treatment with ATHFR induced a hepatosplenomegaly like condition (increased liver and spleen weight); this pathological change was attenuated in the presence of CWE. The ATHFR group exhibited impaired liver function, as evidenced by increased blood levels of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase, fat accumulation in the liver, and lipid profiles. Supplementation of CWE (100 and 200mg/kg) ameliorated these impaired liver functions. Atherogenic diet with fructose increased the protein levels of COX-2 and p38 MAPK, as well as the nuclear translocation of NF-kappaB. These signaling pathways, which are associated with the inflammatory response, were markedly suppressed after CWE treatment (100 and 200 mg/kg). In summary, CWE supplementation reduced high fat and high fructose diet induced fat accumulation and damage in the liver by suppressing COX-2, NF-kappaB, and p38 MAPK. (COI:Properly Declared)

1P-154

SCGB3A2 suppresses pulmonary emphysema by the control of A1AT expression

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Secretoglobin (SCGB) 3A2, which is predominantly expressed in the lung airways, was found to suppress lung inflammation and promote maturation of fatal lungs. Based on the functions of SCGB3A2, we investigated the effect of SCGB3A2 on chronic obstructive pulmonary disease (COPD) using a cigarette smoke (CS)-induced COPD mouse model in Wild-type (WT), *Scgb3a2*-KO (KO), and -TG (TG) mice. The mean linear intercept (Lm) and Destructive Index (DI) of the lungs were significantly increased after CS-exposure in both WT and KO mice. Lm and DI of TG mice lung were not affected by CS-exposure. After CS-exposure, SCGB3A2 expression was decreased in WT mice lungs but not in TG mice lungs. These data indicate that SCGB3A2 suppressed pulmonary emphysema. We hypothesized that the mechanism of action for SCGB3A2 involved alpha-1 antitrypsin (A1AT) which suppress the development of emphysema. SCGB3A2 increased A1AT levels in mouse fibroblast MLg cells and promoted the phosphorylation of STAT3 and STAT1. The knock-down of STAT1 and STAT3 by using siRNAs in MLg cells was performed The downregulation of STAT3 was decreased A1AT expression, while STAT1 has opposing effect. These results suggest the possibility that SCGB3A2 suppresses emphysema by the control of A1AT expression. (COI:No)

1P-155

Hematopoietic stem cells are increased by Cyp2c44 gene deletion: Implication in chronic hypoxia-induced pulmonary hypertension in mice

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Recently, we have demonstrated that deletion of cytochrome P450, CYP2C44, exacerbates chronic hypoxia-induced pulmonary artery remodeling and hypertension. Subsequently, we serendipitously found that Cyp2c44 gene deletion increases hematopoietic stem cell (HSC) number in bone marrow and blood. Therefore, the objective of this study was to investigate whether Cyp2c44 deletion regulates HSC phenotype and whether increased differentiated HSCs contribute to chronic hypoxia-induced remodeling of pulmonary arteries. Our findings demonstrated that deletion of CYP2C44 epoxygenase, which produces epoxyeicosatrienoic acids and hydroxyeicosatetraeonic acid, and increased: 1] HSC numbers as compared to wild type mice, 2] proangiogenic cells, and 3] immunogenic/ inflammatory monocytes and macrophages, in bone morrow and blood. Increased CD133+ and von Willebrand factor positive cells, derived from proangiogenic stem cells, contributed to chronic hypoxia-induced remodeling and occlusion of pulmonary arteries in CYP2C44-deficient mice. In conclusion, our results demonstrated that CYP2C44 and/or CYP2C44-derived lipid mediators play a critical role in regulating HSCs phenotype because deletion of Cyp2c44 gene increased differentiated HSCs, monocytes, and macrophages that contributed to chronic hypoxia-induced pulmonary artery remodeling and occlusion. (COI:No)

1P-156

ABCG2 physiologically mediates intestinal urate excretion in humans: Serum uric acid as a useful marker for impairment of intestinal epithelium

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To investigate intestinal urate excretion via human ABCG2, a high-capacity urate exporter in both intestines and kidney, ABCG2 dysfunctional common variants, Q126X (rs72552713) and Q141K (rs2231142) were genotyped, in hemodialysis and acute gastroenteritis patients, respectively. Serum uric acid (SUA) levels were markedly increased in 106 hemodialysis patients ($P = 1.1 \times 10^{-4}$) by ABCG2 dysfunction, which showed the physiological role of ABCG2 for intestinal urate excretion because their urate excretion almost depends on intestinal excretion via ABCG2. Also, SUA levels were significantly elevated in 67 acute gastroenteritis patients ABCG2 dysfunction ($P = 6.3 \times 10^{-3}$) regardless of the degree of dehydration, which showed the pathophysiological role of ABCG2 in acute gastroenteritis. We demonstrated ABCG2-mediated intestinal urate excretion in humans for the first time, and these findings indicate the physiological and pathophysiological importance of intestinal epithelium as an excretion pathway besides an absorption pathway. Therefore, increased SUA could be a useful marker for epithelial impairment of intestine as well as for dehydration. (COI:No)

1P-157

Novel zebrafish models of Marinesco-Sjogren syndrome

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SIL1 is a nucleotide exchange factor for the endoplasmic reticulum chaperone, BiP. Mutations in the SIL1 gene cause Marinesco-Sjogren syndrome (MSS), an autosomal recessive disease characterized by progressive myopathy, cerebellar ataxia, mental retardation, and congenital cataracts. We analyzed zebrafish sil1 function using antisense morpholino oligos and have started to create MSS knocked-out fish by CRISPR-Cas9 system. At 4 days post-fertilization (dpf), thirty percent of fish injected with morpholinos showed reduced birefringence. Morphants had small diameter of eyes and reduction of purkinje cell numbers in the cerebellar area. Co-injection of zebrafish sil1 mRNA along with the morpholino restored normal development the morphants. Additionally, BiP (a ER stress marker protein), LC3 (an autophagy marker protein) and activated caspase 3 (an apoptosis marker) were increased in these morphants. With CRISPR-Cas9 system, we successfully identified founders that could pass mutations in sil1 gene through the germline. By screening the offsprings from founders, a mutant fish with 13bp deletion in zebrafish sil1 gene has been obtained and the fish line has been established. Our findings suggest that our MSS model fish will be a useful for studies of pathomechanism of MSS and will be a good tool for a therapeutic chemical screening. (COI:No)

1P-159

Comprehensive analysis of gene expression in the lumbar spines from congenital kyphoscoliotic rats

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Scoliosis is a clinical condition that involves an abnormal curvature and deformity of the spinal vertebrae. However, little is known about the genetic background and key responsible genes of congenital scoliosis. To understand the pathogenesis of congenital scoliosis, here we report the results of a comprehensive analysis of gene expression in the lumbar spines from a rat model of congenital kyphoscoliosis (Ishibashi rats, IS). Total RNAs were extracted from the third to fifth lumbar spine segments (L3-L5), where deformities appear most commonly in IS rats. The gene expression of rat lumbar spines was analyzed using DNA microarrays (approximately 20,000 distinct genes). Based on our criteria, 104 genes were found to downregulation in the IS rats compared to the Wild-type rats. According to the results of a gene clustering/pathway analysis, the genes significantly downregulated in IS rats were divided into several functional groups such as the neurotrophin receptor family (Trks) and retinol (vitamin A) metabolism. These findings indicate that the Trks and genes involved in retinol metabolism have an important function in regulating normal bone formation. It will be interesting to investigate further whether abnormality of the lumbar spines is rescued by the increase of expression of downregulated genes. (COI:No)

1P-160

Role of periostin on Knee Osteoarthritis Synoviocytes

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Introduction: Recent studies indicate that periostin is involved in the pathogenesis and progress of knee osteoarthritis (OA). The reaction cascade of periostin, however, remains unidentified. Therefore, we investigated the mediator to secrete periostin and the downstream osteoclasis factor of periostin. Methods: In the first examination, OA patients were classified according to the Kellgren-Lawrence system. The periostin, interleukin (IL)-4, IL-13 and transforming growth factor-β levels in the synovial fluid were measured. Additionally, OA-associated synoviocytes were cultured with different concentrations of IL-13. We measured periostin content in culture supernatants and the level of STAT6 in the culture cells. In the second examination, matrix metalloproteinases (MMPs) or tissue inhibitor of MMPs (TIMPs) with periostin in the culture cells were measured when periostin was added to OA synoviocytes. Results: Periostin and IL-13 levels were up-regulated during the progression of OA. Additionally, periostin content in culture supernatants and the level of STAT6 in the culture cells were significantly increased by IL-13. The increase of periostin was significantly inhibited by dexamethasone and leflunomide. Furthermore, the MMP-2 and MMP-3 levels increased in a periostin concentration-dependent manner. Increases in the MMP-2 and MMP-3 levels were inhibited by dexamethasone. Conclusion: Our research indicates that periostin may be up-regulated via STAT6 downstream of IL-13 in OA synoviocytes. Furthermore, periostin may facilitate MMP production by OA synovial cells. (COI:No)

1P-161

Influence of mechanical force on bone matrix proteins in ovariectomised mice and osteoblast-like cells

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Introduction: Several studies have analyzed the potential role of periostin and semaphorine-3A (SEMA3A) in bone biology and suggest that periostin may be an important regulator of bone formation. However, the influence of periostin on other bone metabolic factors such as SEMA3A when the bone is under mechanical stress still has not been completely verified. Therefore, the aim of this study was to investigate the effects of periostin on SEMA3A expression in a postmenopausal osteoporosis murine model and in osteoblast-like MC3T3-E1 cells. Methods: Female mice were divided into three groups and treated with a sham operation, ovariectomy (OVX) or OVX plus treadmill training (OVX+Run). After 10 weeks, tibias were used for histologic analysis. MC3T3-E1 cells were burdened by mechanical stress using a centrifuge or were treated with periostin, and the production of biologically active SEMA3A was examined in vitro. Results: In OVX+Run group tibias, the number of TRAP-positive osteoclasts was lower than in the OVX group, and the expression of periostin and SEMA3A was higher. In MC3T3-E1 cells, centrifugal stress increased periostin and SEMA3A mRNA expression. Treatment with periostin increased the SEMA3A level. Conclusion: We speculate that mechanical load may increase periostin production in osteoblasts, and periostin may inhibit osteoclast differentiation by its effects on SEMA3A. Our results support the concept of a positive correlation between exercise and inhibition of osteoclasts in post-menopausal osteoporosis. (COI:No)

1P-162

Celastrus orbiculatus extractioninhibits vasculogenic mimicry inhepatocellular carcinoma

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BACKGROUND: Vasculogenic mimicry (VM) occurs in solid tumors and associated with poor outcomes. Notch1 contributes to VM formation in HCC. Celastrus Orbiculatus Extraction (COE) isolated from Celastrus Orbiculatus Vineis active in antitumor. Effects of COE on VM formation in HCC are examined. METHODS: VM formation was obtained using Matrigelin vitro and xenografts in vivo. RT-PCR, W.B. and IHC were used to examine relative genes and their products respectively. Xenograft was monitored using in vivofluorescence imaging. PAS-CD34 and EMS were used to show VM in xenograft. RESULTS: Endogenous increaseof Notch1 in MHCC and increase induced by TGF-β1 in HepG2 are closely associated with VM. COE can inhibit HCC cells to proliferate, invade and form VM in vitroand in vivowith a concentration dependent manner. Notch1 and Hes1 can be downregulated by COE with indicated concentrations. CONCLUSIONS: COE can inhibit VM formation in HCC by downregulating Notch1 signaling and may be considered as a promising candidate for HCC therapy. (COI:No)

1P-163

Steroidogenic acute regulatory protein-related lipid transfer domain containing 10 (STARD10) promotes lipid accumulation and lipid droplet formation

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STARD10 is a member of the START domain-containing lipid transfer protein family. We have previously shown that STARD10 is highly expressed in the liver. When fed a high fat diet, *Stard10* knockout (KO) mice accumulated significantly less cholesterol and triglycerides (TG) in the liver than wild type (WT) mice. The aim of this study was to clarify the role of STARD10 in lipid accumulation. We examined the effect of choline-deficient L-amino acid-defined diet (CDAA) which induces liver specific lipid accumulation. KO mice fed with CDAA gained weight in a manner similar to WT mice. However, the liver of KO mice was smaller in size and area of lipid droplet (LD) in hepatocytes of KO mice was significantly smaller than those of WT mice. Lysophosphatidylcholine acyltransferase 1 (LPCAT1) is the enzyme which catalyzes the reaction of the conversion of lysophosphatidylcholine to phosphatidylcholine (PC). We hypothesized that the interaction between STARD10 and LPCAT1 fine-tunes the balance between PC and TG to promote LD formation. We confirmed the interaction of STARD10 and LPCAT1 in mouse hepatoma cell line. Both STARD10 and LPCAT1 were localized at the surface of LD. Overexpression of STARD10 and LPCAT1 promoted lipid accumulation, suggesting that STARD10 increases LD size through its interaction with LPCAT1. These results indicate that STARD10 is involved in regulating lipid storage and LD formation in the liver. (COI:No)

1P-164

A tRNA methyltransferase FTSJ1 is essential for translational control of the Hippo transducer YAP/TAZ in cancer vells

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Protein synthesis is recently thought to be determinant for the properties of cancer stem cell (CSC), yet the molecular mechanisms that determine the CSC-specific translational machinery are poorly defined. Here, we report that FTSJ1, which catalyzes 2-O-methylation in cytosolic tRNAs, plays essential role in breast and gastric cancer expansion. Expression levels of FTSJ1 mRNA are significantly elevated in cancer tissues. In both breast and gastric cancer cells, FTSJ1 is required to sustain CSC-related traits. Loss of FTSJ1 impairs, while gain of FTSJ1 elevates, the self-renewal capacity, clonogenic potential, drug resistance, and tumorigenic potential in cancer cells. Mechanistically, FTSJ1 controls protein levels of the Hippo pathway transducers YAP/TAZ in a translation-dependent manner, not a transcription-dependent manner. YAP/TAZ are recently recognized as a determinant of CSC properties. Upon FTSJ1 depletion, YAP/TAZ activities are attenuated because of the reduced protein levels, and YAP/TAZ-dependent phenotypic outcomes are declined. These findings demonstrate that tRNA modifications contribute to cancer progression and provide a prospective target for cancer therapy. (COI:No)

1P-165

Molecular mechanism of clearance of modified nucleotides

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RNAs contain a wide variety of posttranscriptional modifications in all domains of life. To date, there are more than a hundred of species of modifications in all RNA species including tRNA, rRNA and mRNAs. These modifications are essential for the biological properties of RNA, such as cellular localization, structural stability and translational activities. In contrast to the increasing knowledge of the biological functions of RNA modifications, however, little is known about the fate of these modified nucleotides. In general, all RNAs are subjected to exoribonuclease-mediated degradation. Degradation of RNA consequently generates numerous amounts of free nucleotides, which are then recycled for new RNA synthesis as well as cellular metabolism. However, degradation of RNA will also inevitably generate a considerable amount of modified nucleotides. Because chemical modifications are structurally bulky and often hinder Watson-Click base-pairing, these modified nucleotides will be potentially harmful for RNA synthesis if they are accidently incorporated during transcription. Therefore, we hypothesized that modified RNA generated by RNA degradation will be rapidly cleared from cytosol to extracellular space. To test this hypothesis, we developed a high-throughput method to examine over 30 species of modified nucleotides in a single analysis by mass spectrometry. We also aimed to identify the transporter for the modified nucleotides in mammalian cells. (COI:No)

1P-166

Chronic exercise with diet restriction prevents diabetes via inactivation of FoxO3 signal in skeletal muscle

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Purpose: WBN/Kob-Fatty (WKF) rats lack leptin receptor, and develop chronic pancreatitis and diabetes with obesity. We recently reported that diet restriction (DR) improves their hyperlipidemia, insulin resistance, and pancreatic dysfunction more effectively when combined with chronic exercise (DR+Ex). In this study, we investigated metabolic profiles and intracellular signals in their skeletal muscle to clarify synergistic effects of chronic exercise on DR. Methods: Male WKF rats (age, 6 weeks) were divided into a fatty-obese (fatty-control), fatty-DR, and fatty-(DR+Ex) groups. WBN/Kob rats were used as lean-control. Food intake of fatty-DR and fatty-(DR+Ex) groups was restricted to 69% and 70% of the fatty-obese group, respectively. The exercise of the fatty-(DR+Ex) group was voluntary wheel running. Results: After 6 weeks of intervention, it was found that chronic exercise increased the expressions of the proteins associated with glucose uptake and phospholylation, mitochondria biomarkers, and autophagy-related proteins in skeletal muscle. In addition, chronic exercise inhibited FoxO3 signal, but not FoxO1 signal, and accelerated PGC-1a protein expression. The phospholylation of down-stream targets of mTORC was, however, inhibited. Conclusion: We concluded that the chronic exercise improved metabolic functions of skeletal muscle at DR condition, and effectively prevent the development of diabetes probably via inactivation of the PGC-FoxO3a signaling pathway. (COI:No)

1P-167

Effect of overweight on Transforming growth factor - β1 after exhaustive exercise

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[Introduction] Some studies have reported an increase in Transforming growth factor - β1 (TGF-β1) after acute strenuous exercise. In this study, our aim was to evaluate whether being overweight affects TGF-β1 after acute strenuous exercise. Being overweight was defined using body mass index (BMI) as a measure of the degree of obesity. [Subjects and Methods] Thirteen healthy young men aged 19 to 23 years old who engaged in daily exercise participated in this study. Seven of these men were categorized in the BMI <25 group, and six were in the BMI >25 group. Venous blood samples were collected from the subjects pre- and post-performance of the Cooper test. This test involved running as far as possible within a 12-minute period. TGF-β1 levels were measured using the collected blood samples. [Results] The TGF-β1 levels increased significantly in the BMI >25 group (pre: 11.9 ± 2.3 ng/mL, post: 20.7 ± 5.3 ng/mL), but these were not significantly increased in the BMI <25 group (pre: 11.5 ± 1.5 ng/mL, post: 12.5 ± 1.1 ng/mL). [Conclusions] Using BMI as an index for evaluation, this study showed that TGF-β1 increase in overweight young men after acute strenuous exercise. (COI:No)

1P-168

Effect of early and late treadmill exercise on motor functional recovery and brain damage after hemorrhage in rats

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This study focused on the effect of early and late treadmill exercise on motor functional recovery, lesion volume, and cortical thickness following intracerebral hemorrhage (ICH) in rats. ICH was induced in rats by an injection of collagenase into the left striatum. They were randomly assigned to no training ICH (ICH), no training placebo surgery (SHAM), early treadmill exercise (ICH + ET), and late treadmill exercise (ICH + LT) groups. The ICH + ET group trained for 7 days from the 2nd to 8th day after surgery. The ICH + LT group trained for 7 days from the 9th to 15th days after surgery. Sensorimotor function was assessed using forelimb placing and horizontal ladder test. Lesion volume and cortical thickness was analyzed using Nissl staining. The ICH + AT group showed significantly improved sensorimotor function compared with the ICH group. The cortical thickness of the ICH + ET group was significantly higher than that of ICH and ICH + LT groups. These results suggest that after cerebral hemorrhage, early treadmill exercise may promote sensorimotor functional recovery by inhibition of cortical atrophy compared with late treadmill exercise. This work was supported by a grant-in-aid for scientific research from the Niigata University of Health and Welfare. (COI:No)

1P-169

The preventive effects of customary exercise on poststroke memory dysfunction

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[Introduction] We have reported that running exercise in the early stage poststroke recovered from the spatial memory dysfunction via elevation of hippocampal level of brain-derived neurotrophic factor (BDNF). In this study, the preventive effects of customary exercise and participation of BDNF in poststroke memory function recovery were investigated. [Methods] After the running on a treadmill for 7 days, 3,000 particles of microspheres (MS) were injected via right internal carotid artery of rats to induce the mild stroke (Ex group). Non-exercise group (NE group) and sham operated group were also examined as control groups. The spatial memory function was evaluated by Morris water maze test that was performed at 8th day after MS injection. BDNF concentration in transected hippocampus were measured at 1, 4 days before and 4, 7 days after MS injection by ELISA. [Results] MS injected rat showed significantly impairment of spatial memory function. However, the Ex group showed significantly recovery of memory function in comparison with that of the NE group. BDNF concentration was elevated 4th and 7th day after starting exercise in the Ex group. [Conclusion] These results suggest that the BDNF elevation in hippocampus by customary exercise might prevent the spatial memory dysfunction after stroke. (COI:No)

1P-170

Respiratory gas response to up ramp load during light and moderate intensity cycling exercise

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Aim: To understand the dynamics of minute carbon dioxide output (VCO2) and minute oxygen uptake (VO2) to up ramp load during light and moderate intensity. Methods: 8 healthy male athletes performed bicycle ergometer tests. Breath-by-breath based respiratory responses to linearly increased up ramp load were tested. The up ramp load lasted for 3 minutes within 2 W and 102 W loads. Steady state load preceded and followed the up ramp load. Linear regression lines were fitted to normalized VCO2 and VO2 responses to the up ramp loads. The slope of the fitted regression lines, half response time of VCO2 and VO2 and the initial delay of these gases were analyzed. Results: To up ramp loads, the slopes of the fitted regression lines differed between VCO2 and VO2 responses. Half response times for VCO2 were longer than those for VO2 in both loads. The half response times showed a small, but significant dependence on load intensity. The initial delays of the responses for VCO2 and VO2 were almost identical in both loads. Conclusion: Linear regression lines fitted to VCO2 and VO2 responses to up ramp load revealed a non-linear property of the respiratory control system. Keywords: up ramp load, minute carbon dioxide output (VCO2), minute oxygen uptake (VO2), nonlinearity (COI:No)

1P-171

A decrease in Th1/Th2 ratio following a prolonged exercise load

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[Introduction] Although the effects of short-term exercise load on immune function have been studied, a few reports have evaluated the effects of prolonged exercise load. Therefore, we focused on a rugby club training camp, in order to examine the effects of prolonged high-intensity exercise load on immune function. [Methods] The subjects were 15 university students who were members of rugby football clubs. The camp lasted 25 days, including 3 days of rest. The subjects underwent immunologic testing before and after the camp. [Results] Th1, Th1/Th2 ratio and natural killer cell activity showed a significantly low value after the camp in comparison with before the camp ($p < 0.01$). But, noradrenaline showed a significantly high value after the camp in comparison with before camp ($p < 0.01$). Th2 showed no significant difference. There was significant correlation between rate of change of Th1/Th2 ratio and rate of change of natural killer cell activity ($r = -0.538$, $p < 0.05$). The rate of change calculated it from the value after the camp before a camp. [Conclusion] We established that the suppression of cell-mediated immunity following prolonged exercise load is caused by natural killer cell activity. (COI:No)

1P-172

Effects of sustained vocalization during exercise on respiratory state and cerebral blood flow

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Kendo kakari-keiko with vocalization (about 80%VO_2max) significantly increased the value of $FetCO_2$ (=$PaCO_2$) compared with that without vocalization. This exercise may increase cerebral blood flow (CBF), because $PaCO_2$ is one of the vasodilatation factors. In this study, we investigated whether intermittent exercise with vocalization, not *kendo*, increased the value of $FetCO_2$ and CBF. 6 male subjects participated in this study. They performed the intermittent exercise (20-sec, 8 times) at 80% and 60%VO_2peak using a cycle ergometer with (Voc) or without vocalization (non-Voc). We measured ventilatory ($FetCO_2$, etc) and CBF (comon carotid artery (CCA) blood flow) variables at before and after exercise. In Voc, the value of $FetCO_2$ tended to be higher than that in non-Voc at 80%VO_2peak. The value of %change of CCA blood flow in Voc tended to be higher at both intensities. Particularly, at 60%VO_2peak, the value of CCA in Voc tended to maintain higher state than that in non-Voc for 3-min after exercise. Intermittent exercise with vocalization at 80%VO_2peak, not *kendo*, increased the value of $FetCO_2$. But, the increase of $PaCO_2$ caused by vocalization during exercise did not necessarily affect CBF. On the other hand, exercise with vocalization at 60%VO_2peak may increase CBF at after exercise. (COI:No)

1P-173

The effect of cognitive-motor dual-task training on cognitive function and plasma amyloid β peptide 42/40 ratio in healthy elderly persons

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We evaluated the effect of dual-task (DT) training on the executive functions and on plasma amyloid β peptide (Aβ) 42/40 ratio, a potent biomarker of Alzheimer's disease, in healthy elderly people. Twenty-seven sedentary elderly people participated in a 12-week randomized, controlled trial. Clinical outcomes were compared between the DT and the control single-task (ST) training groups. Then eight applicants among all participants voluntarily continued DT training for 2 years. In the 12-week trial, an interaction between intervention and group was found on the total score ($p = 0.002$) and the scores in the specific domains, "attention ($p = 0.003$)," and "abstract meaning ($p = 0.049$)" in the Modified Mini-Mental State Exam (3MS). The absolute changes in the total score and in the score of "attention" were greater in the DT group than in the ST group. Plasma Aβ 42/40 ratio similarly decreased in both groups following the training. In the 2-year trial, a significant interaction between intervention and group was found on the total score and the scores of "verbal fluency & understanding," and "attention" in the 3MS. A 2-year continuation resulted in the maintenance in those scores, while impaired in the subjects who discontinued the training. Cognitive-motor DT training was beneficial in improving broader domains of cognitive functions of elderly without modulating Aβ metabolism. (COI:No)

1P-174

Inspiratory muscle training in water improves inspiratory muscle strength more than that on land in healthy young men

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The purpose of this study was to investigate the effect of inspiratory muscle training (IMT) in water on respiratory muscle strength compared with that of on land. Methods: Participants consisted of 12 (water group, WG) and 14 (land group, LG) healthy males. Before starting IMT, we evaluated maximum inspiratory (PImax) and expiratory (PEmax) pressure in the oral cavity as baseline value in both the groups on land as the surrogate indices of inspiratory and expiratory muscle strength, respectively. The WG performed IMT in a sitting position immersing themselves in water up to the clavicles level. The LG performed IMT in a sitting position on land. For the duration of the IMT, the subjects performed 15-min breathing at a respiratory rate of fifteen breaths per min synchronized with a metronome for six weeks, four times per week, during which the inspiratory muscle load was adjusted as 30% of PImax at baseline using an inspiratory loading device. Results: In both, the WG and LG, PImax after six weeks of IMT increased significantly ($p<0.05$) compared with that at baseline. Increase in rate of change in PImax in WG was significantly greater compared with that in LG ($p<0.05$). There was no significant difference in PEmax after six weeks of IMT between the groups. Conclusion: IMT in water up to the clavicles level improves inspiratory muscle strength more than that on land in healthy young men. (COI:No)

1P-175

Effect of environmental factors at high altitude on acute mountain sickness while climbing on Mount Fuji

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Acute mountain sickness (AMS) includes symptoms, such as headache, nausea and fatigue, which mainly develop in altitude above 2500m. One potential factor may relate to environmental factor, e.g., hypobaric hypoxia and cold environmental temperature. To clarify effects of these factors, we performed field study and questionnaire survey for climbers on Mt. Fuji. The first aim of the present study was to investigate whether changes in physiological variables and subjective feelings are associated with AMS or not. Twenty-five healthy young volunteers (7 males, 18 females) participated in the first study. Heart rate, arterial oxygen saturation (SpO_2), sublingual temperature, subjective thermal sensation, thermal comfort and symptoms of AMS assessed by the Lake Louise Questionnaire scoring system were measured at each six point on the climbing route from the fifth station (2305m) to the summit (3776m) on Mt. Fuji. Spearman rank order analysis found that severity of AMS was related to lower sublingual temperature and SpO_2 level ($p<0.05$). In the second study, a questionnaire survey including symptoms of AMS, subjective thermal sensation, thermal comfort, thirst sensation evaluated by visual analog scale, was conducted with a larger population of climbers on Mt. Fuji ($n>1000$). As a result, AMS may be related with a thirst sensation. Collectively, these results suggested that hypothermia and dehydration may relate to AMS on Mt. Fuji. Although, the further studies should be warranted to elucidate the underling mechanisms. (COI:No)

1P-176

Artificial high concentration CO_2-water foot bath facilitates a recovery from gastrocnemius muscle hardness induced by calf raise resistance exercise

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We investigated whether the immersion of extremities including agonist muscles into artificially made high concentration CO_2-water (CO_2>1000 ppm) influences recovery of muscle hardness in fatigue after calf raise resistance exercise. The healthy male college students (n=11, age; 18-19yrs, height; 168.6±4.5cm, weight; 66.2±9.3kg) participated in this study. The subjects were randomly divided into the CO_2-water foot bath group (n=6) and the tap-water group (n=5). A laser Doppler flowmetry probe for recording skin blood flow (BF_{skin}) were attached to the skin over right medial gastrocnemius (MG). The subjects performed 100 times calf raise resistance exercise and immersed lower legs into tap-water or artificial CO_2-water at 35 °C for 10 min after exercise. The hardness of MG dominant muscle was evaluated using ultrasound real-time tissue elastography at pre-exercise, immediately after exercise and 10 min recovery. The strain ratio (SR) of the MG to a reference material was calculated. At 10 min recovery, SR of the CO_2-water group was significantly smaller than that of the tap-water group (0.62±0.07 vs. 1.37±0.38, $p<0.05$). The present results suggested that high concentration artificial CO_2-water bathing may contribute to rapid recovery from the high intensity exercise-induced muscle fatigue. (COI:No)

1P-177

Forearm bathing with CO_2-water between exercises increased muscle blood without an effect on exercise performance

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Bathing in CO_2-hot spring increases blood flow of the skin under water, and the blood flow of skeletal muscle as well. We investigated in this study whether immersion into CO_2-water (CO_2>1g/L) during short time resting between series of exercise influences a muscle blood flow and a deterioration of the muscle performance. Nine healthy male subjects (Ss) participated in this study. Sensors for measuring skin blood flow (Laser doppler flowmetry apparatus) and blood flow of the grip-exercise agonist muscles (NIRS flowmetry) were instrumented onto a dominant forearm of the Ss. The Ss held hand dynamometer at 35% of their maximal grip strength by looking on monitor screen and the time of endurance (35%Gmax time) was measured. Then they took a rest for 10 seconds if the grip-strength had not reached to 35%Gmax level continuously for more than 3 seconds. This protocol was repeated until 35%Gmax time became less than 5 seconds as the end of one set, and totally 3 sets were performed. The forearm was immersed into CO_2-water or tap-water of a same temperature during 10-minutes resting in between the sets. Local blood supply to the agonist muscle of grip exercise was significantly increased by immersion into CO_2-water during inter-exercises rest, whereas no effect was observed by tap-water immersion. The 35%Gmax time shortened with the increase in exercise number of 35%Gmax and in sets number. A degree of the decrease in 35%Gmax time of CO_2-water immersion group did not differ from that of tap-water immersion group. (COI:No)

1P-178

Postural sway evoked by optokinetic stimulation using a head-mounted display device

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We investigated the effects of visual scene motion presented through a head-mounted display device (HMD) on postural control in normal subjects. Eight healthy young adults (aged 19-28 years) were recruited in the study. The subjects were asked to stand quietly on a stabilometric platform. The optokinetic stimulation (OKS) was projected onto the HMD (Oculus Rift, Oculus) at high definition resolution. For the OKS, a pattern of random dots was presented in a virtual space and was rotated continuously around the subject in the horizontal direction. The OKS was performed at a constant velocity of 20, 40, 60, 80, and 100 °/s for 30 s. During each condition, the sway path (SP), circumferential area (CA), and the position of the center of pressure (CoP) along the right-left (x) and antero-posterior (y) axes were examined. There was no significant difference in any of the parameters under the closed eye condition between when the HMD was used and when it was not. When the OKS was performed through an HMD, the SP and CA increased with an increase in the OKS velocity in the 20-60 °/s range, but saturated at velocities over 80 and 100 °/s. We found no significant difference in the CoP during any of the stimulus conditions. Our results suggest that the HMD technique could induce a significant change in body balance during OKS. Therefore, it can be applied to test differences in the strategies used to maintain an upright posture and balance in elderly individuals or in patients with central nervous system disorders. (COI:No)

1P-179

Anthropometry-based estimation of the body heat capacity for individuals aged 7-69 y: the Size Korea Survey 2010

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In previous study, we developed an anthropometric-based calculation of heat capacity (HC) for adult. Although the equation appeared to be precise and valid, its application to children and adolescent may induce bias. In the present study, we employed a large dataset from the Size Korea survey, a national anthropometric survey conducted in 2010, to re-validate our previous HC equation and to develop an equation of HC for children and adolescent. 12766 participants aged 7 to 69 years with body composition measured by multi-frequency bioelectrical impedance analysis were employed. Age strongly determined HC and its related factors including body weight (BW) and body surface area (BSA) in immature individuals, but not in adults. Linear regression was appropriate to describe the relation between HC and BSA in adults, whereas this regression in children and adolescent was quadratic. The predictive equation of HC developed in our previous study revealed a high reliability and predictive power in above-20 age group. The model composed of gender, BW, BSA, and BSA square was appropriate to predict HC in immature individuals aged 7 to 19 years. Percent of fat slightly influences the HC prediction in all age groups. In conclusion, anthropometric-based modeling is a simple, reliable, and useful method for calculation of HC and should be applied differently for mature and immature individuals. This work was supported by the grant (NRF-2015M3A9B6028310; NRF-2014M3A9D7034366) from NRF. (COI:No)

1P-180

Roles of plasminogen in the alterations in bone marrow hematopoietic stem cells during bone repair

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The bone marrow (BM)-derived cells are the source of endothelial cells, macrophages, osteoblasts, and osteoclasts, which are responsible for tissue maintenance and repair. However, the details in the influences of bone repair after bone destruction on hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) in BM remain unclear. We previously revealed that stromal cell-derived factor-1 (SDF-1) is involved in the changes in number of BM stem cells during bone repair process. Moreover, we reported that plasminogen (Plg) deficiency delays bone repair and the accumulation of macrophages at the site of bone damage in mice. In the present study, we examined the roles of Plg on the changes of HSCs in BM during bone repair. We analyzed the HSC and MSC populations in BM form Plg deficient and the wild-type mice after a femoral bone damage by using FACS. Plg deficiency significantly blunted a decrease in number of HSCs after a bone defect. On the other hand, it did not affect an increase in MSCs after a bone defect. Moreover, Plg deficiency significantly reversed number of both SDF-1- and Osterix-positive cells increased by a bone defect in the endosteum around the lesion. In conclusion, our data indicated that Plg and preosteoblast-derived SDF-1 are crucial in the changes in HSCs in BM during bone repair in mice. (COI:No)

1P-181

TAFI-dependent regulation of coagulation-dependent initiation and amplification of fibrinolysis on the surface of activated platelets

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Background: We reported that the process of platelet phosphatidylserine (PS) exposure, fibrin formation and plasminogen (plg) accumulation took place only in the center of the thrombus, suggesting that these events are closely related and finely regulated. Aim: To analyze the role of platelets in spatiotemporal regulatorations of coagulation and fibrinolysis. Methods: Fibrin network formation and the following lysis were analyzed in an *in-vitro* experiment employing diluted platelet-rich plasma supplemented with fluorescently labeled coagulation- and fibrinolytic- factors using Confocal Laser Scanning Microscopy. Results: Tissue factor triggered PS exposure on platelets' surface and the following fibrin network formation. Fibrin network structure was uneven and denser at the sites of coagulation initiation regions (CIRs) on PS exposing platelets. When tissue type plasminogen activator (tPA) was supplemented, labeled plg as well as tPA accumulated at CIRs from where fibrinolysis started. Recombinant human soluble thrombomodulin (rhsTM) attenuated CIR-dependent plg accumulation, and strongly delayed fibrinolysis at CIRs (approx. 7 times). Carboxypeptidase inhibitor dose-dependently enhanced CIR-dependent fibrinolysis initiation especially in the presence of rhsTM. Conclusion: Our study for the first time directly presents the crosstalk between coagulation and fibrinolysis, which takes place on the surface of activated platelets and is further controlled by thrombin activatable fibrinolysis inhibitor (TAFI). (COI:Properly Declared)

1P-182

The role of hepatocyte tissue factor in the hypercoagulable state observed in chronic liver injury

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BACKGROUND: Patients with chronic liver disease have a dysregulated coagulation system and are prone to thrombosis. The basis for this hypercoagulable state is not completely understood. Tissue factor (TF) is the primary initiator of blood coagulation. The aim of this study was to determine the contribution of TF to the hypercoagulable state in a mouse model of chronic liver injury. METHODS: We measured levels of TF activity in the liver, white blood cells (WBC) and circulating microparticles (MP), and a marker of activation of coagulation (thrombin-antithrombin complexes (TATc)) in the plasma of mice subjected to bile duct ligation for 12days. We used wild-type (WT) mice, mice with a global TF deficiency (low TF mice), and mice deficient for TF in either myeloid cells ($TF^{flox/flox}$,LysM-Cre mice) or in hepatocytes ($TF^{flox/flox}$,Alb-Cre). RESULTS: WT mice with liver injury had increased levels of TF activity of WBC and MP, and TATc compared to sham mice. Low TF mice and mice lacking TF in hepatocytes had reduced levels of TF in the liver and in MP and exhibited reduced TATc without a change in liver fibrosis. In contrast, mice lacking TF in myeloid cells had reduced WBC TF but no change in MP TF activity or TATc. CONCLUSIONS: Hepatocyte TF activates coagulation in a mouse model of chronic liver injury. TF may contribute to the hypercoagulable state associated with chronic liver diseases in patients. (COI:No)

1P-183

Glycinergic thoracic interneurons are not involved in the rostro-caudal gradient in the thoracic inspiratory motor activity

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The inspiratory motor outputs are larger in the intercostal muscles at more rostral segments. Such rostro-caudal gradient is kept in the *in vitro* preparation from neonatal rat. Anatomical studies showed no evidences that the inspiratory bulbospinal neurons have systematic patterns of connections to different segments. Therefore, such gradients should be generated by the propriospinal neurons. To clarify the involvement of the inhibitory thoracic interneuron, the effects of blockade of the glycine receptor were examined. The respiratory-related neuronal activities were optically recorded from thoracic segments in the isolated brainstem-spinal cord preparations from neonatal rats stained with voltage-sensitive dye. Inspiratory-related signals were detected from ventral surface of all spinal segments examined (T1-T13). Bath application of 10 μM strychnine to the thoracic cord caused the seizure-like activity in the C4 ventral root. The amplitude of the seizure-like optical activity was more than ten times larger than the amplitude of the inspiratory signals. The amplitude of the inspiratory optical signals were not so affected by strychnine, and the inspiratory signals in the rostral thoracic segments were always larger than those in the caudal thoracic segments. These results suggest that the thoracic inspiratory inhibitory interneurons are not involved in the rostro-caudal gradients of the inspiratory motor activity. These neurons may be involved in shaping the expiratory motor activity. (COI:No)

1P-184

Locomotor activities in the external and internal intercostal muscles in the neonatal rat *in vitro*

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The major roles of the external and internal intercostal muscles (EIM, IIM) are respiration. However, it has been suggested that these muscles have also roles in stabilization of the trunk during locomotion. The neuronal mechanisms to adjust these muscle activities to both respiration and locomotion are not fully understood. It is well documented that bath application of NMDA and 5-HT evokes locomotor activities in the hind-limb muscles in the *in vitro* preparations from neonatal rat. In the present study, we studied the pattern of the motor activity in EIM and IIM at sixth-eighth thoracic segments (T6-8) during the locomotion evoked by NMDA and 5-HT. All dorsal roots were sectioned to eliminate afferent feedback. The hind-limb flexor muscle activity was monitored from the second lumbar ventral root (L2VR), and inspiratory activity was monitored from the fourth cervical VR (C4VR). The recording from L2VR was also used as the abdominal expiratory motor activity. EIM consistently showed inspiratory activity under normal pH solution. In most of preparations, IIM showed expiratory activity under normal or low pH solution. When the locomotor activity was induced by NMDA and 5-HT, EIM and IIM showed flexor- and extensor-related activity, respectively. These results suggested that the inspiratory and expiratory intercostal muscles play as flexor and extensor muscles during locomotion. However, since the abdominal muscles at T13-L2 showed expiratory and flexor-related activities, the expiratory muscles do not always play a role as extensor muscles. (COI:No)

1P-185

Respiratory activities of medullary neurons and astrocytes in the isolated brainstem-spinal cord analyzed by calcium imaging

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Astrocytes play active roles by communicating with neurons in various brain functions. Indeed, we discovered respiratory astrocytes in medullary slices (Okada et al. 2012). We aimed to reveal the participation of astrocytes in respiratory rhythm generation in the isolated brainstem-spinal cord, which has a more integrated network structure than slices. The preparation was bent so that the rostral cut surface was horizontal. A calcium indicator Oregon Green was pressure-injected into the ventrolateral medulla. Cellular activities with inspiratory C4 output were firstly recorded in the normal condition, and were secondly investigated in the presence of TTX with a confocal calcium imaging system. Then, they were classified into neurons and astrocytes by lowering the superfusate potassium to 0.2 mM with TTX. Astrocytes were spontaneously active with TTX, and lowered potassium induced intracellular calcium rise in astrocytes but not in neurons. Cross correlation with inspiratory C4 output revealed that activities of a number of astrocytes were inspiratory-modulated. So far, respiratory astrocytes have been encountered at the level rostral to the rostral portion of the pre-Botzinger complex. Further studies are needed to clarify the precise role of astrocytes in rhythm generation. (COI:No)

1P-186

Respiratory control by the inhibitory neurons in the ventrolateral subnucleus of the nucleus of the solitary tract in the mouse

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The ventrolateral subnucleus of the nucleus of the solitary tract (vlNTS) contains vesicular GABA transporter (VGAT)-positive neurons, and these neurons are activated by respiratory facilitation in hypercapnia. Here we first demonstrated that rostral ventral respiratory group (rVRG) projecting vlNTS neurons are activated by hypercapnia using retrograde tracing combined with Fos labeling. Next, we showed by whole body plethysmography that Cre recombinase-dependent deletion of VGAT neurons in the vlNTS increases tidal volume (TV) and decreases respiratory rate (RR) during hypercapnia and even in a normal condition in unrestrained conscious mice. In contrast, pharmacogenetics activation of VGAT neurons in the vlNTS using DREADDs system induced a decrease of TV and an increase of RR. We further showed that axon terminals from VGAT neurons in the vlNTS are apposed to rVRG neurons projecting to the phrenic nucleus, using conditional anterograde tracing combined to traditional retrograde tracing. These results suggest that inhibitory neurons in the vlNTS regulate TV and RR through their projection to the rVRG in normal breathing, and these VGAT neurons are activated during large inflation of the lung in hypercapnia. (COI:No)

1P-187

Developmental changes in the intracellular Cl^- concentration in the perinatal hypoglossal motoneurons related with respiration-related activities

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GABA is one of main inhibitory neurotransmitter in adult central nervous system but an excitatory neurotransmitter during early postnatal development. Such GABA action shift from excitatory to inhibitory is caused by decreasing of intracellular chloride ion concentration ($[Cl^-]_i$) which is determined by balance of K^+-Cl^- co-transporter-extrusion system (KCC2) and Na^+, K^+-$2Cl^-$ co-transporter-accumulation system (NKCC1). Role of GABAergic transmission in regulation of medullary respiration-related rhythmic activity (RRA) perinatally, however, is yet to be determined. We previously reported that mean numbers of RRA recorded from mouse hypoglossal nucleus (12N) were significantly increased from embryonic day (E) 16 to postnatal day (P) 0 but there were no significant changes in RRA from P0 to P7. Here, we measured the $[Cl^-]_i$ in the motoneurons of 12N using gramicidin-perforated patch clamp recording method during perinatal development. The $[Cl^-]_i$ of prenatal motoneurons in 12N was higher than that of postnatal motoneurons. These results suggested that decreasing $[Cl^-]_i$ caused by increasing KCC2 expression levels might induce inhibitory GABAergic actions in 12N until P0. (COI: No)

1P-188

Relationship between anxiety behavior and respiration control as determined using optogenetics

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Breathing patterns vary based on emotions, including anxiety and fear. However, the mechanism whereby emotion influences respiration has not been fully elucidated. Recent reports examining fear and anxiety mechanisms mediated by monoaminergic systems indicate that selective acute excitation of serotonin (5-HT) neurons in the median raphe nucleus (MRN) increase innate anxiety. Thus, we used optogenetic methods to study the effects of MRN 5-HT neurons on the relationship between innate anxiety and respiration. We generated Tph2-tTA::tetO-ChR2[C128S]EYFP double transgenic mice in which a channelrhodopsin-2 variant (ChR2[C128S], a step-function opsin, was expressed under the control of Tph2 promoter (Tanaka et al., Cell Rep. 2012). As expected, MRN 5-HT neurons expressed ChR2. We inserted an optical fiber to target MRN and stimulated 5-HT neurons via a Teleopto stimulator during ongoing behavior.We used an elevated plus-maze to examine innate anxiety (time spent in open arms) and whole-body plethysmograph to measure respiratory variables (respiratory rate, tidal volume, etc.). Our results suggest that MRN 5-HT neurons affect the relationship between innate anxiety and control of respiration. (COI:No)

1P-189

Neuronal mechanisms of inhibitory effects of eugenol on respiratory neuron activity in the brainstem-spinal cord preparation

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Eugenol is contained in several plants including clove and is used as an analgesic drug. In peripheral and central nervous system, this compound modulates neuronal activity through action on voltage-gated ionic channels and/or on TRP channels. We previously reported that eugenol induced inhibitory effects on burst generation of respiratory neurons in the medulla of the brainstem-spinal cord preparation. To elucidate the detailed mechanisms, we examined effects of TRPV1 or TRPA1 antagonists on eugenol-induced burst depression. We also examined effects of eugenol on persistent sodium current of respiratory neurons. The brainstem-spinal cord preparation were isolated from newborn rats (P0-P3) and superfused by modified Krebs solution at 25-26C°. The inspiratory C4 ventral root activity was monitored. Membrane potentials of respiratory neurons were recorded in the caudal parafacial region of the rostral medulla. Shortening of C4 burst duration by eugenol was not reversed by application 10 μM capsazepine or 10 μM HC030031. We analyzed the negative slope conductance in response to depolarizing voltage-ramp stimulation under voltage clamp conditions. The application of eugenol (1 mM) significantly reduced the negative slope conductance of the respiratory neurons, suggesting the blockade of persistent sodium current. Present results suggested that eugenol exerted the inhibitory action on respiratory neuron burst generation by inhibiting the persistent sodium current of respiratory neurons via pathways independent of TRPV1 or TRPA1. (COI:Properly Declared)

1P-190

Dexmedetomidine sedation and imidazoline 1 receptor activation in spontaneously breathing newborn rats

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Dexmedetomidine may activate not only alpha-2 (α_2) adrenoceptor but also imidazoline 1 (I_1) receptor. We examined a possible involvement of I_1 receptor activation in physiological changes, including those related to cardiorespiratory function, under dexmedetomidine sedation in spontaneously breathing newborn rats. We implanted into newborn rats an abdominal catheter to administer drugs and measured heart rate and respiratory indices. Each rat was administered dexmedetomidine (50 μgkg^{-1}) and, 5 minutes later, administrated normal saline or 1 to 10 $mgkg^{-1}$ atipamezole (selective α_2-adrenoceptor antagonist) or efaroxan (α_2-adrenoceptor/I_1 receptor antagonist). Dexmedetomidine administration alone significantly changed most of the cardiorespiratory indices and the addition of atipamezole and efaroxan fully or partially ameliorated the dexmedetomidine-associated reduction in heart rate and respiratory frequency. Mean inspiratory flow (V_T/T_I ; V_T is tidal volume and T_I is inspiratory time), which is an index of respiratory drive, was not significantly affected by the administration of dexmedetomidine alone or dexmedetomidine + atipamezole, but was significantly decreased after the dexmedetomidine + efaroxan administration. In newborn rats druing dexmedetomidine sedation, the I_1 receptor is likely to be activated and play a significant role in maintaining respiratory drive. The results are going to be discussed with blood analysis. (COI:No)

1P-191

Major dissociation between wake- and respiration-promoting activities: Modafinil promotes wakefulness but not respiration in rodents

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Background: Modafinil is a wake-promoting agent and has been widely used for daytime sleepiness in patients with narcolepsy and other sleep disorders. A recent case series reported that daily oral modafinil alleviated hypercapnic respiratory failure in patients with COPD. However, the precise action of modafinil on respiration such as hypercapnic and/or hypoxic ventilatory responses remains unclear. Methods: We investigated the hypothesis that modafinil enhances resting ventilation as well as the stimulatory ventilatory responses to hypercapnia and hypoxia. Minute ventilation, respiratory rate and volume components using plethysmography, combined with a concurrent EEG monitoring of the level of wakefulness before and after administration of modafinil in two doses in unanesthetized mice were measured. Results: Wakefulness, locomotor activity and variability of the breathing pattern in tidal volume were promoted by both doses of modafinil. Neither dose of modafinil increased the absolute values of resting ventilation or promoted the ventilatory responses to hypercapnia and hypoxia. Conclusions: Modafinil is conducive to the state of wakefulness but does not augment resting ventilation or the hyperventilatory responses to chemical stimuli in unanesthetized rodents. (COI:Properly Declared)

1P-192

Quantitative analysis of respiratory central chemoreflex system in human

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Background: Central chemoreflex is the principal system responsible for respiratory homeostasis. It is also well known that failure of central chemoreflex leads to respiratory abnormalities in patients with heart failure. The aim of this study is to develop a quantitative and minimally invasive method to characterize central chemoreflex function. Methods: In 6 healthy volunteers, we measured end tidal CO2 (EtCO2), and minute ventilation (VE). We divided the respiratory system into two subsystems: the controller (EtCO2 to VE) and plant (VE to EtCO2). To identify the controller, we randomly switched inhaling CO2 between 2 levels (0 or 5%). To evaluate the plant, subjects were instructed to randomly change their respiration according to commanding visual sequences. We modeled both systems as $H(s)=G\times\exp(-s\times Td)/(\tau s+1)$ (G=gain, τ=time constant and Td=dead time) and identified these parameters by fitting the model to the time-series.Results: We found the parameters of controller as G=0.89±0.41 L/min/mmHg, τ=45±15 sec and Td=3.0±9.3 sec, and those of the plant as G=-0.58±0.1 mmHg/L/min, τ=18.4±8.0 sec and Td=-0.9±1.2 sec. Time series back calculated from parameters in each subject matched well with those measured (R2=0.72±0.12 in controller and 0.78±0.11 in plant), indicating the accuracy of the models and parameter estimations. Conclusions: The proposed method accurately characterizes the central chemoreflex function. (COI:No)

1P-193

Effects of a visual feedback of thoracoabdominal motion on the respiratory muscle oxygen consumption during diaphragmatic breathing exercise in healthy humans

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Diaphragmatic breathing (DB) exercise is often used for COPD patients to improve breathlessness. It has been reported that a visual feedback of thoracoabdominal motion (VF) enhances the movement of abdomen compared to rib cage during DB (Sackner et al.,1984). We hypothesized that DB with VF is more effective than DB without VF, and as a result the oxygen consumption of respiratory muscles (VO2 resp) during DB with VF is lower. Seven healthy males were used in the present study. The subjects sit on a chair with a backrest reclined at an angle of 60 degrees. Displacement of rib cage and abdomen was obtained using a respiratory inductive plethysmography, and these two signals were displayed in real time on a liquid crystal monitor as the X-Y diagram for VF. The experimental protocol was as follow; (a) the subjects performed quiet breathing for 3 min, (b) breathed with 12 liters of dead space to increase respiratory drive for 3 min, (c) performed DB without VF for a minute, and (d) DB with VF for a minute. Effects of DB with/without VF on VO2 resp was compared by values obtained with the following formula; (VO2 at c or d - VO2 at a) / body weight / (VE at c or d - VE at a) [ml/kg/L]. The obtained value was -0.487 ± 0.708 for DB with VF and was significantly lower than that for DB without VF (-0.057 ± 0.202, P<0.05). These results suggest that DB with VF is an effective technique for rehabilitation. (COI:No)

1P-194

A trial of anomaly detection from biological time-series data by using machine learning

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Machine learning has been used for detecting anomaly from long period of time-series data in many fields such as economics. Recent progress of the technology makes it possible to obtain our individual daily biological data such as ambulatory activities. If machine learning automatically detects an unusual trend of these time-series data, it may be helpful to determine the inappropriate behavior related to our health problem. In this study, instead of using human data, we used ambulatory activity and body temperature data of mouse to examine whether machine learning can detect deviations of data after four hours of sleep deprivation. As a baseline, the body temperature and ambulatory activity of mouse were recorded for one week every ten seconds. After the baseline recording, we performed four hours of sleep deprivation expecting to alter the trends of these data. We applied k-nearest neighbors algorithm by using R programming language to obtain the distance measure as the level of anomaly. The calculated distance value of temperature data of light period on the day of sleep deprivation was apparently higher than that of other days. It is suggested that machine learning may be a powerful tool to automatically detect unusual deviation of the biological time-series data. Further basic research may be needed to apply machine learning on the analysis of human biological time-series data. (COI:No)

1P-195

Quantitative analysis for crystallin changes by Raman spectroscopy

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Purpose: We identified the molecular lever of component in tissue by Raman scattering spectroscopy. Tissue Raman scatting spectrum going to be determined with more week laser irradiation and in short time period, it will become possible due to advance in spectrometer and lazar technique. This is the basic research for clinical quantitation of cataract formation. We report the results of Raman spectra in rat lens by using portable Raman spectrometer. Methods: Sprague-Dawley rat (7-week-old) was anesthetized with pentobarbital sodium (50 mg/kg, i.p.), fixed a head and determined Raman spectrum of rat lens by portable Raman spectrometer (ProRaman-L, Enwave Optronics Inc., USA): applied a 785 nm laser with 130 mW, acquisition time 30 seconds. Results: The Raman spectra of rat lens showed a band at 3300 cm^{-1} due to an OH stretching mode and bands at 2935 cm^{-1} and 3065 cm^{-1} assigned to crystallin CH group. Conclusion: These results suggest that Raman spectra of cataract may be measurable by using potable Raman spectrometer in clinic. We will measure Raman Spectra of human nucleated lenses and nucleated eyes, will cooperate with development of spectrometer and laser irradiation device, and lead to clinical use. (COI:No)

1P-196

Gene-labeled cultured tumor model inspection for anticancer drugs using acoustic impedance microscope

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High-frequency ultrasonic microscope is useful to observe intracellular structure of cultured living cells. We have observed the dynamical change of intracellular actin filaments in several cells, and reported the difference of stabilities between cancer cells and ordinary cells. The dynamics of actin filaments are deeply related to cancer cell viability. We have observed the effect of DNA targeting anticancer drugs on co-cultured glia-glioma tumor model using acoustic impedance microscope. In this study, we have applied this acoustic microscopy to breast cancer research. Breast cancer is the most commonly but highly proliferative and uncontrolled cancer. In cancer treatment, many of different targeted chemotherapy have been approved for clinical use, although many of them are toxic even on normal cells. Therefore, it is required to check the function and reliability of each drug to living cells viability. We conduct a quantitative observation of different anticancer drugs on fluorescent-gene labeled breast cancer cells. Through this study we attempt to build a quantitative assay that could reveal the relationship of structure-mechanical property-biological function-disease state relationships that could guide the development of novel tools for disease diagnostics and therapeutics, as well as drug efficacy assays. (COI: No)