Poster Presentations

Day 3

(March 24, 13:00 - 14:00)

3P-001 – 3P-016	Ionic Channel, Receptor (3)
3P-017 – 3P-042	Cell Physiology, Molecular physiology (2)
3P-043 – 3P-076	Heart, Circulation (3)
3P-077 – 3P-099	Environmental Physiology
3P-100 – 3P-108	Drug Actions
3P-109 – 3P-116	Membrane Transport
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3P-139 – 3P-144	Digestion, Absorption
3P-145 – 3P-169	Nutrition, Metabolism, Thermoregulation
3P-170 – 3P-188	Pathophysiology
3P-189 – 3P-195	Physical Fitness, Sports Medicine
3P-196 – 3P-202	Respiration
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3P-212 – 3P-219	Others

3P-001

Independent activation of the two subunits of the TMEM16 calcium-activated chloride channel

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The TMEM16 family encompasses Ca^{2+}-activated Cl^- channels (CaCCs) and lipid scramblases. These proteins are formed by two identical subunits, and the structure of a TMEM16 molecule has recently been solved. Mutagenesis studies in TMEM16A have identified several acidic amino acids, the mutation of which reduces the apparent Ca^{2+} affinity of the channel by 100-1000-fold. The crystal structure of a TMEM16 scramblase supported that these acidic residues indeed coordinate bound Ca^{2+}. Here, we construct a TMEM16A channel with linked TMEM16A subunits of different Ca^{2+} affinities by mutating a glutamate residue critical for Ca^{2+} affinity in only one subunit. The dose-response curve of such a heterodimer can be described by the weighted sum of the wild-type and the mutant TMEM16A dose-response curves, as if activations of two subunits are independent. The independent activation of the two subunits was also revealed upon washing out Ca^{2+} due to different Ca^{2+}-unbinding rates in different subunits. Furthermore, the Ca^{2+} activation curve of the heterodimer at the [Ca^{2+}] range of < 5-10 μM is nearly the same as that of the wild-type channel; the Hill coefficients in both cases are significantly greater than 1. These results suggest that a single TMEM16 subunit may consist of more than one Ca^{2+} binding site, and that Ca^{2+} binding to one subunit of TMEM16A is sufficient to activate the channel, in contrast to the scenario in voltage-gated cation channels where all subunits must be activated before the channel can be opened significantly. (COI:No)

3P-002

Functional interaction between TRPV4 and anoctamin 1 in salivary and lacrimal glands

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Calcium is the primary intracellular factor in the regulation of fluid secretion in salivary and lacrimal glands. Several ion channels and transporters, water channels, as well as polarized calcium signaling events participate to that regulation. The major ion channel involved in fluid secretion has recently been identified in salivary glands as Anoctamin 1 (ANO1), which constitutes the apical Ca^{2+}-activated Chloride channel (CaCC) efflux pathway required for Ca^{2+}-dependent fluid secretion. The understanding of the mechanisms of activation and molecular interactions of ANO1 could offer new leads for the treatment of exocrine gland diseases such as the Sjogren syndrome. Our recent work has led to the characterization of a functional interaction between ANO1 and some members of the TRP channels superfamily, exposing new regulators of the ANO1-mediated functions. One of them was the Transient Receptor Potential Vanilloid 4 (TRPV4), a calcium-permeable channel. Interestingly, this ion channel is reported to be highly expressed in the membrane of secretory acinar cells, while its function remains unclear. Here, we report a functional interaction between TRPV4 and Anoctamin 1 in salivary and lacrimal acinar cells, suggesting a role of TRPV4 in the regulation of fluid secretion. (COI:No)

3P-003

Lack of TRPV2 impaired BAT thermogenesis in mice

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Brown adipose tissue (BAT), a major site for mammalian non-shivering thermogenesis, could be a target for prevention and treatment of human obesity. Transient receptor potential vanilloid 2 (TRPV2), a Ca^{2+}-permeable non-selective cation channel, plays vital roles in the regulation of various cellular functions. TRPV2 was functionally expressed in brown adipocytes and involved in their differentiation. mRNA levels of thermogenic genes were significantly lower in both cultured brown adipocytes and BAT from TRPV2 knockout (TRPV2KO) mice, and increases in the genes to β-adrenergic receptor stimulation were significantly lower in TRPV2KO brown adipocytes and significantly suppressed by reduction in intracellular Ca^{2+} concentrations in WT brown adipocytes. In addition, TRPV2KO mice have significantly heavier white adipose tissue and larger sizes of brown adipocytes. And TRPV2KO mice showed cold intolerance and less increases in *in vivo* BAT temperature to β-adrenergic receptor stimulation. Furthermore, TRPV2KO mice showed significantly heavier body weight and fat upon high fat diet treatment. Based on these findings, we concluded that TRPV2 is involved in BAT thermogenesis and could be a human obesity therapy target. (COI:No)

3P-004

Rab3 interacting molecule 3 mutations associated with autism alter regulation of voltage-dependent Ca^{2+} channels

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We have previously demonstrated that Rab3 interacting molecule 3 (RIM3) physically and functionally interacts with voltage-dependent Ca^{2+} channels (VDCCs) expressed in neurons via the β subunits, and increases neurotransmitter release. Here, by introducing corresponding autism-associated mutations that replace glutamic acid residue 176 with alanine (E176A) and methionine residue 259 with valine (M259V) into the C_2B domain of mouse RIM3, we demonstrate that both mutations partly cancel the suppressive RIM3 effect on voltage-dependent inactivation of Ba^{2+} currents through P/Q-type $Ca_V2.1$ recombinantly expressed in HEK293 cells. In recombinant N-type $Ca_V2.2$ VDCCs, the attenuation of the suppressive RIM3 effect on voltage-dependent inactivation is conserved for M259V but not E176A. Slowing of activation speed of P/Q-type $Ca_V2.1$ currents by RIM3 is abolished in E176A, while the physical interaction between RIM3 and β subunits is significantly attenuated in M259V. Moreover, increases by RIM3 in depolarization-induced Ca^{2+} influx and acetylcholine release are significantly attenuated by E176A in rat pheochromocytoma PC12 cells. Thus, our data raise the interesting possibility that autism phenotypes are elicited by synaptic dysfunction via altered regulation of presynaptic VDCC function and neurotransmitter release. (COI:No)

3P-005

Theoretical analysis of inositol 1,4,5-trisphosphate receptor-mediated Ca^{2+} mobilization in mouse pancreatic β-cells

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GLP-1 is an intestinal hormone that potentiates glucose-stimulated insulin secretion from pancreatic β-cells. The secretagogue action of GLP-1 is explained, at least in part, by its ability to stimulate cAMP signaling pathway and subsequently facilitate the release of Ca^{2+} from IP_3 receptor (IP_3R)-regulated Ca^{2+} stores. However, the molecular mechanisms and dynamic processes linking GLP-1-stimulated cAMP production to Ca^{2+} mobilization remain elusive. Here, we performed simulation studies to investigate how GLP-1 alters the abilities of Ca^{2+} and IP_3 to act as co-agonists at IP_3Rs. A new dynamic model was constructed, which demonstrates dual steady state allosteric regulation of the IP_3R by Ca^{2+} and IP_3. Data obtained from β-cells were then analyzed to understand how GLP-1 facilitates IP_3R-mediated Ca^{2+} mobilization when UV flash photolysis is used to uncage Ca^{2+} and IP_3 intracellularly. After the dynamic model for IP_3R activation was incorporated into a minimal cell model, the Ca^{2+} transients and oscillations induced by GLP-1 were successfully reconstructed. Simulation studies indicated that transient and oscillatory responses to GLP-1 were produced by sequential positive and negative feedback regulation due to fast activation and slow inhibition of the IP_3R by Ca^{2+}. Interestingly, the slow rate of Ca^{2+}-dependent inhibition was revealed to provide a remarkable contribution not only to the time course of the decay of cytosolic Ca^{2+} transients, but also to the frequency of Ca^{2+} oscillations. (COI:No)

3P-006

Protein kinase C regulates human cardiac Kv1.5 potassium channels

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Human cardiac potassium Kv1.5 channel (hKv1.5) is the main contributor to the cardiac ultra-rapid delayed rectifier potassium current (I_{Kur}), which plays an important role in the early repolarization of the atrial action potential. We herein, investigate using hKv1.5-expressing HEK cells whether the expression, degradation and intracellular localization of hKv1.5 are modified by chronic activation of protein kinase C (PKC) and/or its downstream signaling pathways. Western blot analysis indicated that the protein levels (both immature and mature proteins) of hKv1.5 were significantly elevated by 12 h pre-treatment with phorbol 12-myristate 13-acetate (PMA) a PKC activator at 10~100 nM. Staurosporine an inhibitor of PKC attenuated PMA-induced enhancement of hKv1.5 protein expression. In addition, hKv1.5 current was also significantly increased when pre-incubated with 100 nM PMA. Moreover, PMA pre-treatment markedly increased the hKv1.5 fluorescence signal in the cell surface. Experiments using tunicamycin, an inhibitor of N-glycosylation, indicated that the N-glycosylation of hKv1.5 is more pronounced after chronic PMA exposure. Our results suggest that the chronic PKC activation could stabilize the channel protein in the cellular organelles or on the plasma membrane, and modify its maturation and/or trafficking, thus enhancing the amplitude of the current. (COI:No)

3P-007

Localization of the concentration-sensitive sodium channel in the mouse lung

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Background: Concentration-sensitive sodium channel (Na_C) expressed in the brain plays a role as a sodium sensor for the body fluid balance. On the other hand, little is known about the physiological role of Na_C expressed in the lung. Aims: Since various channels, including epithelial sodium channels (ENaCs) and cystic fibrosis transmembrane conductance regulator (CFTR) contribute to ion transport across the alveolar epithelium to maintain alveolar functions, we hypothesized that Na_C, expressed in the lung, is responsible for Na^+ transport in the alveolus. The aim of this study was to determine Na_C localization in the lung. Methods: Na_C distribution in the mouse lung was examined by immunofluorescence with antibodies against Na_C, aquaporin 5 (AQP5), CFTR, and γENaC. Na_C subcellular distribution was examined by immunoelectron microscopy. The expression of Na_C was analyzed by RT-PCR and immunostaining in pulmonary microvascular endothelial cells (PMVECs) isolated from the mouse lung. Results: Immunofluorescence studies showed that Na_C localized in the alveolar wall. Na_C did not colocalize with aquaporin 5 and CFTR, but partially colocalized with γENaC. Immunoelectron microscopy revealed that Na_C localized in the basolateral membrane of PMVECs as well as in the apical membrane and in the cytoplasm of alveolar epithelial type II cells (AECII). Results of RT-PCR and immunostaining showed that Na_C was expressed in PMVECs isolated from the mouse lung. Conclusions: Na_C is mainly expressed in the basolateral membrane of PMVECs and localizes in the apical membrane and cytoplasm of AECII. (COI:No)

3P-008

Postnatal developmental changes in sensitivity of cardiac L-type Ca^{2+} channel to inhibition by verapamil

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L-type calcium (Ca^{2+}) channel blockers (verapamil, dihydropyridines, diltiazem) are widely used for the treatment of hypertension and tachyarrhythmias in clinical settings. However, intravenous administration of verapamil for tachyarrhythmias is considered to be contraindicated in neonates and infants, due to the perceived risk of hypotension or bradycardia. We investigated the postnatal developmental changes in the sensitivity of L-type Ca^{2+} current ($I_{Ca,L}$) to their blockers 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 similar Langendorff-perfusion methods. Whole-cell patch-clamp technique was applied to ventricular myocytes of various postnatal ages to record $I_{Ca,L}$ and to examine the sensitivity of $I_{Ca,L}$ to inhibition by three groups of L-type Ca^{2+} channel blockers. IC_{50} for the inhibition of $I_{Ca,L}$ by verapamil was significantly smaller in day-0, day-7, day-14 and day-21, compared with day-28 and adult (10-15 weeks) ventricular myocytes. On the other hand, there were no significant differences in IC_{50} for the inhibitory action of nifedipine or diltiazem in all postnatal developmental ages. $I_{Ca,L}$ in neonates and infants exhibits a higher sensitivity to inhibition by verapamil compared with that in child and adult stages, which may contribute at least partly to the development of verapamil-induced hypotention in neonates and infants. (COI:No)

3P-009

Interaction of an antiarrhythmic drug bepridil with human Kv1.5 channel through specific amino acids within the pore region

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Because human Kv1.5 (hKv1.5) channels are highly expressed in cardiac atria but are scarce in ventricle, pharmacological blockade of hKv1.5 has been regarded as effective strategy for prevention and treatment of reentry-based atrial tachyarrhythmia. Although the antiarrhythmic drug bepridil has been reported to inhibit hKv1.5 channel, there is little information as to the amino acids in the pore region of hKv1.5 which bepridil interacts with. Thus, this study was undertaken to examine the effect of bepridil on hKv1.5 channel and to elucidate the underlying molecular determinants. Site-directed mutagenesis was carried out to introduce single point mutants (T480A, A501V, I502A, I508A, L510A and V516A) into hKv1.5 cDNA by using a Quikchande-II-XL Kit. Whole-cell patch-clamp technique was used to record membrane currents from hKv1.5 wild type (WT) and mutant channels heterologously expressed in Chinese hamster ovary (CHO) cells. Bepridil concentration-dependently (IC_{50}, 1.2 μM±0.40) blocked hKv1.5 current. In addition, bepridil-induced current block gradually progressed during the depolarizing pulse, suggesting that bepridil preferential block the channels as an open-channel blocker. Moreover, the degree of current block by bepridil was significantly attenuated in I502A, I508A and V516A, but not in T480A, A501V and L510A mutants. Our results indicate that, several amino acids within pore S6 domain (Ile502, Ile508 and Val516) are critically involved in bepridil-induced inhibitory effect on hKv1.5 channel. (COI:No)

3P-010

Structure-function relationship of small-molecule binding site in Kir1.1 channel

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Inwardly rectifying potassium (Kir) channels play fundamental roles in diverse physiological process and may be unexploited drug targets. VU590 and VU591 are the first small molecules that can potently inhibit Kir1.1 channel, but not Kir2.1 or Kir4.1 channel. In this study, we carried out electrophysiological analyses and molecular dynamics simulation to explore the molecular basis of drug-channel interactions required for selective Kir1.1 channel inhibition. The effects of VU590 and VU591 on chimeric and site-directed mutants of Kir1.1 expressed in Xenopus oocytes were examined using the two-electrode voltage clamp technique. Val140 and Asn171 of Kir1.1 were indispensable for the inhibition of the current. The closed and open conformation models of the Kir1.1 pore suggested that both residues faced the central cavity and they were positioned within a geometrical range capable of interacting with the drugs. Docking simulation showed that a drug used Val140, Asn171 and other pore-lining residues for interaction with Kir1.1. Mutations of the corresponding residues (Thr127 and Glu158) in Kir4.1 affect the susceptibilities of the channels to the drugs. These results indicate that VU590 and VU591 interact with Kir1.1 channel pore residues, which may account for their selective inhibitory action on Kir1.1 channel. (COI:No)

3P-011

Monocarboxylate transporter 9 is a novel urate transporter in kidney

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Monocarboxylate transporter 9 (MCT9) is an orphan transporter member of the 16th solute carrier family (SLC16). Its amino acid sequence has only 30-35% identity to other members and distinguishes from MCT1 to 4. MCT9 has been suggested to be involved in the onset of hyperuricemia or gout by genome-wide association studies (GWAS). Since no experimental data about MCT9 function has been reported, we tried to clarify its physiological roles. The quantitative RT-PCR (qRT-PCR) against human tissue cDNAs revealed that the mRNA expression of MCT9 is quite higher in kidney than other tissues. Using Xenopus oocyte expression system, MCT9 mediated the transport of [^{14}C]urate in Na^+-, Cl^- and voltage-independent manner. The common missense variant of MCT9 (T258K), which was suggested to be related with gout, did not alter the urate transport capability. Urate lowering drug, benzbromarone and febuxostat, but not allopurinol could inhibit MCT9-mediated urate transport. Here, our study is the first evidence for MCT9 function as a urate transporter in kidney. (COI:No)

3P-012

Functional expression of purinergic P2X7 receptors in rabbit articular chondrocyte

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ATP is released by articular chondrocytes under physiological and pathological conditions, which activates plasma membrane purinergic (P2Y and P2X) receptors to modulate the cellular function in an autocrine and paracrine manner. It has been reported that various classes of P2X ion channel receptors are expressed, but little is known about the electrophysiological effects of extracellular ATP in articular chondrocytes. In this study we show that P2X7 ion channel receptor is functionally expressed in rabbit articular chondrocytes using whole-cell patch-clamp technique. Rabbit cartilage was collected from the joints of male animals, and freshly isolated chondrocytes were employed for current recordings. Extracellular application of ATP readily and reversibly activated a non-selective cation conductance that exhibited an inwardly rectifying current-voltage relationship. ATP and Bz-ATP (a potent agonist for P2X7 receptor) activated the membrane current in a concentration-dependent manner with a half-maximal excitatory concentration (EC50) of 1.04 mM and 1.15 uM, respectively ATP-induced activation of the membrane current was almost completely abolished by the P2X7-selective antagonists A438079 (300uM) and A740003 (30uM). These findings provide evidence that P2X7 ion channel receptors function in rabbit articular chondrocytes. (COI:No)

3P-013

Functional expression of P2X receptors in an odontoblast cell line

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Stimuli to odontoblasts induce intercellular communication between neurons and adjacent odontoblasts. Purinergic receptor antagonists suppress these intercellular communications, thereby demonstrating that the communication is mediated by extracellular ATP as the transmitter. Purinergic receptors are subdivided into two structurally distinct subfamilies: P2X and P2Y receptors. P2Y receptors show higher selectivity for nucleotides other than ATP. In this study, we examined the functional expression of ionotropic ATP-selective receptors, P2X receptors, in a mouse odontoblast cell line. Membrane currents induced by P2X receptor activation were recorded by whole-cell patch-clamp recordings with a holding potential of -70 mV. Extracellular application of 100 μM K^+-ATP as the non-selective P2X receptor agonist, 300 μM BzATP as the $P2X_7$ selective agonist, and α,β-MeATP as the $P2X_{1,2/3,3}$ selective agonist evoked inward currents with amplitudes of -31.8 ± 5.1 pA, -26.4 ± 0.7 pA, and -1.1 ± 1.6 pA, respectively. Inward currents induced by K^+-ATP were significantly inhibited by 5-BDBD as a selective $P2X_4$ antagonist and KN62 as a $P2X_7$ selective antagonist, but not by NF449 as a $P2X_1$ selective antagonist and NF110 as a $P2X_3$ selective antagonist. In addition, reversal potentials of the currents showed a positive shift to +56 mV by K^+-ATP and +53 mV by BzATP. The results indicated that odontoblasts express $P2X_4$, and $P2X_7$ receptors, but do not functionally express $P2X_{1,2,3}$ receptors. (COI:No)

3P-014

Role of STIM1 in the heteromer formation of TRPC channels in PC12 cells

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Muscarinic receptor stimulation induces the activation of nonselective cation (NS) channels in adrenal medullary (AM) cells of guinea pigs, but not rats, and STIM1, which is suggested to be involved in gating of store-operated channels including TRPC channels, is expressed in the former, but not the latter. The electrophysiological and pharmacological properties of the muscarinic receptor-regulated NS channel resemble those of the heteromer of TRPC1 and TRPC4 channels. These results raise the possibility that STIM1 is somehow involved in the functional expression of the heteromer channel. To explore this possibility, TRPC1 and TRPC4 or TRPC5 channels were expressed with or without STIM1 in PC12 cells, an immortalized cell line of rat AM cells. The proximity ligation assay was used to examine heteromer formation. Overexpressed TRPC1 and TRPC4, but not TRPC5, were found to from a heteromer in the cytoplasm in a STIM1-dependent manner. The muscarinic stimulation resulted in translocation of heteromers to the cell periphery, probably the plasma membrane. The heteromer formation occurred with the expression of a C-terminal truncated-mutant of STIM1 that lacks the last 237 residues; however, the heteromer in cells expressing such a STIM1 mutant was not translocated to the cell periphery upon muscarinic stimulation. These results suggest that STIM1 is essential for the formation of TRPC1/4 heteromer channels and their translocation upon muscarinic stimulation, but different domains of STIM1 are involved in the two effects. (COI:No)

3P-015

Evaluation of alteration in intracellular Ca^{2+} mobilization with MaxiK channels in the endoplasmic reticulum

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Ion channels working at plasma membrane are assembled in endoplasmic reticulum, then they are transported to their destination via Golgi apparatus. But it is unclear that whether they are conferred function and physiological role(s) in the intracellular membrane.We observed large conductance Ca^{2+}-activated K^+ (MaxiK) currents in nuclear envelopes (peri-nuclear ER membrane) using the patch-clamp technique. Consequently we tried to elucidate the roles of ion channels in ER membrane with this channel.In this study, to evaluate alteration in intracellular Ca^{2+} mobilization with MaxiK channel in the ER membrane. We employed Ca^{2+} imaging technique using a genetically encoded and ER targeted Ca^{2+} indicator, G-CEPIA. The probe was expressed in wild type or MaxiK-expressing HEK293 cells. And the Ca^{2+} dynamics was recorded in response to thapsigargin or P2Y receptor stimulation. We assessed the difference in magnitude of $[Ca^{2+}]_{lumen}$ change and speed of Ca^{2+} release / replenishment between two type of cells. (COI:No)

3P-016

Potassium channels contribute to motor regulation of esophageal striated muscle in rats

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Physiological roles and properties of potassium channels in gastrointestinal and arterial smooth muscles have been reported. However, it is unclear whether potassium channels can contribute to motor regulation of the esophageal striated muscle. Therefore, the aim of the present study was to clarify regulatory roles of potassium channels in the motility of the esophageal striated muscle in rats. An isolated segment of the rat esophagus was placed in an organ bath and the mechanical responses were recorded using a force transducer. Electrical stimulation of the vagus nerve evoked the contractile response in the esophageal segment. The vagally mediated contraction was enhanced by application of inhibitors of potassium channels: glibenclamide, an ATP-dependent potassium channel blocker, and TEA and 4-aminopyridine (4-AP), non-selective blockers of voltage-gated potassium channels. However, application of apamin, an Ca^{2+}-dependent potassium channel blocker, did not affect the vagally mediated contractions of the esophageal striated muscle. On the other hands, nicorandi, an opener of ATP-dependent potassium channels, attenuated the esophageal striated muscle contractions induced by vagal stimulation. These findings suggest that voltage-gated potassium channels and ATP-dependent potassium channels might contribute to regulation of the motor activity in the esophageal striated muscle. (COI:No).

3P-017

Exercise training attenuate the development of diabetic cardiomyopathy through modulating inflammation and oxidative stress

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Background: Over-nutrition and sedentary lifestyle has led to a worldwide increase in obesity, insulin resistance, and type 2 diabetes associated with an increased risk of cardiovascular disorders. Diabetic cardiomyopathy (DCM), independent of hypertension or coronary disease. Pathogenesis of DCM is complex and multifactorial, and it will eventually lead to reduced cardiac working capacity and ischemic injury. We evaluated whether chronic exercise could attenuate the development of obesity-induced DCM. Methods: Lean WT & genetically diabetic (ob/ob) mice were subjected to sedentary (SED) or treadmill exercise regimens. Exercise training (EXT) consisted of low-intensity/long-duration (6W) protocols. Metabolic & cardiac abnormalities were thoroughly analyzed with ECHO, histopathology, IHC & immunoblots. Further, we employed Azan-Mallory & HE staining to demonstrate cardiac fibrosis & hypertrophy respectively. Results: After 6W of training exercise improved cardiac function and decreased food consumption, water intake significantly. EXT obese mice decreased fasting blood glucose, plasma inflammatory cytokines (interleukin-6 & TNF-alpha) and oxidative stress when compared to SED obese mice. Staining revealed that EXT mice improved fibrosis and hypertrophy than the SED control mice. Conclusion: Our results indicate that chronic exercise improves cardiac function & reverses abnormal cardiac structural remodeling, through the suppression of inflammation and oxidative stress. (COI:No)

3P-018

Comparison of the effect of current therapeutic agents for diabetes in Cdkal1-deficient mice

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Genetic variations in the Cdk5 regulator associated protein 1-like 1 (CDKAL1) gene have been associated with type 2 diabetes (T2D). Cdkal1 catalyzes 2-methylthio modification at position 37 adenosine in cytosolic tRNALys(UUU). The 2-methylthio modification is critical for the accurate decoding of lysine codon. Deficiency of 2-methylthio modification in pancreatic beta-cell-specific cdkal1-deficient (KO) mice show aberrant proinsulin translation, and resulted in impaired insulin secretion and glucose intolerance. Given the similarity of symptoms between Cdkal1 KO mice and T2D patients carrying risk CDKAL1 variation, we utilized Cdkal1 KO mice as T2D model to investigate the long-term effects of anti-diabetic drugs. We treated Cdkal1 KO mcie with glibenclamide, glucagon like peptide 1 (GLP1) agonists including exendin-4 and liraglutide, and sitagliptin, an inhibitor of GLP-1 protease (DDP-4) up to 8 weeks. Glucose metabolism and gene expression were then investigated. Long-term application of glibenclamide impaired the glucose tolerance and insulin secretion in KO mice. In contrast, GLP-1 agonists and sitagliptin significantly improved the glucose tolerance and insulin secretion. Examination of gene expression showed that both liraglutide and sitagliptin, but not glibenclamide, decreased the expression of unfolded protein response-related genes as well as L-Myc, a dedifferentiation-related gene. Our results suggest that the GLP-1 related drugs will be beneficial for T2D patients carrying risk Cdkal1 variation. (COI:No)

3P-019

Cytoskeleton-dependent regulation of volume-sensitive anion channel is involved in resistance to anticancer drugs

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Although cancer cells are normally sensitive to anticancer drugs, they gradually acquire tolerance for the drugs during the treatment. We have previously found that volume-sensitive anion channels are functionally expressed in human epidermoid carcinoma KB cells, but not in the cisplatin-resistant KCP-4 cells derived from KB cells. In the present study, we investigated how volume-sensitive anion channels are down-regulated in KCP-4 cells. Two-dimensional gel electrophoresis combined with MALDI-TOF MS showed that expression level of β-actin in KCP-4 cells was much lower than that in KB cells. Immunocytochemistry using an anti-β-actin antibody showed clear staining of actin filaments in KB cells, but not in KCP-4 cells. To investigate whether actin filaments regulate volume-sensitive anion channels in KB cells, we performed whole-cell patch-clamp recordings and cell volume assays. Knockdown of β-actin in the cells decreased the swelling-induced current of volume-sensitive anion channel and inhibited the regulatory volume decrease after cell swelling. Similar effects were observed in the cells treated with cytochalasin D which disrupts actin filaments. Interestingly, cytochalasin D inhibited cisplatin-triggered caspase-3/7 activation in KB cells. These results suggest that volume-sensitive anion channels are down-regulated by disruption of actin filaments, and that decreased activity of the channels is associated with cisplatin resistance of KCP-4 cells. (COI:No)

3P-020

Contribution of Na^+ / H^+ exchanger1 to lymph node metastasis of head and neck squamous cell carcinoma by participating in collective migration

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Recent studies elucidated cancer metastasis can be initiated by cellular migration in collectives, namely collective migration. We observed enhanced protein expression of Na^+ / H^+ exchanger 1 (NHE1) in human head and neck squamous cell cancer (HNSCC) tissue. Aiming at to investigate the role of this overexpression, we compared the phenotypes of human HNSCC cell lines SAS and its metastatic subline SASL1m which express NHE1 in higher level than SAS. SASL1m showed higher rates of metastasis to lymph node in mouse metastasis model and of in vitro invasion in modified Boyden chamber Matrigel invasion assay. Moreover, time-lapse video analysis revealed these human HNSCC cells migrate collectively, while the randomness of the migration of SASL1m was higher than SAS. Knockdown experiments demonstrated the participation of NHE1 in lymph node metastasis in SASL1m, together with the severe impairment of invasive activity and motility in the collective migration. We propose NHE1 as a possible target for anti-metastasis therapy in HNSCC as a responsible factor for invasiveness of metastatic SCC by participating in collective migration. (COI:No)

3P-021

Isoamericanol A from *Jatropha curcas* seed extract shows anti-cancer activity by cycle arrest at G2/M in the human breast cancer cell MCF-7.

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Our study reports on the anti-carcinogenic activity of isoamericanol A (IAA) as *Jatropha curcas* (*J. curcas*) seed extract. The results showed that IAA is capable of inhibiting cell proliferation in a dose-dependent manner on the human cancer cell lines of MCF-7, MDA-MB231, HuH-7, and HeLa. The molecular mechanisms of 25μg/ml IAA on MCF-7 were investigated by DNA-microarray analysis, flow cytometry, TUNEL assay, western blot, and quantitative real-time PCR. The results showed increased expression of BTG2 (B-cell translocation gene 2, $p<0.05$), p21 ($p21^{WAF1/CIP1}$, $p<0.05$), and GADD45A (growth arrest and DNA-damage-inducible, alpha, $p<0.001$), in addition to decreased expression of CDK1 (cyclin-dependent kinase 1, $p<0.05$) and cyclins B1 ($p<0.001$) and B2 ($p<0.001$). These expressional changes indicate a inhibition of the cyclin B/CDK1 complex, the major activator of G2/M cell cycle progression, resulting in an inhibition of further cancer cell growth. In comparison to the non-treated cells, cell division processes were significantly impaired in IAA treated cells using immunofluorescence staining of aurora B kinase, α-tubulin, and the chromosome. These findings suggest that IAA has great effects in halting cancer cell growth by G2/M cell cycle arrest. (COI:No)

3P-022

Involvement of TRPC6 in Na^+/H^+ exchanger NHE1-induced enhancement of calcineurin-NFAT signaling

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Calcineurin (CaN), a Ca^{2+}-dependent phosphatase, is a key molecule to govern pathological cardiac hypertrophy. CaN dephosphorylates a downstream transcription factor NFAT, which in turn induces hypertrophic gene expression. We have previously reported that a pH-regulating transporter NHE1 activates CaN and leads to cardiomyocyte hypertrophy, via direct binding of CaN to the cytosolic domain of NHE1. Since this NHE1 function requires both CaN binding and ion transport activity of NHE1, we hypothesized that a local sub-plasma membrane pH increase produced by NHE1 enhances the activity of the CaN bound to NHE1 by sensitizing CaN to intracellular Ca^{2+}. In the present study, we obtained several data suggesting that TRPC6, one of transient receptor potential (TRP) channels, is an additional factor regulating this NHE1-mediated activation of CaN. 1) Co-immunoprecipitation analysis revealed that NHE1 and TRPC6 interacts each other in heterologous expression system. 2) Fractionation of cardiomyocytes lysate using sucrose density gradient centrifugation showed that a part of both TRPC6 and NHE1 proteins exist in lipid raft where CaN is also present. 3) A TRP inhibitor ruthenium red abolished NHE1-mediated enhancement of NFAT reporter activity. From these results, we consider that TRPC6, NHE1, and CaN form a complex in lipid raft in cardiomyocytes, and the Ca^{2+} near the complex supplied through TRPC6 may further activate CaN enhanced by NHE1. We are now examining whether change in NHE1 activity modifies TRPC6 activity. (COI:No)

3P-023

The effect of Yuzaki-nebuka (Allium vegetables) on migration of liver cancer cell in the presence of adipocyte.

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It is reported that obesity is the deterioration factor for the prognosis of cancer. The migration of cancer cells causes cancer metastasis and is followed by bad prognosis. This study is aimed to investigate the influence of adipocytes on migration of cancer cells and the effect of Yuzaki-nebuka (Allium vegetable), which is the traditional vegetable of Nara prefecture, on migration of cancer cells in the presence of adipocytes. To assess the migration abilities of liver cancer cells in the presence of adipocytes, HepG2 cells were co-cultured with 3T3-L1 preadipocytes by using Boyden chamber. 3T3-L1 preadipocytes were cultured in the lower chamber and differentiated into adipocytes, prior to insertion of upper chamber in which HepG2 cells were disseminated. More migration of HepG2 cells was observed in the presence of adipocytes which had more accumulated lipid. However, Yuzaki-nebuka extracts significantly prevented the migration of HepG2 cells in the presence of adipocytes. Since gelatin degradation activity and urokinase-type plasminogen activator (u-PA) activity were involved in the migration, both activities in the co-culture medium were measured by zymography. Depending on the lipid accumulation in adipocyte, both gelatin degradation and u-PA activities were increased. However, Yuzaki-nebuka extracts did not affect both activities. It was suggested that adipocytes induced migration of liver cancer cells and Yuzaki-nebuka inhibited the migration ability of them in the presence of adipocytes. (COI:No)

3P-024

Quantitative evaluations of reactive oxygen cascade based on exogenous H2 consumption in the adult humans

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Inhaled or ingested hydrogen gas (H2) inactivates hydroxyl radicals (OH) in the biological system. We previously reported that the H2 consumption rate (VH2) after the ingestion of H2 water was compatible with that during inhalation of low levels of H2 gas. Pretreatment with antibiotics did not affect VH2. H2 loss from the skin surface was negligible. Administration of vitamin C decreased VH2 in a dose-dependent manner. These pieces of evidence lead us to speculate that exogenous H2 binds to OH radical and that VH2 reflects the OH production rate in the human body. Physicochemical studies reported the temperature dependence of rate constants in the reaction of H2 + OH -> H2O + H. The activation energy for this reaction is low. Furthermore, we recently found that inhalation of H2 gas decreased emanation of OH(H2O)n clusters from the human skin, suggesting that OH(H2O)n cluster have longer lifetime than monomer OH, and it may play a key role for the H2 consumption in the biological system. Based on these results, we quantitatively evaluated the reactive oxygen cascade in the adult humans. (COI:Properly Declared)

3P-025

Functional Analysis of TRPM7 in Odontoblasts

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Transient receptor potential (TRP) ion channel family is well known to play a role in a sensor such as temperature, osmotic pressure, and redox status. Among TRP channel family, TRPM7 has a unique structure organization that contains a TRP channel domain with 6 transmembrane segments fused to an atypical serine-threonine kinase domain at its C terminus. Genetic deletion of TRPM7 in model systems demonstrates that this channel is critical for cellular growth and embryonic development. In this study, we found that TRPM7 is highly expressed in odontoblasts in the dental pulp by in situ hybridization of mouse embryo. Quantitative real-time PCR analysis revealed that expression of TRPM7 in the tooth was remarkably higher than any other tissue of adult mouse. We also confirmed that TRPM7 protein is expressed in odontoblasts by immunohistochemistry. To investigate the physoloical function of TRPM7 in odontoblasts, we examined TRPM7 channel activities using a mouse odontoblast cell line. These results suggested that higher expression of TRPM7 plays as an important role in odontoblasts. We will also show our recent results of physiological role of TRPM7 in odontoblasts. (COI:No)

3P-026

Roles of bone marrow stem cells during bone repair process

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The bone marrow (BM)-derived cells might be the source of endothelial cells, osteoblasts, and osteoclasts, which are responsible for tissue maintenance and repair. However, the influences of the bone repair process after bone destruction or fractures on BM cells, particularly hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), remain unclear. In the present study, we examined the effects of bone defect on the number of HSCs and MSCs in BM and spleen in mice and its mechanism. We analyzed the HSC and MSC populations in BM tissues form mice with a femoral bone damage by using flow cytometry. The numbers of HSCs and MSCs in the BM of mice with damaged femurs were significantly lower and higher, respectively, than the numbers of these cells in the BM of the contralateral intact femurs on day 2 after injury. Both intraperitoneal administration of AMD3100, a C-X-C chemokine receptor 4 (CXCR4) antagonist, and local treatment with an anti-stromal cell-derived factor-1 (SDF-1) antibody blunted the decrease and increase in HSC and MSC populations, respectively, in the BM of injured femurs. In conclusion, the present study revealed that there is a concurrent decrease and increase in the numbers of HSCs and MSCs, respectively, in mouse BM during repair after a femoral bone damage. Furthermore, the SDF-1/CXCR4 system was implicated in the changes in these stem cell populations during bone repair. (COI:No)

3P-027

Functional analysis of Endothelial cells derived from Plasminogen activator inhibitor-1 (PAI-1) deficient patients

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[Background] Plasminogen Activator inhibitor-1 (PAI-1) is the principle regulator of PA system which is related between enhanced PAI-1 expression levels and diseases such as thrombosis and malignant tumors. We have identified PAI-1 deficient patients having apparent phenotypes of severe bleeding and impaired wound healing, both of which are not seen in the PAI-1 KO mice. We are investigating the intrinsic function of PAI-1 in endothelial cells using iPS cells established from the patients. [Methods] iPS cells from patients were generated, then we differentiated iPS cells to endothelial cells. Endothelial cells were isolated by magnetic sorting with anti-VEGFR-2 antibody, and their functions and the related gene expressions were analyzed. [Results] (1) Endothelial cells derived from PAI-1 iPS cells (PAI-1 iPS-ECs) detached from dish bottom easier than control when the cells were cultured for longer period of time on gelatin-coated dishes. (2) PAI-1 iPS-ECs migrated faster in migration assay. (3) The expression of Dll4 gene, known to be associated with the region of angiogenic-edge, was enhanced and the branching in tube formation assay was reduced in PAI-1 iPS-ECs.[Conclusion] These results suggest that PAI-1 could play critical roles in differentiation and maturation of endothelial cells. (COI:No)

3P-028

Regulation of vesicle rupture and cell death caused by blue-light irradiation on acridine orange stained cells

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It is known that many kind of stress cause cell death with vesicle and nuclear membrane rupture. Acridine orange (AO) has been widely used in many method such as the assessment of sperm quality. On the other hand, blue-light irradiation with AO maintains intracellular vesicle rupture and to go into cell death. We previously found that ion replacement of extracellular fluid could suppress cell death with lysosome stabilization. In this study, we examined vesicle behavior and events relating to cell death during and after blue-light irradiation with AO on HeLa cells. Vesicles started to rupture from about 30 seconds of irradiation, that caused enzyme dispersion and cell death. And the cytotoxic effect of blue-light irradiation depended on time and concentration of AO. Bafilomycin A1 treatment suppressed rupture through inhibition of AO accumulation in lysosome. As known to an inducer of HSPs, Geranylgeranylacetone (GGA) inhibited rupture events during blue-light irradiation too. Cells performed cell death after overnight culture as aftereffects of irradiation, but viability of GGA-treated cells was higher than that of untreated cells. Because of extracellular chloride ion replacement could suppressed cell death after overnight culture, it implies the adjustment of ion flux is widely effective on cell death pathway. (COI:No)

3P-029

Effects of 385 nm UVA LED light irradiation on cultured RAW 264.7 cells

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We studied effects of ultraviolet A (UVA) irradiation using light-emitting diode on RAW 264.7 cells. The peak wavelength of the diode was 385 nm when performed from the reverse side of the dish with UVA LEDs at 145.8 W/cm^2. These cells were irradiated for varied time (0-2 hr), but lactate dehydrogenase (LDH) release into medium was not found for 2 hr at least. Then, we tried to measured reactive oxygen species (ROS) by addition of 2,7-dichlorodihydrofluorescein diacetate(DCFH$_2$ DA) in medium. Medium ROS initially increased and attained a constant level, and intracellular ROS was also increased with time, but the revel started to decrease after 1 hr. The initial increase in ROS in both cell and medium within 1hr were suppressed by addition of histidine or NaN_3 (scavenger of $^1O_2$), and stimulated when intracellular glutathione (GSH) content decreased by addition of 1-chloro-2,4-dinitrobenzene. Next, to detect ROS induced in the medium irradiated by UVA light, we measured EPR signals in the presence of spin trapping agents (TPC and DMPO) by EPR(electron paramagnetic resonance) spectrometer. NaN_3 decreased the spin peaks formed by TPC and histidine decrease the peaks by DMPO. These results suggest that ROS induced by UVA-irradiation is mainly composed of $^1O_2$, and GSH acts as a factor in protecting the cells from these ROS. (COI:No)

3P-030

ERK inhibition accelerates bone marrow adipogenesis despite the existence of estrogen

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Postmenopausal osteoporosis is associated with estrogen deficiency, because loss of estrogens leads to reduction in bone formation, increasing in bone resorption, and high levels of marrow adipogenesis. Both direct and indirect effects of estrogens on osteoblastogenesis has been widely studied. Estrogens also suppress the adipogenesis of bone marrow stromal cells (BMSCs). However, there are few reports on signaling pathways of the estrogen-induced adipogenic suppression. In the present study, we investigated the relationship of estrogen and extracellular signal-regulated kinases (ERK) pathway on the bone marrow adipogenesis.Mouse primary BMSCs were cultured in standard medium, with or without 17-beta-Estradiol (E_2, 10 nM). After 7 days, adipogenesis was introduced by adipocyte differentiation medium. The cells were fixed after 14 days, the value of lipid accumulation was assessed by staining for lipid accumulation and extraction with Oil Red O. E_2 suppressed the lipid accumulation in BMSCs and suppressed the mRNA levels of C/EBPα and PPARγ, both of which are important adipogenic transcription factors. We next determined the relationship of estrogens and ERK. Under inhibition of ERK activity by MEK inhibitors, E2 did not show the suppression of lipid accumulation in BMSCs. E_2 did not affect phosphorylation of ERK. These data suggest that decreased ERK activity in BMSCs might increase adipocyte accumulation in spite of under normal concentration of estrogens. (COI:No)

3P-032

Electrical stimulations affect the proliferative and migratory potentials of bone marrow stromal cells (BMSCs)

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It has been recognized that bioelectrical stimuli effectively modulate some physiological events such as proliferation, differentiation, and migration. However, the mechanism(s) underlying them rather remain equivocal. We recently found that increased TRPC6 channel activity critically drives the G_1/S cell cycle progression of BMSCs via a depolarizing shift of resting membrane potential (RMP). An independent study also reported that artificially induced de-/hyper-polarization can inhibit/facilitate BMSC differentiation, respectively. These findings point to the ability of RMP to control the cell cycle progression and differentiation of BMSCs. To gain more insights about this possibility, we tested the effects of artificial electrical manipulation of RMP on BMSC proliferation. Sustained culture in an elevated K^+-medium (by 100μM) induced a BMSC death within six days. In contrast, electrical stimulation induced BMSC migration and facilitated its proliferation. More specifically, BMSCs were periodically stimulated by a monophasic and squared waveform (0.1V, 0.2msec, 1 pulse/min). 24 hr later, BMSCs migrated toward the electrode, the direction of which turned to opposite by reversing the polarity of electrical stimulation. Furthermore, prolonged application of electrical stimulation promoted BMSC proliferation, whereas excessive electrical stimulation (1 pulse/10sec) killed BMSCs. These observations suggest that varying strengths and patterns of electrical stimulation differentially affect the fate of BMSC, and when they are optimal, facilitating its migration and proliferation. (COI:No)

3P-033

Involvement of MARCKS phosphorylation and translocation in amylase secretion in rat pancreatic acini

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Cholecystokinin (CCK) induces amylase release in pancreatic acinar cells. In this process, activation of protein kinase C (PKC) is thought to be an essential step. Myristoylated alanine-rich C kinase substrate (MARCKS) is a major substrate of PKC. The phosphorylation of MARCKS by PKC results in the translocation of MARCKS from the membrane to the cytosol. PKCδ has a strong affinity for MARCKS and can phosphorylate MARCKS. Here, we investigated the involvement of MARCKS phosphorylation and translocation by PKCδ in CCK-induced amylase release in rat pancreatic acini. MARCKS, phosphorylated-MARCKS and PKCδ were detected by Western blotting. Amylase activity was measured by Bernfeld's method. MARCKS protein was detected in pancreatic acini. CCK induced MARCKS phosphorylation. CCK induced MARCKS translocation from the membrane to the cytosol. When acini were lysed by a detergent, Triton X-100, CCK partially induced displacement of the MARCKS from the GM1a-rich detergent-resistant membrane fractions (DRMs). CCK induced PKCδ activation. A PKCδ inhibitor, rottlerin, inhibited the CCK-induced MARCKS phosphorylation and amylase release. A MARCKS-related peptide inhibited the CCK-induced amylase release. These findings suggest that MARCKS phosphorylation by PKCδ and then MARCKS translocation from the GM1a-rich DRMs to the cytosol are involved in the CCK-induced amylase release in pancreatic acini. (COI:No)

3P-034

Ambroxol-stimulated increases in CBA and CBF via pH_i increase and $[Cl^-]_i$ decrease in airway ciliary cells of mice.

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Ambroxol (ABX), a mucolytic agent, is known to be a drug stimulating the ciliary beating. However, the mechanisms of ABX actions on the ciliary beating remain uncertain. In this study, we examined the effects of ABX on CBA and CBF using bronchiolar ciliary cells of mice.Ciliary cells isolated from mouse lungs by an elastase treatment were observed using videomicroscopy equipped with a high speed camera (500 fps) at 37&degC. We measured two parameters from video images; ciliary beat frequency (CBF) and ciliary bend angle (CBA), which increase the rate of mucociliary transport.ABX increased CBA and CBF via two pathways: 1) pH_i elevation caused by ABX-activated NBC increases CBA and CBF; 2) $[Cl^-]_i$ diminution associated with ABX-evoked cell shrinkage mediated via nifedipine-sensitive increase of $[Ca^{2+}]_i$ elevates CBA. Removal of Cl^- in the incubating solution elevates CBA mimicking the ABX action via $[Cl-]_i$ diminution associated with ABX-evoked cell shrinkage on CBA.In conclusion, ABX stimulates the ciliary beating coupled with transepitherial HCO_3^-/Cl^- secretion by modulating pH_i and $[Cl^-]_i$. (COI:No)

3P-035

Glucagon-like peptide-1 stimulates K^+ channel activity in cultured human proximal tubule cells

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Glucagon-like peptide-1 (GLP-1) is a well-known incretin hormone that enhances insulin secretion in a cAMP-dependent manner. In addition to the pancreatic action, it was reported that GLP-1 affected renal functions, such as glomerular filtration and sodium reabsorption in the proximal tubule. The sodium reabsorption in renal tubule cells depends on the electrochemical gradient which is provided by K^+ channels, as well as Na^+/K^+-ATPase. Therefore, it is intriguing to know whether GLP-1 would affect K^+ channel activity in renal tubule cells. In cultured human proximal tubule cells (RPTECs), an inwardly rectifying K^+ channel with an inward conductance of 40 pS was most frequently observed under the control condition, and this K^+ channel contributed to the formation of cell-negative potential. In this study, we investigated the effects of GLP-1 on the activity of the 40 pS K^+ channel in RPTECs, using the patch-clamp technique. In cell-attached patches, GLP-1 (100 nM) acutely stimulated channel activity. This stimulatory effect was blocked by a GLP-1 receptor antagonist, exendin(5-39) (500 nM). Furthermore, a PKA inhibitor, KT5720 (500 nM) abolished the GLP-1-induced stimulation of channel activity. We also confirmed that the mRNA of GLP-1 receptor was actually expressed in RPTECs, using RT-PCR. These results suggested that GLP-1 stimulated the activity of the 40 pS K^+ channel in RPTECs through binding to its specific receptor, and that the stimulatory effect was dependent on the cAMP/PKA pathway. (COI:No)

3P-036

A swelling-induced Ca^{2+} entry pathway in $MrgprB4^+$ dorsal root ganglion neurons

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MrgprB4 is a member of MAS-related G protein-coupled receptors (Mrgpr), which are predominantly expressed in primary sensory neurons in dorsal root ganglia (DRG). Recently, $MrgprB4^+$ neurons have been reported to detect a massage-like pleasant tactile sensation in mice. However, it was also reported that electrophysiological analysis of isolated $MrgprB4^+$ neurons failed to reveal responses to mechanical stimuli. Therefore, the mechanisms of how the neurons detect tactile stimuli are unknown. As a response to a type of mechanical stimuli, we found that hypotonic-induced cell swellings increased intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) of $MrgprB4^+$ neurons. Therefore, the aim of this study is to characterize the mechanism of the swelling-induced $[Ca^{2+}]_i$ increase. DRG neurons were dissociated from genetically-modified mice, whose $MrgprB4^+$ neurons express mtdtomato, a red fluorescent protein. $[Ca^{2+}]_i$ was measured using Fura2. When the neurons were perfused with a hypotonic solution, from which 50 mM NaCl was omitted, the $[Ca^{2+}]_i$ was gradually increased. However, a low-NaCl isotonic solution, which contains mannitol instead of the omitted NaCl, did not increase $[Ca^{2+}]_i$. The swelling-induced $[Ca^{2+}]_i$ increase was dependent on the presence of extracellular Ca^{2+}. The $[Ca^{2+}]_i$ increase was inhibited by ruthenium red, Gd^{3+}, and FM1-43, but neither by tranilast (a TRPV2 blocker), HC030031 (a TRPA1 blocker), nor HC067047 (a TRPV4 blocker). In conclusion, it is suggested that $MrgprB4^+$ neuron express a swelling-activated Ca^{2+}-permeable channel, which is not the TRP channels mentioned above. (COI:No)

3P-037

The molecular mechanism of intracellular Cl^- in cancer progression by regulating Src kinase signal cascades

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A malignant tumor is a serious health problem because cancer cells can spread to distant parts of the body, so-called metastasis. For example, gastrointestinal cancer cells arising in the gastrointestinal tract enter the bloodstream and spread to distant organs such as the lung. Since metastasis is the most common cause of death from cancer, this process is an important therapeutic target. However, the molecular mechanisms of the metastasis are not fully understood. Our recent studies show that the cytosolic Cl^- plays important roles in fundamental cellular functions. If there are differences between the cytosolic Cl^- concentrations ($[Cl^-]_c$) of primary and metastatic tumor cells due to ionic environments surrounding primary and metastatic tumor cells, and the activity of Cl^- transporters and/or Cl^- channels of primary and metastatic tumor cells, the change in $[Cl^-]_c$ of primary and metastatic tumor cells would be a candidate causing the cell migration and the cell invasion. Therefore, in this study, we investigated the effect of $[Cl^-]_c$ on the cell migration and the cell invasion in several gastrointestinal tumor cell lines. Our study indicates that cytosolic Cl^- is a key factor regulating Src kinase signal cascades involved in tumor migration and invasion. These results strongly suggest that changes of $[Cl^-]_c$ would play important roles in cancer migrations and invasion. (COI:No)

3P-038

Activation peptide of coagulation factor IX regulates endothelial permeability

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Hyper-permeability of endothelium are involved in critical illness. Recently it has been reported that activation peptide of coagulation factor IX (AP) enhances cell-matrix and intercellular adhesion. The aim of this study is to investigated its function on intercellular adhesion of endothelial cells and evaluate its effect on endothelial permeability. In the presence of AP, cells spread with lamellipodia-like broad protrusions multi-directionally and adhesion area to matrix increased by 16%. In intercellular adhesion, the treatment with AP induced overlapping of adjacent cell edge and remodeling of intercellular adhesion with colocalization of adherens junctional proteins, VE-cadherin and β catenin, and a marker protein of lateral border recycling compartment, PECAM. AP suppressed the permeability of endothelial cell monolayer induced by interleukin-1βin a dose-dependent manner. The treatment with AP decreased eNOS protein expression and changed its subcellular localization, decreasing intracellular cGMP. An analog of cGMP suppressed the effects of AP on cell spreading. Finally, the effect of AP on hyper-permeability was investigated in mice injected with lipopolysaccharide (LPS). LPS increased lung weight by 28% and the treatment with AP significantly suppressed it to 5%. Our study indicates that AP regulates endothelial intercellular adhesion and it could be applied to the treatment of vascular hyper-permeability. (COI:No)

3P-039

Identification of novel substrates of plasmin on endothelial cell

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Plasmin is a serine protease to dissolve fibrin, a main component of blood clots. Plasminogen, an inactive precursor of plasmin, is activated by tissue plasminogen activator (tPA). Plasmin has broad substrate specificity and regulates cell function by degrading fibrin or other extracellular proteins, but its substrates are still largely unknown. We tried to find novel plasmin substrates, especially from endothelial cells in this study.bEnd.3, a mouse brain capillary endothelial cell line, was treated in 4 different condition, exposure to normoxia with glucose over 6h (Nor/-Plg/-tPA), exposure to oxygen glucose deprivation (OGD) alone over 6h (OGD/-Plg/-tPA), exposure to OGD 6h followed by treatment of tPA (OGD/-Plg/+tPA) or exposure to OGD 6h followed by treatment of plasminogen (Plg) and tPA (OGD/+Plg/+tPA). After the treatments, whole membranes of bEnd.3 were extracted and assessed by SDS-PAGE or 2-dimensional electrophoresis (2D-PAGE). Furthermore, the bands in SDS-PAGE were excised from the stained gel and analyzed by LCMS-IT-TOF.A database analysis was then performed by using MASCOT search method.It was found that there were several appeared or disappeared bands or spots in whole membrane extract under OGD/+Plg/+tPA in comparison with OGD/-Plg/-tPA in SDS-PAGE or 2D-PAGE, indicating that proteins in disappeared bands were cleaved by plasmin and their degraded products were observed as appeared bands. Further, a part of vimentin was detected in newly apparent bands in both membrane extracts and reaction supernatants under OGD/+Plg/+tPA, suggesting that vimentin is a novel plasmin substrate in endothelial cells. (COI:No)

3P-040

Investigation of molecules related to the antiproliferative effects of hesperetin on the cancer cells

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Hesperetin (3', 5, 7-trihydroxy-4'-methoxyflavanone) is found in citrus fruits and is used in Chinese medicine. Previous studies have shown that it has anticancer effects in vivo and in vitro, but its action and molecular mechanism remain unclear. On the other hand, induced NO synthase (iNOS), stathmin1, and galectin-1 have been known to be potential targets to cancer therapy. Moreover currently glyceraldehyde-3-phosphate dehydrogenase (GAPDH) is highlighted not only as a glycolytic enzyme, but also as a multifunction protein, which plays roles in apoptosis, DNA repair, and cytoskeletal organization. Therefore it is suggested to be a potential target to care some kinds of cancer.
This study aimed to reveal the action and molecular mechanism underlying anticancer effect of hesperetin to the cancer cells, especially GOTO cells, IMR-32 cells and U2OS cells. Trypan blue staining showed decrease in cell number after treatment with 100 μM hesperetin to all cells at 24 h. Therefore, these results indicate that hesperetin has an antiproliferative effect to the cancer cells. In addition, immunocytochemistry with GOTO cells also indicates that only a subpopulation of the iNOS-positive cells were also stained by an antibody against GAPDH strongly. Moreover we are analyzing the GAPDH expression of GOTO cells treated with various concentrations of hesperetin for 24 h and 48 h by Western blotting. (COI:No)

3P-042

The new topical drug delivery system with Cell-Penetrating Peptide (CPP)

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Topical therapy is the most favored form of treatment for whitening against hyper-pigmentation, sunburn and skin cancer. However, high-molecular-weight, hydrophilic chemicals are difficult to use as transdermal delivery drugs and the use of topical drugs has been highly limited. We applied 11-arginine (11R), a cell-membrane-permeable peptide, as a transdermal delivery system with a skin delivery enhancer, pyrenbutyrate. We performed intracellular peptide screening for melanogenesis inhibitors with several kinds of tyrosinase inhibitory peptides from natural sources. Next, we performed daily repetitive topical application of this LILVLLAI peptide found in gliadin protein, a wheat component, for two weeks against a UV-induced sun-tanning guinea pig model and confirmed significant melanogenesis inhibition in suntan model. And we applied anti-tumor peptide screened from p53 fragment peptide library against mouse and human melanoma cell lines. We administrated anti-tumor peptide against mouse melanoma model and showed the apoptotic cell death effect in melanoma histology. We summarized that 11R using transdermal drug delivery system with melanogenesis inhibitory peptide and anti-tumor peptide against melanoma is a very safe and promising method for applications from cosmetics to the anti-cancer drug. (COI:No)

3P-043

Polymorphisms of phospholipase A2 receptor 1 gene alter its functions and are a genetic risk of an increased intima-medial thickening of the carotid artery

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Background: Phospholipase A2 receptor 1 (PLA2R) has multiple biological functions beyond a receptor for secretory PLA2s. Two nonsynonymous polymorphisms in C-type lectin-like domains (CTLD) 1 of PLA2R gene were associated with idiopathic membranous nephropathy. This study examined whether the same polymorphisms in PLA2R may alter functions of PLA2R in cells expressing the variant PLA2R. And, clinical relevant of the experiment was examined.
Methods and Results: Two nonsynonymous polymorphisms (T/C at rs3749117 and G/C at rs35771982) in CTLD1 of PLA2R gene were completely linked. HEK293 cells expressing mutant type of PLA2R had lower migratory and proliferative responses to collagen I compared with cells expressing wild-type of PLA2R. In 580 male patients, the polymorphisms of PLA2R gene were associated with an increase in maximum intima-media thickening (maxIMT) of the carotid artery. Multivariate regression model showed that the polymorphisms of PLA2R were a risk factor for an increased maxIMT that was independent of the conventional risk factors (OR = 1.93, 95% CI: 1.17-3.19, $p<0.01$).
Conclusions: The nonsynonymous common variants of PLA2R gene altered biological functions in cells expressing the variant PLA2R. The polymorphisms of PLA2R gene are a genetic risk of an increased IMT of the carotid artery. The functional changes in the variant PLA2R may be partly responsible for its association with an increased IMT of the carotid artery.. (COI:No)

3P-044

The development of Simultaneous observation of single sarcomere length and local intracellular calcium in rat neonatal cardiomyocytes via expression of Yellow Cameleon-Nano140 in Z-discs

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In the present study, we developed a novel experimental system for simultaneous measurement of changes in the intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) at local areas of the Z-discs and single sarcomere length (SL) via expression of FRET-based Ca^{2+} indicator Yellow Cameleon (YC)-Nano140 in Z-discs (α-actinin) in rat neonatal cardiomyocytes. Under dual-view fluorescence microscopy, local $[Ca^{2+}]i$ was determined by the YFP/CFP fluorescence ratio and SL by the peak-to-peak distance of the YFP fluorescence profile. During spontaneous beating, although $[Ca^{2+}]i$ changed uniformly in a localized area (5 consecutive sarcomeres) of a myocyte, sarcomere shortening / lengthening was not synchronized, but rather occurred in an asynchronous fashion, suggesting that the length of each sarcomere is determined via tug-of-war forces of sarcomeres along the myofibril. Furthermore, α-actinin-YC-Nano140 enabled high-precision simultaneous measurements of local $[Ca^{2+}]i$ and single sarcomere contraction under electric field stimulation at 5 Hz. The present experimental system has a broad range of application possibilities for analyzing cardiac excitation-contraction (E-C) coupling in detail at the single sarcomere level. (COI:No)

3P-045

Visualization of excitation spread pattern using the cMOS image sensor and a voltage-sensitive absorption dye

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Using the cMOS image sensor together with a fast voltage-sensitive absorption dye (NK2761), we have visualized the excitation spread pattern in the isolated rat atrial preparation. We have been recorded optical action potentials simultaneously from many sites and mapped the excitation spread pattern in the isolated rat atrial preparation, especially during the event of experimental tachyarrhythmia (tachycardia-like excitation, TE) using 16 X 16-element photodiode array as the photodetector. Recently, the cMOS camera with relatively large well depth has become available. In order to improve the spatial resolution of the optical mapping and to visualize the spatiotemporal pattern of the excitation spread, we tried to use two cMOS cameras (Hamamatsu Photonics ORCA flash4.0, and Andor Zyla 5.5 10-tap) as the photodetector of the optical recording. Using both cameras, we could recorded the optical action potentials with the temporal resolution of 10 frame per second and the S/N ratio of >3, which were inferior to the optical signals detected by the photodiode array (PDA). However, using the digital image processing techniques, we could make movies of the excitation spread of the action potentials from the stimulation site to the whole area of the preparation. Furthermore, we made the movies of the excitation spread during TE with the circus movement of the excitatory wave (i.e. "micro re-entry"). The author deeply thanks Hamamatsu Photonics K. K. and Andor Technology Ltd. for free lending of cMOS cameras and their technical supports. (COI:No).

3P-046

Quantitative analyses of sarcomeric dynamics of transplanted rat neonatal cardiomyocytes in the mouse heart

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To realize the effectiveness of regenerative medicine using cardiac cell therapy, quantitative analyses on the excitation-contraction coupling of transplanted cardiomyocytes are imperative. However, no such studies have yet to be reported. In the present study, we performed *in vivo* analyses of sarcomere dynamics of transplanted rat neonatal cardiomyocytes in the mouse heart, because their features are reportedly similar to those of stem cell-derived cardiomyocytes. We recently developed a high precision measurement of sarcomere dynamics in cultured rat neonatal myocytes (Shintani et al., J Gen Physiol 2014) as well as in adult cardiomyocytes in the beating mouse heart *in vivo* (Kobirumai-Shimozawa et al., J Gen Physiol in press), via expression of α-actinin-AcGFP in Z-discs. By fully taking advantage of these technologies, we are analyzing sarcomere dynamics of rat neonatal cardiomyocytes transplanted into the mouse heart. (COI:No)

3P-047

Development of non-staining imaging technique for evaluating safety using human iPSC-derived cardiomyocytes.

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It is highly expected in the safety assessment to develop nonclinical pharmacological test methods for detecting proarrhythmic risk using human iPSC-derived cardiomyocytes. Imaging techniques with voltage sensitive or calcium dyes have been applied for cardiac safety assessment. However, fluorescent dyes have problems of bleaching, toxicity, and interaction with drugs, etc., thus it is difficult to use as a routine observation and evaluation tool for cardiomyocytes.In the present study, we have developed non-staining imaging technique using polarizer. Optical signals synchronized with heart beats could be obtained from cardiomyocytes without staining using the method. In order to evaluate the properties of the optical signals, cardiomyocytes on a multi-electrode array (MEA) probe were simultaneously recorded by the extracellular field potential and non-staining imaging methods. As a result, the rise time of optical responses synchronized with 1st peak of field potential due to sodium channel activities, indicating that beats from cardiomyocytes could be accurately detected. Furthermore, we found that triggered activities (TA) induced by 100 nM of E-4031 that is IKr channel blocker could be selectively detected, suggesting that the novel technique could distinguish early afterdepolarization (EAD) and TA easily. (COI:No)

3P-048

Facial skin blood flow responses during exposures to emotionally charged negative and positive pictures

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While it is known that emotional arousal affects skin sympathetic nerve activity of a limb, an influence of emotional challenges on facial skin blood flow regulated by sympathetic and parasympathetic nerve are still unclear. We examined the facial skin blood flow responses to visually-elicited emotional interventions using two dimensional laser Doppler imaging. Facial skin blood flows, as well as forearm and dorsum of the hand skin blood flows, heart rate (HR), and mean arterial blood pressure (MAP), were measured in 5 females and 4 males (22 ± 0.4 yrs) during 2 min viewing of positive and negative pictures, which were selected from the database of International Affective Picture System. The extents of pleasantness and consciousness were estimated using subjective rating scores [for example, from -5 (the most unpleasant) to 5 (the most pleasant)]. The average facial skin blood flow increased ($P < 0.05$) during viewing the negative pictures, while the facial skin blood flow did not change during viewing positive pictures. On the other hand, the forearm and hand skin blood flows, HR, and MAP did not change significantly during exposure to any emotionally charged pictures examined. These results suggest that facial skin blood flow may serve as a more sensitive tool to assess an emotional or mood status rather than limb skin blood flow and systemic cardiovascular variables. (COI:No)

3P-049

Resuscitation by transfusion with Hemoglobin Vesicles in Trauma Hemorrhagic Shock / Coagulopathy Rabbits.

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We have developed Hemoglobin vesicle (HbV) as an artificial substitute for Red blood cells (RBC).Objective: To evaluate the efficacy of HbV for alternative treatment of massive transfusion protocol (MTP) in traumatic hemorrhagic shock / coagulopathy rabbits.Methods: Hemorrhagic shock / thrombocytopenia were induced in rabbits by repeated blood withdrawal (total 400ml) and isovolemic transfusion of autologous washed RBC. Liver penetrating injury led lethal oozing hemorrhage. Balloon compression and Platelet rich plasma (PRP) administration stopped bleeding. Thereafter, allogenic RBC transfusion, HbV or 5% Albumin was administered to the animals (n=30). Acute prognosis was compared among them for 24 hr. Hemodynamic and hematologic parameters were periodically recorded for first 1hr.Results: In anemic and thrombocytopenic rabbits (Hb < 6.0 g/dl, platelets < 40,000 /μL, mean arterial pressure (MAP) < 50 mmHg), liver penetrating injury caused additional bleeding and animals suffered class IV shock (Hb < 5.0 g/dl, MAP < 40 mmHg). PRP administration restored platelets > 70,000 /μL, which stopped bleeding within 40 min. Subsequent administration of HbV as well as RBC transfusion regained MAP more than 50 mmHg, and they rescued 70 % animals from liver hemorrhage, although rabbits receiving 5% albumin showed 10% survival in the first 24 hours. HbV administration did not affect the coagulation parameters as well as RBC transfusion.Conclusions: HbV may be effectively instead of RBCs for acute hemorrhagic shock and trauma induced coagulopathy. (COI:No)

3P-050

Oxygen supply for foot tissue by the arterialization of venous network in PAD assumingthe cylindrical diffusion of oxygen

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Peripheral arterial disease (PAD) is caused by reduced blood flow in hind limbs. The peripheral artery is destroyed in PAD and oxygen transport to muscular tissues is reduced. The reduction in oxygen supply causes serious muscle destruction and ulcer in foot, causing intolerable pain and loss of locomotive function. To restore the reduced arterial perfusion in the peripheral foot tissue, a small arterial branch is connected with a peripheral venous branch. Arterial blood flows retrogradely into the small venous branch and further spread into the venular network. Soon after the start of retrograde perfusion of distal veins, the skin color of the affected foot recovers pinky. The improvement of tissue oxygenation was confirmed using the assumption of the one dimensional diffusion. In the present study cylindrical diffusion equation was applied to obtain more exact estimation of the oxygen diffusion. In the present study the cylindrical diffusion equation was used for the more precise estimation of oxygen supply. Also by the revised estimation we came to the conclusion that the oxygen front would be established in the tissue cylinder. Oxygen delivery from venular network to the surrounding tissue may seem rather difficult. However, where oxygen gradient exists, oxygen diffuses according to the partial pressure gradient. The calculated oxygen front covers the venular tissue cylinder. (COI:No)

3P-052

Responses of the rostral nucleus of the solitary tract neurons that receive cardiovascular afferent inputs to stimulation of the lingual-trigeminal nerve

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The rostral part of the nucleus tractus solitarius (NTS) is well known as first order gustatory relay center. We previously demonstrated that a majority of rostral NTS neurons receive cardiovascular and respiratory afferent inputs. We examined responses of the rostral NTS neurons that receive cardiovascular afferent inputs to stimulation of the lingual-trigeminal nerve (LTN) in urethane-chloralose anesthetized rats. Many of the rostral NTS neurons that receive cardiovascular afferent inputs exerted excitatory effects, showing orthodromic spike responses and/or increasing firing rate, on the LTN stimulation at 1 Hz. The latencies of the orthodromic spike responses were 2.0-7.8 ms, suggesting monosynaptic and/or polysynaptic spike responses. Some of these neurons were excited by mechanical stimulation applied to the tongue. On the other hand, some neurons exerted inhibitory effects, decreasing their activity, by the LTN stimulation at 1 Hz. The inhibitory effects were observed during 4-80 ms in the post stimulus time histograms. These results suggest that many of the rostral NTS neurons that receive cardiovascular afferent inputs also receive lingual-trigeminalsomatic afferent inputs. These neuronal activities may be involved in the lingual-trigeminal induced cardiovascular reflex responses in part. (COI:No)

3P-053

Impact of long term spaceflight on arterial pressure regulation upon head-up tilt

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The vestibular system is known to have an important role in controlling arterial pressure (AP) upon posture transition. However, this system is known to be highly plastic, i.e., if subjects are exposed to a different gravitational environment, sensitivity of this system is altered. Thus, it is possible that sensitivity of vestibulo-cardiovascular reflex (VCR) is diminished after spaceflight, and then orthostatic intolerance is induced after spaceflight. To test this hypothesis, role of VCR in maintaining AP upon head-up tilt (HUT) was examined before and after 4-6 months stay in the International Space Station. The vestibular-mediated AP response was transiently interrupted by galvanic vestibular stimulation (GVS), which was externally applied electrical stimulation using surface electrode bilaterally placed over the mastoid processes. Thus, magnitude of VCR was evaluated by a difference in HUT-induced AP response between without GVS and with GVS. HUT experiments were performed 4 times: 2 months before spaceflight (Pre), 1-2 days (P1), 2 weeks (P2), and 2 months (P3) after return to the earth. The magnitude of VCR was 146 ± 20 mmHg× s at Pre, significantly decreased at P1 and P2 (-51 ± 20 and -50 mmHg×20 s, respectively), and recovered at P3 (94 ± 49 mmHg×20 s). Furthermore, AP was well maintained upon HUT at Pre and P3, however it fell at P1 and P2. These results indicate that a long-term exposure to microgravity induces a suppression of VCR, and this may be involved in a mechanism of spaceflight-induced orthostatic intolerance. (COI:No)

3P-054

Brain blood flow during anaphylactic hypotension in anesthetized rats

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The cerebral blood flow (CBF) autoregulation during anaphylactic hypotension was studied by comparing with that during systemic hypotension of similar magnitude, which was induced by acute hemorrhage, vasodilator (sodium nitroprusside; SNP 3.5 mg/kg, s.c.) or caval occlusion in anesthetized rats. Slow hemorrhage (0.18 ml/min) was also examined. Anaphylactic hypotension was induced by 0.6 mg intravenous ovalbumin in sensitized rats, and caval occlusion by balloon inflation of the Forgarty catheter (3F) placed in the vena cava inferior. Mean arterial pressure (MAP) and carotid blood flow were recorded, and CBF was measured by a laser Doppler technique. MAP decreased similarly to about 70 mmHg at 1 min after the start of MAP fall in all groups except the slow hemorrhage group. The CBF also decreased in parallel with the MAP fall in all groups except the slow hemorrhage, in which CBF was preserved at the early stage. The MAP at which CBF decreased by 10% of the baseline was 71&plusmn5 mmHg in the slow hemorrhage group, which was significantly greater than that in the other groups (105&plusmn13, 113&plusmn8, 109&plusmn5, and 112&plusmn8 mmHg in the anaphylaxis, SNP, acute hemorrhage, and caval occlusion groups, respectively). In conclusion, the response of CBF to anaphylactic hypotension is similar to other types of systemic hypotension. CBF autoregulation seems not to be observed during anaphylactic hypotension due to a rapid decrease in MAP in anesthetized rats. (COI:No)

3P-055

Impaired right coronary vasodilator function in pulmonary hypertensive rat heart assessed by synchrotron microangiography

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Pulmonary hypertension (PH) causes right ventricular (RV) hypertrophy, often leads to RV failure. We hypothesized that the mechanical stress of RV associated with increased afterload impairs vasodilator function of the right coronary artery (RCA) in PH. Using synchrotron microangiography, we compared the vasodilator function of RCA in two different models of PH rats. Rats were divided into 3 groups: (1) control, (2) MCT (monocrotaline; 60mg/kg s.c.), (3) SuHx (Su5416; 20mg/kg s.c. with exposure to hypoxia (10%) for 3wk followed by reexposure to normoxia for 5wk). Changes in RCA vessel number and caliber during endothelium-dependent or endothelium-independent vasodilator stimulation before and after combined blockade of nitric oxide synthase (NOS) and cyclooxygenase (COX) were compared among groups. MCT treatment decreased RCA vessel calibers at baseline but maintained vasodilator responses. MCT group displayed focal stenoses and segmental constrictions during NOS/COX blockade. On the other hand, SuHx treatment attenuated endothelium-independent vasodilator response. Note that most of rats in SuHx had cardiac arrest after NOS/COX blockade. In conclusion, the observed impaired vasodilator function of RCA in these two PH models suggests that impaired RCA function may lead to RV failure in patients with PH. (COI:No)

3P-056

Mechanism of positive inotropic action of a new myosin activator, omecamtiv mecarbil on LV mechanical work and energetics

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A novel myosin activator, omecamtiv mecarbil (OM) is a cardiac inotropic agent with a unique new mechanism of action. The inotropic action of OM is due to an increase in the number of myosin heads interacting with actin filament without changing myosin ATPase activity and calcium transient. In this study, we investigated the effects of OM on LV mechanical work and energetics in isoproterenol-induced heart failure (ISO-HF). We analyzed the LV end-systolic pressure-volume relation (ESPVR) and the linear relation between the myocardial oxygen consumption per beat (VO_2) and systolic pressure-volume area (PVA; a total mechanical energy per beat) in isovolumically contracting rat hearts at 240-bpm pacing in the absence or presence of OM in normal hearts and failing hearts treated with isoproterenol (1.2 mg/kg/day) for 4weeks. The mean ESP_{mLVV} did not significantly change by OM treatment not only in the control hearts (CTL) but also in the ISO-HF. But some of hearts in both of CTL and ISO-HF showed the increase of LV contractility by treatment with OM. The mean VO_2 intercept, which is composed of each myocardial oxygen consumption for calcium handling in excitation-contraction coupling and for basal metabolism, of VO_2-PVA relations was not significantly different in the OM-treated hearts. The slope was decreased by OM in both CTL and ISO-HF. These results suggested that OM improved the oxygen cost of PVA (contractile efficiency), although OM did not show obvious positive inotropic action in both CTL and ISO-HF. (COI:No)

3P-057

Electropharmacological characteristics of human iPS cell-derived cardiomyocytes sheet

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Human iPS cell-derived cardiomyocytes have been expected to be useful to analyze the cardiac safety profile of drugs. In this study, we pharmacologically characterized the electrophysiological properties of human iPS cell-derived cardiomyocytes sheets using electrical stimuli at pacing cycle length (PCL) of 600-1,000 ms, which is considered to simulate 2 dimensional tissue of human heart. In basal condition at PCL of 1,000 ms, field potential duration (FPD), effective refractory period (ERP) and conduction speed (CS) were 330-379 ms, 434-468 ms and 0.11-0.15 m/s (n=18), respectively. The FPD and ERP of the cell sheets were 1.6 times or longer, but the CS was about the half compared to those reported in the intact human ventricle. By shortening the PCL from 1,000 to 600 ms, FPD was shortened, ERP was increased but CS was decreased in a frequency-dependent manner, suggesting that the action potentials may be evoked before sufficient repolarization was accomplished at shorter PCL. Disopyramide (n=5), lidocaine (n=4) and flecainide (n=5) prolonged FPD and ERP but decreased CS. These results indicate that excitation-conduction may depend on Na^+ channel activities in the cell sheets like in human ventricle. On the other hand, verapamil (n=4) shortened FPD and ERP but increased CS, suggesting that verapamil may inhibit Ca^{2+} channel more than hERG K^+ channel in the cell sheets. (COI:No)

3P-058

Low cardiac output leads hepatic fibrosis in right heart failure model rats

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Background: Hepatic fibrosis progresses with right heart failure. Although its causal factor still remains unclear. Here we evaluated the progression of hepatic fibrosis using a pulmonary artery banding (PAB)-induced right heart failure model and investigated whether cardiac output (CO) is responsible for the progression of hepatic fibrosis.Methods and Results: Five-week-old Sprague-Dawley rats divided into PAB and sham-operated control groups. After 4 weeks from operation, we measured CO by echocardiography, and hepatic fibrosis ratio by pathological examination using color analyzer. In the PAB group, CO was significantly lower by 48% than that in control group . Hepatic fibrosis ratio and serum hyaluronic acid, an index of hepatic fibrosis, significantly increased in the PAB group than those in control group . Notably, the degree of hepatic fibrosis significantly correlated a decrease in CO. Immunostaining analysis revealed that hepatic stellate cell activation and tissue hypoxia were markedly observed in the PAB group. Furthermore, by real-time PCR analyses, transcripts of profibrotic factors and hypoxic factors were increased in the PAB group than those in control group. Conclusions: Our study demonstrated that low CO and tissue hypoxia were responsible for hepatic fibrosis in right failure heart model rats. (COI:No)

3P-059

Dynamic effects of spinal cord injury on splanchnic vascular functions and roles of regenerative medicine

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The aims of this study were to investigate the effects of spinal cord section on regional blood flow and sympathetic tone in order to understand the influences of spinal cord injury on regional vascular functions. Celiac and mesenteric flows in conscious control and hypertensive rats (NCR and SHR) were observed using an implanted electromagnetic flowmeter under anesthesia with thiamylal sodium. Arterial pressure (AP) was measured in the terminal aorta. The spinal cord was transected at thoracic 1 (Th1) under ether anesthesia. In a neuraxis-intact state, sympathetic tone (vascular resistance = AP/flow) was significantly decreased by hexamethonium (C6) in the celiac bed but not in the mesenteric bed. However, celiac tone in either spinal NCR or SHR was not decreased significantly by C6, while mesenteric tone was significantly decreased in both spinal rats. Mesenteric tone was suppressed reflexively in a neuraxis-intact state. Sympathetic tone in the celiac bed was supraspinal origin like that in the hindquarter bed, but in the mesenteric bed was spinal origin like that in the carotid and renal beds. Vasopressin sensitivity increased in both spinal rats. Total mean flows in the main five vascular beds decreased (-28.8%) after spinal transection. This indicates that venous return volume and right atrial pressure were decreased by dilation of capacitance vessels accompanying upon spinal transection. Portal collateral flows appear to be enhanced by portal hypertension. Application of human iPS cells to spinal cord injury is expected to recover splanchnic dysfunction. (COI:No)

3P-060

Effect of neuronal activation in the midbrain on cardiovascular response in the frog (*Rana catesbeiana*).

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We have previously shown that physiological stressor such as a pinching stimulation produces significant rises in blood pressure (BP) and heart rate (HR) in the frog. Also, the pinching stressor expressed *c-Fos* protein, a marker of neuronal excitation, in neurons at the ventral region of the aqueduct of the midbrain. The aim of study is to investigate effect of neuronal stimulation in the ventral region of the aqueduct of the midbrain in anesthetized frogs (*Rana catesbeiana*). The frogs were anesthetized with MS-222 (0.2% in distilled water), were cannulated in a branch of an abdominal artery to measure BP and HR, and then were placed on the stereotaxic apparatus for microinjection of glutamate (containing a stain to confirm the injection site). After the stimulation, the brain was fixed and then removed. The brain was sectioned (50μm thick) and the injection sites were checked. Microinjection of glutamate (50mM, 20-50nl) to the ventral region of the aqueduct in the rostral midbrain caused decent increases in BP and HR. The injection site where the profound pressor and tachycardic responses were observed was the same area in the rostral midbrain that a lot of *c-Fos* immunoreactive neurons were found after the pinching stress. Taken together with these results and our previous results, the neurons in the ventral region of the aqueduct in the rostral midbrain participate at least in part on the cardiovascular response evoked by the physiological stress. (COI:No)

3P-061

Increased prefrontal oxygenation at the early phase of voluntary exercise is not derived from transient changes in end-tidal CO2

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Regional cerebral blood flow (rCBF) is modulated by arterial carbon dioxide tension (PaCO2). We recently reported that prefrontal oxygenated hemoglobin (Oxy-Hb) concentration increases at the early phase of voluntary exercise in association with central command (Matsukawa et al. J Appl Physiol, 2015). However, it cannot be neglected that the increased rCBF estimated with the Oxy-Hb may be evoked by a transient increase in PaCO2 like Valsalva maneuver. To test this hypothesis, we simultaneously measured end-tidal CO2 (EtCO2) and the Oxy-Hb signals of the prefrontal cortices during voluntary one-legged cycling exercise as well as respiratory interventions (hypocapnia by voluntary hyperventilation and hypercapnia by asphyxia). The relative concentration of Oxy-Hb in the prefrontal cortices was used as an index of rCBF. Voluntary one-legged cycling increased Oxy-Hb in the prefrontal cortices at the early phase of exercise, while EtCO2 decreased at that period. Respiration rate increased prior to the voluntary exercise and tidal volume and ventilation began to increase immediately after the start of exercise. Hypocapnia decreased Oxy-Hb, while hypercapnia increased Oxy-Hb. These findings suggest that rCBF in the prefrontal cortices increases at the early phase of voluntary exercise, as opposed to the rCBF response to a decrease in EtCO2. The increased rCBF, independent of the changes in EtCO2, may reflect neural activity in relation to central command. (COI:No)

3P-062

Risk factors of patients with cerebral infarction in the elderly:Differences based on ages and types of ischemic stroke

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[Background] To determine whether the risk factors of the patients with cerebral infarction were different or not between the elderly patients with an age of >75 and <75 and types of ischemic stroke, we compared clinical parameters between them in a cross-sectional study.[Methods] The total subjects were 300 acute stroke ; 150 with an age of >75 and 150 of <75 (both 150 included atherosclerotic :ATI, cardiogenic :CEI and lacunar stroke :LS of 50 each). [Results] The comparison among the 3 types of ischemic stroke (n=100 for each) showed the highest atheromatous factors such as HbA1c (p=0.001) and LDL (p<0.001) in ATI, the largest cardiac load such as shown larger LAD dimension (p<0.001) and cardiothoracic ratio (CTR) (p<0.001) in CEI and the highest triglyceride level (p=0.05) in LS. The comparison with an age of >75 and < 75 (n=150 for each) showed higher cardiovascular load indicated by higher blood pressure (p<0.02), lager LVD dimension (p<0.001), LVWth (p=0.027) and aortic dimension (p=0.007) in the latter. The former patients are characterized by decreased renal function by lower eGFR (p<0.001) and larger CTR (p<0.001), possibly due to atrial enlargement.[Conclusions] The risk factors were quite different among 3 types of stroke, and hemodynamic loads seemed to be decreased in the patents with age of >75. (COI:No)

3P-063

Maintenance of normal plasma colloid osmotic pressure suppresses the inflammatory response during cardiopulmonary bypass

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Cardiopulmonary bypass (CPB) preserve the patient's life by providing adequate oxygen supply and blood flow to vital organs. However, previous studies have suggested that the interaction of hemodilution and vascular hyperpermeability induces inflammatory response and tissue edema during CPB. In this study, we developed a hypothesis about suppression of inflammatory response and tissue edema during CPB by maintaining normal plasma colloid osmotic pressure (COP). We used the blood plasma substitute (hydroxyethyl starch: HES) for maintaining normal plasma COP. The SD rats (400-450g) were divided into acetate ringer's (AR) CPB group (n=7) and HES CPB group (n=7). In the AR CPB group the CPB circuit was primed with AR solution and in the HES CPB group it was primed with HES formulation (priming volume: 15ml). Blood samples were collected before, at 60 min and 120 min after initiation of CPB. We measured COP and TNF- α level as a the pro-inflammatory marker. Moreover, we also measured the wet-to-dry weight (W/D) ratio of the lung 120 min after the initiation of CPB. During CPB, it was possible to preserve normal plasma COP (23-27mmHg) in the HES CPB group. The increased levels of TNF- α (1054 ±101 pg/ μl, 1564 ±192 pg/ μl ,respectively) and W/D ratio (5.43 ±0.09, 6.16 ±0.10, respectively) of the lung in the HES CPB group were significantly attenuated compared with those in the AR CBP group. The data suggest that the maintenance of normal plasma COP is effective for suppressing the inflammatory response during CPB. (COI:No)

3P-064

Gender differences of gene expression profiles in the nucleus tractus solitarius

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It is well known that the level of arterial pressure (AP) is lower in pre-menopausal women than in men of similar age. The brainstem cardiovascular control areas are believed to be responsible for the underlying mechanisms. Indeed, our previous studies revealed that the gene expression levels of some inflammatory molecules in the nucleus of the solitary tract (NTS), a pivotal region in regulating AP, are strongly associated with the basal levels of AP (e.g. Waki H et al. Hypertension 2007 & 2012, Gouraud S et al. Auton Neurosci 2011, J Hypertens 2011, & Acta Physiol 2015). Thus, we hypothesized that the NTS is one of important central areas in determining the gender differences of AP levels. Since females of spontaneously hypertensive rat (SHR) exhibit an obvious lower AP levels than their male counterparts, we investigated whether the NTS of SHRs exhibit gender differences in gene expression by using high throughput transcriptomics techniques. Interestingly, our cDNA microarray analysis showed that, among others, a large cluster of inflammatory molecules was differentially expressed between the NTS of female and male SHRs. Such inflammatory factors are involved in IL 6, IL10 and FAS pathways that were previously found to be linked with hypertensive condition. Although further validation studies will be required, gene expression profiles in inflammatory factors seem to be associated with the gender differences of AP levels. (COI:No)

3P-065

Development of a couplon model based on data from experimental and simulated Ca^{2+} sparks

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Couplons are functional elementary units, which are composed of multiple RyRs facing to the L-type Ca^{2+} channels and favourable for the efficient Ca^{2+} induced Ca^{2+} release (CICR) from the sarcoplasmic reticulum (SR) in cardiomyocytes. We have recently developed a CICR model (Himeno *et al. Biophys J*, 2015), in which the local [Ca^{2+}] referred to by couplons are given by instantaneous equations based on the Hinch formalism (Hinch *et al. Biophys J*, 2004). The kinetics of the couplon was determined based on experimental and simulated data of single RyRs and couplons. Firstly, the opening and closing kinetics of a single RyR was determined from reported single channel recordings obtained by the lipid bilayer method. It was confirmed that the obtained rates of RyR were within a range of various single RyR models used in other spark models. Secondly, we assumed a two-state transition scheme for a couplon and approximated overall opening and closing rates by fitting to the time course of simulated Ca^{2+} sparks in previous report by Laver *et al*. (*JMCC*, 2013) and to the behavior of a cluster of several independent RyRs calculated stochastically as a couplon. Our novel CICR model could reproduce behavior of Ca^{2+} sparks within a whole cell model successfully in deterministic simulation. It was concluded that deterministic calculation of the couplon activity using a mean nanodomain [Ca^{2+}] given by instantaneous equations is a good approximation for the reproduction of CICR in a cell model. (COI:No)

3P-066

A Teaching Material for Cardiac Physiology - systematic compilation of biophysical simulation models of cardiac cellular function (e-Heart project)

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Through experimental research activity, physiologists have been deeply impressed by the fine and beautiful harmony of various cellular functions toward the maintenance of homeostasis of the internal environment. However, it is generally very difficult to interest students in understanding the mechanisms of the cellular function in physiology. Students tend to avoid time-consuming and effort-demanding understanding of complex physiological system and are not well prepared to consider the physiological functions of life. Modelers, who assemble a variety of information of each functional unit of the cell using explicit mathematical equations with satisfactory visualization, could be a strong candidate as a member of physiological education team. In this presentation, we aim at giving an outline of our simulation programs, e-Heart, to be used for education in "Cardiac and Circulatory Physiology" at various levels. This teaching material consists of many biosimulation models for each functional unit of the cell, namely, ion channels, membrane excitation, excitation-contraction coupling, cell volume regulation, muscle contraction, ATP consumption and generation, cardiac cell models, arrhythmia, Laplace heart, the capillary model, etc. This material is still not arranged in a final format, and we appreciate constructive comments from potential users. Source codes with instructions are available from our website (http://www.eheartsim.com/). (COI:No)

3P-067

Dynamic simulation of energy metabolism in fetal guinea pig ventricular cells

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The developmental program of the heart requires accurate regulation to ensure continuous circulation and simultaneous cardiac morphogenesis because any functional abnormalities may progress to congenital heart malformation. Energy metabolism in fetal ventricular cells is regulated differently from that in adult ventricular cells: fetal cardiomyocytes generally have immature mitochondria, and fetal ventricular cells show greater dependence on glycolytic ATP production. Here, we integrated various characteristics of fetal ventricular cells based on a mathematical model and predicted the contribution of each characteristic to maintenance of intracellular ATP concentration and sarcomere contraction under anoxic conditions. Our simulation results showed that higher glycogen content, higher hexokinase activity, and lower creatine concentration helped prolong the time that contraction of ventricular cells was maintained under anoxic conditions. The integrated model enabled us to quantitatively address the contributions of factors related to energy metabolism in ventricular cells. Because fetal cardiomyocytes show similar energy metabolic profiles to stem cell-derived cardiomyocytes and cardiomyocytes in the failing heart, an improved understanding of fetal cardiomyocytes will contribute to understanding processes in stem cell-derived cardiomyocytes and cardiomyocytes under pathological conditions. (COI:No)

3P-068

Developmental changes in ventricular cell contraction: a simulation study

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As the heart develops, it gains new functions while continuously pumping blood; abnormalities during this developmental period may cause congenital heart malformations. Therefore, the developmental program of the heart, including expression of genes encoding various ion channels, is likely to be tightly regulated. Previously,we demonstrated that developmental changes in action potentials were well represented the Na^+ current (I_{Na}) increased before the disappearance of the funny current (I_f) followed by a 10-fold increase in inward rectifier K^+current (I_{K1}) via simulation of the 512 combinations between early embryonic (EE) and late embryonic (LE) stages. Here, we constructed a model of the middle embryonic (ME) stage of a guinea pig ventricular cell basedon experimental data and considered structural changes during embryonic development. We then shifted relative current densities from EE, ME, to LE stages to determine factors that contribute to developmental changes in the contraction of a ventricular cell. We demonstrated that a decrease in I_f density was necessary for proper excitation- contraction coupling after the disappearance of spontaneous activity. Furthermore, we showed that representation of developmental changes in transverse tubules in a ventricular cell is necessary to quantitatively describe the contraction of a developing ventricular cell. (COI:No)

3P-069

Cardiac sympathetic nerve activity does not contribute to centrally-induced suppression of cardiac baroreflex at the start of spontaneous motor activity in decerebrate cats

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Our laboratory has reported that central command blunts the sensitivity of arterial baroreceptor-heart rate (HR) reflex at the onset of voluntary static exercise in conscious cats and spontaneous contraction in decerebrate cats. The purpose of this study was to examine whether cardiac sympathetic nerve activity (CSNA) mediates the centrally-induced supression of cardiac baroreflex at the onset of spontaneous motor activity. Using paralyzed, decerebrate cats, CSNA, HR, and mean arterial blood pressure (MAP) were measured during spontaneous motor activity that was monitored by discharge of the tibial nerve. When MAP was raised by brief occlusion of the abdominal aorta at rest, baroreflex bradycardia was observed. The same aortic occlusion failed to induce baroreflex bardycardia at the start of spontaneous motor activity, despite the identical or greater pressor response. However we found the similar baroreflex inhibition of CSNA by the pressor response not only at rest but also at the start of spontaneous motor activity. Atropine abolished the baroreflex bradycardia evoked by the pressor response at rest. Taken together, it is concluded thatCSNA does not mediate the centrally-induced supression of cardiac baroreflex at the onset of spontaneous motor activity in paralyzed, decerebrate cats. Centrally-induced inhibition of the baroreflex activation of cardiac parasymapthetic nerve activity should be involved in the phenomenon. (COI:No)

3P-070

Difference of cerebral blood flow (CBF) during acceleration between non-treated and treated hypertensive rats

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During high sustained acceleration of fighter aircraft, maintain blood pressure level of the aortic valve is the most important to avoid unconsciousness. Sequela of uncontrolled hypertension is increased risk of aviator's sudden incapacitation. In the 91st meeting, we have reported that hypertension was not an advantage against high sustained acceleration. This year we report about the effects of antihypertensives on cerebral perfusion of spontaneously hypertensive rats (SHR) during high sustained acceleration. We used 10-week-old male SHR. Eight SHR were given normal diet and tap water for 2 weeks; non-treated-SHR (No-Tr-SHR). Eight SHR were fed a diet include 0.1 % nifedipine and normal tap water; nifedipine treated SHR (Ni-Tr-SHR). Other eight were fed normal diet and tap water include 0.025 % losaltan potassium; losaltan treated SHR (Lo-Tr-SHR). Rats were anesthetized and exposed to acceleration (4.5 Gz) for 5 sec using our centrifuge. Blood pressure at the level of the brain (BPLB) and cerebral blood flow (CBF) were continuously monitored. During acceleration, the decline in the BPLB was greater in No-Tr-SHR than in Ni-Tr-SHR or Lo-Tr-SHR. This finding is interested to discuss with antihypertensive effects to systemic and local regulation of the arterial pressure. No significant difference decline of CBF was observed among those groups. These results suggest that CBF is regulated and maintained even if great decrease of BPLB occurs. (COI:No)

3P-071

Mechanisms of spontaneous vasomotion in postcapillary venules of the rat stomach

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Background: We have reported spontaneous rhythmic constrictions (vasomotion) of venules in the bladder, distal colon and stomach, which are expected to prevent blood stagnation in microcirculations during organ wall distension. Here we further examined venular vasomotion mechanisms. **Methods:** Changes in postcapillary venule (PCV) diameter and intracellular Ca^{2+} dynamics in PCV mural cells (smooth muscles or pericytes) were monitored by video imaging and Ca^{2+} imaging, respectively, in the rat stomach submucosa. **Results:** Stellate-shaped PCV mural cells exhibited synchronised spontaneous Ca^{2+} transients that caused PCV vasomotion at 2-6 cycles/min; these activities were disrupted by gap junction blocker (3 μM carbenoxolone). Blockers of Ca^{2+}-activated Cl^- channel or L-type Ca^{2+} channel abolished vasomotion. Low Cl^- solution disrupted spontaneous Ca^{2+} transient synchrony and abolished vasomotion, while Na^+-K^+-Cl^- cotransporter inhibitors (10 μM bumetanide, 30 μM furosemide) suppressed the vasomotion. A phosphodiesterase type 5 (PDE5) inhibitor (1 μM tadalafil) disrupted spontaneous vasomotion in an NO-dependent manner. **Conclusion:** Star-shaped PCV mural cells exhibited synchronised spontaneous Ca^{2+} transients, causing PCV vasomotion. Cl^- accumulation into mural cells partly via Na^+-K^+-Cl^- cotransport appears to underlie Cl^- efflux (depolarisation) upon the opening of Ca^{2+}-activated Cl^- channels that induces Ca^{2+} influx via voltage-dependent L-type Ca^{2+} channels to cause spontaneous vasomotion. Continuous PDE5 activity (cGMP degradation) maintains vasomotion by counteracting endothelial NO-cGMP signalling. (COI:No)

3P-072

The evidence of abdominal respiration-induced hemodilution and a related reduction in the ADH concentration of blood is applied to evaluate of lymph flow from thoracic duct

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To establish effective lymph drainage methods and develop concise and accurate clinical techniques for evaluating lymph flow from the thoracic duct in healthy individuals and patients with cancer treatment-related lymph edema, we investigated the numbers of red (RBC) and white (WBC) blood cells, and platelet in blood, hematocrit (Ht), and the blood concentrations of total protein (TP), albumin (Alb), and anti-diuretic hormone (ADH) before and after 5 minutes' lymph drainage followed by 30 minutes' rest with or without abdominal respiration in the supine position. The 5 minutes' facial, upper and lower extremities lymph drainage followed by 30 minutes' rest in the supine position induced significant reductions of the TP and Alb in all subjects, and their RBC and Ht levels in some subjects. The 30 minutes' rest only in the supine position produced also significant reductions of blood TP and Alb. In addition, abdominal respiration in the supine position without manual lymph drainage caused more significant hemodilution, being significant reductions of TP, Alb, RBC, Ht, and ADH. These findings may be related to effective lymph drainage from the chylocyst. In conclusion, abdominal respiration during 30 minutes' rest in the supine position is effective at inducing lymph flow from the thoracic duct, and the associated induction of hemodilution and lowering of the blood ADH concentration may be useful for assessing the extent of lymph flow rate. (COI:No)

3P-073

The responses of internal and external carotid arteries blood flow to acute hypertension during resistance exercise

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An inappropriate intracranial cerebral over-perfusion does not occur during heavy resistance exercise despite an exercise-induced acute hypertension over the cerebral autoregulation range (50-160 mmHg). The purpose of the present study was to test our hypothesis that vascular bed at the external carotid artery (ECA) play a role of buffering system to prevent a large and acute increase in the internal carotid artery (ICA) blood flow. Twelve healthy participants performed one-legged static knee extension exercise at 30 % maximal voluntary contraction for 2 min. The ICA and ECA blood flows were evaluated by duplex ultrasonography. During the resistance exercise, the ICA blood flow increased and reached to the peak at 60 sec, and thereafter it unchanged despite a large increase in cardiac output and perfusion pressure. ICA conductance significantly decreased by -14.4 ± 13.8 % at the end of exercise ($P<0.01$). In contrast, ECA blood flow increased larger than ICA blood flow ($P<0.01$), and ECA conductance increased to 20.0 ± 30.5 % ($P<0.05$). The larger increase in ECA blood flow and ICA vasoconstriction may protect the intracranial cerebral vasculature against the resistance exercise-induced hypertension. This heterogeneous cerebral vascular responses may prevent over-perfusion to the intracranial cerebral region during resistance exercise with acute hypertension. (COI:No)

3P-074

To what extent stiffness parameter β depend on blood pressure level? Experimental study in normal and KHC rabbits

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We investigated to what extent stiffness parameter β (Stf β) was dependent on blood pressure (BP) level in normal and Kurosawa and Kusanagi-hypercholesterolemic (KHC) rabbits aged 10-12 months. Two catheter-tip transducers were placed at the ascending (AA) and distal abdominal (DAA) aortas under pentobarbital anesthesia. Pressure waves were measured at AA and DAA when BP level was altered by intravenous infusion of angiotensin II and sodium nitroprusside. Stf β was determined by PWV between AA and DAA, systolic (SBP) and diastolic (DBP) blood and pulse (PP) pressures at mean arterial pressure level of 60, 80, 100, 120 and 140 mmHg. Stf β distributed within a relatively narrow range while SBP and DBP varied in a wide range in the control rabbit group. Although Stf β showed weak but significant correlation with SBP and DBP in the two strains, the correlation coefficient was significantly smaller in the control rabbit group than in the KHC rabbit group. Correlation coefficient of Stf β with SBP and DBP was also significantly smaller than that of PWV with SBP and DBP in the two strains, respectively. We can conclude that Stf β is less dependent on BP level than PWV in the two strains, which is particularly prominent in the control rabbit group. (COI:No)

3P-075

Can emotional stress induce atrial fibrillation in mice?

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In a previous study, we have demonstrated that our custom designed multi-stripe-electrode electrocardiogram (ms-ECG) plate is able to record the heart rate (HR) response to psychological (emotional) stimuli by utilizing emotional sweating, which lowers the skin-electrode contact impedance, from eccrine sweat grands on soles of intact C57BL/6J mice. In addition, although the mechanism is unknown, we also found that mice exhibited sweating from the beginning of sleep, which enabled us to record ECG of a sleeping mouse by using the ms-ECG plate. A psychological stress during active state by hitting the mouse cage with a stick caused the emergence of ECG for a short duration (7.4 ± 6.2 s; average HR 754 ± 29 bpm; n = 7) with a small HR change. In contrast, when the psychological stress was applied during sleep, mice exhibited a dynamic HR response, which was unexpectedly induced for several minutes when an experimenter entered the mouse's room and only gazed at the mouse in a cage. The low HR during sleep jumped up (340 ± 12 to 608 ± 55 bpm; n = 6) and then followed by a bradyarrhythmia with unstable HR fluctuation, which surprisingly accompanied small pulses at a period of ~30ms similarly to the f-wave induced by atrial pacing as reported by other research groups. As it is believed for now that reentry hardly happens in tiny mouse heart, further study is needed to clarify whether the bradyarrhythmia induced by the psychological stress in mice is atrial fibrillation or not. (COI:No)

3P-076

Measurement of the cardiac time interval using a piezoelectric transducer sensor in a hypertensive cardiac hypertrophy rat

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The cardiac time intervals (CTIs), which is the time of each phase in a cardiac cycle, change in diseased hearts such as heart failure and cardiac hypertrophy. We have previously shown that the thoracic biosignal (TBS) obtained by a piezoelectric transducer sensor corresponded to local vibrations of the chest wall associated with the cardiac movements and displayed characteristic features during cardiac cycles. In this study, we have made an attempt to evaluate the CTIs with the TBS, and examined how the TBS in the cardiac cycle changed in a hypertensive cardiac hypertrophy model animal. All animal experiments adhered to the regulation for animal experimentation of Akita university. DIS/Eis rats were used in this study. The CTIs such as isovolumic contraction time, ejection period, isovolumic relaxation time were identified using the TBS. Also, hemodynamic, histological, and echocardiographic parameters were measured and compared between the high salt diet (HSD) group and the normal diet (ND) group. The changes in the TBS in the HSD group were characterized by prolongation of early systole and early diastole periods in comparison to the ND group. Blood pressure, the heart-body weight ratio and the cross sectional area of the myocardium cell increased in the HSD group. Thus, the changes in the CTIs measured with the TBS correlated with those of hemodynamic and morphological parameters during the course of development of cardiac hypertrophy. These findings suggest that the TBS might be useful to estimate the CTIs. (COI:No)

3P-077

Neonatal isolation augments social dominance by altering actin dynamics in the medial prefrontal cortex

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Social maltreatment early in life can lead to the development of impaired interpersonal relationships and profound social disorders. However, the underlying cellular and molecular mechanisms involved are largely unknown. Here, we found that isolation of neonatal rats induced social dominance over non-isolated control rats from the same litter in juveniles that was glucocorticoid-dependent. Furthermore, neonatal isolation inactivated the actin-depolymerizing factor (ADF)/cofilin in the juvenile medial prefrontal cortex (mPFC). Isolation-induced inactivation of ADF/cofilin resulted in the decrease of glutamate synaptic AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor (AMPAR) contents by the increase of stable actin fractions at dendritic spines in the juvenile mPFC. The expression of constitutively active ADF/cofilin in the mPFC rescued the effect of isolation on social dominance. Thus, neonatal isolation traumatizes spines in the mPFC by altering actin dynamics, leading to abnormal social behavior later in life. (COI:No)

3P-078

Effects of bathing in CO_2-enriched water on muscle fatigue produced by intermittent resistance exercise

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Bathing in artificially made CO_2-enriched water (CO_2 concentration > 1g/L) induces vasodilation not only in the skin but also in the skeletal muscle under the skin of immersed part, even under relatively low water temperature of 30-35 °C. To investigate the hypothesis that the CO_2-water bath may affect muscle fatigue, effect of pretreatment of the forearms with CO_2-water bath on changes in hand grip strength measured continually was investigated in 11 healthy subjects (Ss). Ss griped two hand dynamometers by each hand simultaneously for 10 seconds in maximum effort, then rested for 5 s. One set of exercise consisted of 25 times of this grip strength measurement. Ss performed 3 sets of this exercise, and in resting for 10 minute between the sets they immersed the forearm of one side into CO_2-water and forearm of another side into tap-water. Blood flow in the flexor digitorum superficialis muscle (MBF) was estimated by means of near-infrared spectroscopy. Hand-grip strength was reduced by about 30 % at the end of each set. In the dominant hands, the forearm pretreatment with CO_2-water significantly prevented the reduction of grip strength compared to the no-treatment case. In comparison with tap-water, CO_2-water pretreatment tended to reduce the fatigue in grip strength. CO_2-water pretreatment tended to increase MBF in dominant hand while Tap-water and no-immersion did not affect. Forearm pretreatment with CO_2-water bath may suppress the exacerbation of muscle fatigue by means of increase in muscle blood flow. (COI:No)

3P-079

Effects of osmotic and non-osmotic stimuli on drinking behavior in mice: Possible involvements of TRPV1 and TRPV4

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The detection of plasma osmolality and body fluid volume and sensation of thirst related to drinking behavior may be mediated in part by TRPV1 and TRPV4 channels. In the present study, we examined the effects of osmotic and non-osmotic stimuli that cause drinking behavior on accumulating water intake in TRPV1-deficient (TRPV1-/-) and TRPV4-deficient (TRPV4-/-) mice in comparison with wild type (WT). Intraperitoneal injection of hypertonic saline (HS) and water deprivation (WD) for 24 hours was performed as osmotic stimuli. Subcutaneous injection of 20% polyethylene glycol (PEG) and intracerebroventricular injection of angiotensin II (AII) was also performed as non-osmotic stimuli. HS and WD caused drinking behavior and drank similar accumulating water volume in all types of mice. PEG-induced water intake in TRPV4-/- mice was significantly smaller than that in TRPV1-/- and WT mice. AII-induced water intake in TRPV1-/- mice was significantly smaller than that in TRPV4-/- and WT mice. These results suggest that TRPV1 and TRPV4 may be involved potently in AII- and hypovolemia-induced drinking behavior, respectively. (COI:No)

3P-080

Psychiatric and psychological challenges and countermeasures of astronauts for human interplanetary explorations

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Introduction By 2030s, a human interplanetary exploration of Mars (HIEM) is anticipated. But it will be quite different from current manned space exploration programs. Astronauts will reach so deep space where additional supplies and real-time communication are impossible. We discussed psychiatric and psychological challenges and proposed countermeasures for HIEM. **Methods** Through comparison of anticipated HIEM and the current programs and literature review of ground simulations and analogue environments, psychiatric and psychological challenges were discussed and their countermeasures were proposed. **Results and Conclusion** It is considered that astronauts will face the following three major challenges in future HIEM; severer isolation environment, more need for autonomy, and need for self-helping stress coping system. We propose the following three countermeasures. Firstly, we should optimize psychiatric and psychological selection criteria for astronauts, including " mental stability " , " interpersonal stability " and " satisfaction with limited supports " . Secondly, the training program should deal with more psycho-social factors to enhance their self-management ability in stressful environment. Thirdly, more on-site psychological support activities should be introduced using robots or virtual reality materials. (COI:No)

3P-081

Salivary melatonin level seems to be related with the depression scores than the sleep quality scores

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Comparisons of salivary melatonin levels with different factors including quality of sleep, anxiety, and depression, were conducted to examine whether there is a relationship between melatonin, presumably associated with sleep, and psychological stress. A decrease in the quality of sleep is believed to cause anxiety and worsen depression. The saliva of healthy young females was collected during the daytime and night time, and salivary melatonin levels were measured. The quality of sleep was scored using the Pittsburgh Sleep Quality Index. The anxiety and depression were scored using the State-Trait Anxiety Inventory and the Self-Rating Depression Scale, respectively. The following findings were obtained: 1) Salivary melatonin levels measured during the daytime and night time were higher in females with a high depression score, compared to those with a low score; and 2) salivary melatonin levels measured before night time (in a sitting position) were higher in females with a high state anxiety score, suggesting a correlation between state anxiety scores and salivary melatonin levels during the night. Both depression and a sense of anxiety are forms of psychological stress. Therefore, it is assumed that, when a person is under psychological stress, the action of melatonin as a ligand on its receptor is reduced. Meaning psychological stress may induce oxidative stress in the body. On the other hand, no correlation was noted between the quality of sleep and salivary melatonin levels during the night. (COI:No)

3P-082

Thermogenetic factors affecting torpor patterns in mice

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Torpor is a short-term hibernation-like state characterized by low metabolic rate and reported in many small mammals. In the case of mice, fasting in a quiet environment induces torpor within several hours. Once torpor is induced, the core body temperature drops to ca. 20°C. Owing to a large proportion of heat dissipation in small mammals, persistent thermogenesis plays an important role to regulate body temperature. Thermogenesis consists of shivering and uncoupling protein 1 (UCP1)-mediated non-shivering thermogenesis. In this study, we examined the role of two forms of thermogenesis to regulate torpor expression pattern. Torpor was induced in the following mice: cool-acclimated (CA; 18°C), warm-acclimated (WA; 28°C) wild type and cool-acclimated UCP1 knockout (KO; 18°C) mice. All mice expressed torpor by fasting at 18°C ambient temperature. During torpor, the minimum core body temperature was 20.9 ± 0.9°C in WA, significantly lower than CA (23.9 ± 1.8°C), whereas the temperature in KO was not different from CA. Torpor duration and the number of torpor expressions did not show differences among the groups. When fasting, the blood concentration of free fatty acid was significantly higher in WA than CA and KO, suggesting low consumption of free fatty acid by shivering in WA. These results indicate that the activation of thermogenesis affects the depth of torpor, but neither torpor expression itself nor the torpor duration. They also suggest shivering plays a major role in controlling minimum body temperature during torpor rather than non-shivering thermogenesis. (COI:No)

3P-083

Brain regions involved in thermal perception

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The aim of the present study was to find brain regions involved in the process of thermal perception. Sixteen subjects had thermal stimulus of either 41.5°C or 18.0°C at the forearm. during whole-body stimulus of 47.0°C, 32.0°C, or 17.0°C. The local stimuli were delivered on the right forearm with the Peltier device, and the whole-body stimuli were conducted with a water-perfusion suits. The local stimulation with the same temperature was conducted 5 times with a 30-s interval. Brain activity was assessed by functional magnetic resonance imaging (fMRI), and the subjects were asked to report thermal sensation and pleasantness/unpleasantness ratings following each local stimuli ended. There were no differences in local thermal sensation among the three whole body temperatures, although the local thermal pleasantness/unpleasantness was different. BOLD signals for perception showed significant activation of medial prefrontal cortex extending to anterior cingulate cortex, bilateral insula and the right inferior parietal lobe, which overlapped with across the 6 experimental conditions. Regions including insula were related to discriminative component evaluated, whereas medial prefrontal cortex was activated while the hedonic component was estimated. Although the brain activation were not specific to hot and cold sensations and feelings, the insula and medial prefrontal cortex may be responsible for evaluating discriminative and hedonic components of thermal perception. (COI:No)

3P-084

Hypergravity affects muscle and bone through the vestibular system in mice.

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Microgravity concurrently induces muscle atrophy and osteopenia with changes of vestibular signaling and sympathetic outflow in a space flight. We examined roles of the vestibular system in the alterations of muscle and bone induced by hypergravity in mice. Bilateral inner vestibules were surgically lesioned (VL) in C57/BL6J mice. After the surgery, mice were exposed to 1 g or 3 g environment induced by centrifuge for 4 weeks. The tibial muscle and bone masses were quantified by quantitative computed tomography (QCT) followed by adjusting for body weight. QCT analysis revealed that VL attenuated hypergravity-induced increases in tibial muscle and trabecular bone masses. In contrast, hypergravity did not affect tibial cortical bone and fat masses. VL decreased myofiber size and mRNA levels of myogenic genes (MyoD, Myf6 and myogenin) enhanced by hypergravity in the soleus muscle, suggesting that the vestibular system contributes to the hypergravity-induced increase in muscle mass through an enhancement of myogenic differentiation. Propranolol, a β-blocker, significantly antagonized the hypergravity-induced muscle changes. In conclusion, we demonstrated that hypergravity affects muscle and bone through vestibular signaling and the subsequent sympathetic outflow in mice. (COI:No)

3P-085

Asynchronous fluctuations of skin blood flow in different sites of finger during mild cold exposure

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The aim of this study was to compare finger skin blood flows (SBFs) at different sites which are hypothesised to vary in the content of Arteriovenous anastomoses (AVA). Twenty-one healthy young women participated in this experiment with their written consent. The subjects sat in a climatic chamber set at 32 °C with the relative humidity of 50 %. Thirty minutes after attaining thermal equilibrium, the temperature was progressively decreased to 20 °C for 60 minutes. SBFs were measured using laser-Doppler flowmetry (LDF) every second at the dorsal middle phalanx and dorsal and palmar distal phalanx in the second finger of the right hand. Skin temperatures were measured by thermistors at the dorsal middle phalanx and palmar distal phalanx in the third finger and dorsal distal phalanx in the fourth finger of the right hand. At the condition of 32 °C, skin blood flows were comparatively large in the 3 sites in all subjects. SBFs spontaneously fluctuated and exhibited an occasional greater fall. In some subjects, SBFs fluctuated asynchronously in one site against the other two sites, whereas asynchronous changes disappeared with advancing mild cold exposure. The combinations of sites showed asynchronous changes were not necessarily consistent over the subjects. These observations suggest that the threshold for vasoconstriction of finger skin observed by LDF might not be uniform over the finger, which might be related to individual differences in distribution of AVA. (COI:No)

3P-086

Mistimed exercise interfere re-entrainment of circadian rhythms in behavior and *Per1* expression in lung and skeletal muscle to shifted light-dark cycle

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Previously, we demonstrated timed wheel-running (WR) activity after an abrupt shift of a light-dark cycle (LD) accelerates re-entrainment of circadian behavior and *Per1* rhythms in the lung and skeletal muscle in mice. In that study, the WR activity was started from the onset of shifted dark phase. In the present study, effects of WR at the end of shifted dark phase were examined on the re-entrainment in mice. LD was advanced by shortening and was delayed by lengthening the first light period in the advance and delay protocol, respectively. Shifted LD was continued for 4 days, which was followed by constant darkness (DD). Per1 expression was measured in the cultured tissues obtained on the first day of DD from mice carrying a bioluminescence reporter of Per1 expression. In the advance protocol, re-entrainment was not influenced by WR in any circadian rhythm examined. In the delay protocol, re-entrainment of the circadian locomotor rhythm was not affected by WR. However, re-entrainment of circadian Per1 rhythm was significantly decelerated in the skeletal muscle and lung. These findings indicate that the effects of WR on re-entrainment depend on the time of day and the peripheral tissues. Mistimed exercise interfere re-entrainemnt of circadian rhythms in the lung and skeletal muscle. (COI:No)

3P-087

Sweat ion concentrations and sweating rate responses to passive heating dependent on age

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It is known that the secretion abilities of sweat glands in response to heat decrease with age. We investigated the changes in the sweat ion concentrations (Na^+, K^+, Ca^{2+} and Cl^-) and the sweat rate (SR) in response to the passive heating stress for 60 minutes in elderly and younger men. Seven elderly (71.0±4.9yrs) and seven young (22.4±1.1yrs) healthy men participated as volunteers. The sweat ion concentrations were analyzed for sweat samples 30, 45, and 60 minutes after the start of the passive heating. Changes in the SR of young men, in response to a >0.3 degrees Celsius (ºC) increase of the core temperature (DTe) from the base line, were greater than those of elderly men in response to a >0.5 ºC increase in the DTe. The slope of SR/DTe in the young men was steeper than that of the elderly men. The sweat concentrations of Na^+ and Cl^- in the young men were lower than those in elderly men during the heating stress. However, they displayed an increasing trend proportional to the SR. In contrast they displayed a decreasing trend in the elderly. The sweat K^+ and Ca^{2+} concentrations decreased with increasing SR in the both age groups expect for K^+ in the elderly. It is possible that in the eccrine sweat gland of the elderly, molecules responsible for the Na^+ and Cl^- reabsorption, as well as Na-K-Cl cotransporter and Na-K-ATPase are reduced. (COI:No).

3P-088

Investigation of mechanisms for the resistance to Ischemia-Reperfusion stress in kidney during hibernation period

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Hibernation is an amazing strategy to survive the harsh conditions of winter by suppressing energy demand and lowering body temperature, heart rate and oxygen consumption. Especially, several mammalian hibernators including ground squirrels and hamsters dramatically reduce body temperature and heart rate during deep torpor (DT) phase, and sharply recover to the normal state during the periodical arousal (PA) phase. These DT-PA cycles occur repeatedly throughout the hibernation period. Such DT-PA cycles can cause various stress and damages such as Ischemia-Reperfusion (I/R) stress, which may result from rapid re-oxygenation at PA after lowered oxygen supply at DT. Indeed, it has been suggested that hibernating ground squirrels have resistance to experimental I/R stress in gut and liver. However the molecular mechanisms for the resistance to I/R stress has not been elucidated. To investigate resistance mechanisms in hibernators to I/R stress, we first established the system for I/R model experiment in the kidney of Syrian golden hamster (*Mesocricetus auratus*), whose hibernation can be induced in laboratory condition. We are examining whether hamsters at PA phase also have resistance to experimental I/R stress. By transcriptome analysis using next generation sequencer, we have found many genes that were up-regulated specifically during hibernation period and may contribute to pathways or processes involved in the resistance to I/R stress. The data will be discussed. (COI:No)

3P-089

The relationship between sleep parameter and subjective evaluation about sleep quality during follicular and luteal phases of the menstrual cycle

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The aim of this study is to compare the sleeping state between follicular (FP) and luteal (LP) menstrual cycle phases in the daily life, by eighteen healthy women aged 19-49 years. They participated in this study on one or two nights each in above two phases. This study was approved by the institutional ethics review committee and all subjects gave their consent to this study. The subjects spent daily times as usual. This experiment was carried out in each subject's home and measured the ECG using heart rate monitor (Mybeat, Union tool Co.). After the night experiment, subjects answered the following questionnaire such as sleep onset latency, total sleep time (TST), awaked time during sleep, sleep quality by using visual analog scale.The mean TST and sleep onset latency of the FP and LP was 406± 64.6 and 385± 72.6 min and 12.9± 7.1 and 17.4± 11.8 min, respectively. The mean sleep quality of the FP was higher than that of the LP, but not significantly. The RR interval before sleep onset in the FP was significantly longer than that in the LP. However, the RR interval during the time after sleep onset did not show the significant difference between the FP and LP. The correlation between the sleep quality and the TST revealed statistically significance ($r=0.525$, $p<0.05$) in the LP, but not in FP ($r=-0.232$, $p>0.05$).We concluded that the TST could influence the sleep quality in the LP, but not in the FP. (COI:No)

3P-090

Effects on blood or CSF S100B-protein concentration to brain ischemia due to high +Gz acceleration

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Pilots of high performance aircraft are exposed to high head-to-foot acceleration (+Gz), which may induce temporary severe brain ischemia under lack of proper anti-G protection system. We have previously reported that the brain injury by strong laser irradiation causes an increase in plasma S100B concentrations, which is derived from glial cells, in rats. In this study, we tried the possibility of brain damage at +Gz acceleration using blood or cerebrospinal fluid (CSF) S100B as a brain damage marker in Sprague-Dawley rats. Under intraperitoneal pentobarbital anesthesia (50 mg/kg), a stainless steel cannula was implanted into the cisterna magna a week before the experiment and another polyethylene catheter was put on the left femoral artery on the day of the experiment. Rats were exposed to acceleration load for 3 minutes at intensity of +2 Gz, +3 Gz or +4 Gz using a centrifuge for small animals. The S100B concentration was measured by the ELISA (BioVendor Laboratory Medicine, Inc., Human S100B ELISA Kit). In the +2 Gz load, blood S100B concentration increased from 130 ± 32 to 1170 ± 173 pg/ml (mean ± SE, $p < 0.01$) at 3 hours later after the acceleration, followed by returning to control levels at 6 hours later after the acceleration. But no significant increases were observed in blood or CSF S100B concentration at +3 Gz and +4 Gz accelerations. We will challenge other morphological evidences and discuss the brain damages in the high acceleration exposure. (COI:No)

3P-091

Platelet-derived growth factor receptor alpha (PDFGRα) expression are involved in feeding-related events in the male mouse hypothalamus.

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NG2-positive cells are known as oligodendrocyte precursor cells. However, their functions are not only limited as oligodendrocytes: it is suggested that they develop into neurons. NG2-positive cells are also discovered to express PDGFRα for their survival and expressed in the hypothalamus which is an important site for feeding regulation. In the present study, we firstly confirmed that PDGFRα-positive cells were also positive for NG2 by immunocytochemistry. This suggested that PDGFRα-positive cells in the hypothalamus were oligodendrocyte precursor cells. We next examined whether feeding status affected the expression of PDGFRα protein in the mouse hypothalamus. In addition, we checked whether high-fat diet feeding altered the PDGFRα expression. In the chow fed groups, fasting significantly increased the PDGFRα expression in the hypothalamus. This increase was impaired by the high-fat fed groups either short time (10 days) or long time (5 and 12 weeks). We finally determined which types of PDGFRα ligands were involved in feeding status-related change in the PDGFRα expression. Our results suggested that PDGF-A, -C, -D, but not -B, were canditates. We hypothesize from the present study that PDGFRα-mediated cellular signals are involved in the regulation of feeding behavior by the hypothalamus. (COI:No)

3P-092

Mechanical stimulation causes Ca^{2+} rises in mouse brown adipocyte

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Brown adipocytes have a lot of mode to carry out rising intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$). Mitochondrial uncoupling by β_3-adrenergic activation or an uncoupler (FCCP) causes Ca^{2+} release from mitochondria and subsequently Ca^{2+} release from the endoplasmic reticulum (ER) and further evokes plasmalemmal Ca^{2+} entry in rodent brown adipocytes. These rises of $[Ca^{2+}]_i$ promote thermogenesis via the activation of Ca^{2+}-dependent dehydrogenase. In this study we indicate a new mode of $[Ca^{2+}]_i$ rises in brown adipocytes elicited by mechanical stimulation using dynamic water pressure measured by fluorometry of $[Ca^{2+}]_i$. Mechanical stimulation evoked Ca^{2+} rises in brown adipocytes. These rises in $[Ca^{2+}]_i$ induced by mechanical stimulation were depressed by ML-9 (an inhibitor of phosphokinase C (PKC)) and not by a nominally Ca^{2+} free, GsMTx-4 (a blocker of TRPC6). These rises were promoted by the application of R59022 (a PKC activator). RT-PCR revealed the expression of mRNA type of PKC. Furthermore, immunoblotting showed the expression of PKC protein. Thus, mechanical stimulation causes Ca^{2+} release from intracellular directly linked to PKC activation. These modes of Ca^{2+} entry would provide in part the basis for heat production. (COI:No)

3P-093

Responses of serotonin release in the nucleus accumbens to tactile stimulation in rats

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We have shown that dopamine (DA) release in the nucleus accumbens (NAc) increases in response to tactile stimulation in both anesthetized and conscious rats (Maruyama et al., 2012). The NAc is also innervated by serotonergic neurons, and the infusion of serotonin (5-HT) into the NAc stimulates DA release in the NAc (Parsons and Justice, 1993). In the present study we examined the responses of 5-HT release in the NAc to tactile stimulation in both anesthetized and conscious rats. The 5-HT release was measured with the use of microdialysis technique and HPLC. A coaxial microdialysis probe was stereotaxically implanted in the NAc and perfused with modified Ringer's solution at a speed of 2 µl/min. Tactile stimulation was applied to the bilateral back for 5 min. Tactile stimulation significantly increased the 5-HT release in both anesthetized and conscious rats. These results demonstrate that tactile stimulation can directly stimulate 5-HT release in the NAc in the absence of conscious perception or emotion. Furthermore, the results in the present study and those by Parsons and Justice (1993) suggest that the increases in 5-HT release in the NAc mediate the increased responses of the DA release in the NAc to tactile stimulation. (COI:No)

3P-094

Masculinization of the rat SDN-POA is induced by cell-dispersive effect of estrogen

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The sexually dimorphic nucleus in the preoptic area (SDN-POA) is larger in males than in females, however the mechanism of sexual differentiation of this structure remains largely unknown, except that aromatizable androgen and estrogen during perinatal period cause the masculinization of this nucleus. We have shown recently that EGFP is expressed in the SDN-POA under the control of an estrogen receptor α gene promoter 0/B (0/B-SDN). In the present study, we observed the sexual differentiation of the 0/B-SDN in vitro using the organotypic brain slice cultures, which were prepared from embryonic day 18 brains. Estrogen or aromatizable androgen in the culture medium masculinized the 0/B-SDN at 200 hours in vitro, but non-aromatizable androgen or antagonist of estrogen receptor could not. These results suggest that sexual differentiation of the 0/B-SDN could be established in vitro as well as in vivo. Next, we visualized the nucleogenesis of the 0/B-SDN in the organotypic slice culture using time-lapse imaging. Outline of the 0/B-SDN appeared in the slice at 48 hours in vitro and many EGFP expressed cells aggregated prior to 100 hours in vitro, even in the absence or presence of estrogen. On the other hand, after the aggregation of EGFP cells, further diverging migration was observed in the 0/B-SDN using the culture medium including estrogen, however, 0/B-SDN cells further migrated in a converging fashion in the control medium without estrogen. These results propose the diverging migration by estrogen is important for the masculinization of the SDN-POA. (COI:No)

3P-095

Acute running exercise enhances sleep in mice

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Many studies suggest that acute or chronic exercise is beneficial for not only metabolic regulation but also brain functions such as sleep. However, the mechanisms are still unclear because neural function of physical activity on the sleep are complicated. In present study, we focused the effect of acute treadmill exercise on sleep function in mice. Male mice (aged 10 weeks) were subjected to treadmill exercise for 0 (control), 5, 15, 30, or 60 minutes. We selected an exercise starting point at light-onset. Running exercise was performed at the speed of 100 rpm/min. During non-running period, mice in each group were deprived sleep to unify the duration of sleep deprivation. After the running, we carried out sleep recording for 14 hours. Mice exercised for 30 and 60 minutes showed markedly increase in the amount of non-rapid eye movement (NREM) sleep and an enhancement of slow-wave activity (SWA) during NREM sleep which is known as the index of sleep depth, while running for 5 and 15 minutes did not change these parameters. Ketone bodies of plasma were increased in mice exercised for 30 and 60 minutes compared with control mice. We also observed an increase in mRNA expression of Hmgcs2, an enzyme of ketone bodies production, in liver and cortex tissue. These results indicate a possibility that acute exercise would elicit deep sleep and enhance ketogenesis depending on running duration. (COI:No)

3P-096

Monitoring fast neuronal activities and circadian rhythms of intracellular calcium ions in the suprachiasmatic nucleus

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The master circadian clock in mammals locates in the suprachiasmatic nucleus (SCN) of the hypothalamus of the brain. The SCN consists of around 20,000 neurons in rats and mice, each of which is thought to contain autonomous circadian oscillator. Previous studies have found that intracellular calcium concentrations of SCN neurons fluctuate in the circadian manner. These studies were investigated by using fluorescent imaging with exposure time of few seconds, so that the fast neuronal activity underlying circadian calcium rhythms remains unknown. Generally, neurons show various calcium activities with time course of sub-seconds, such as action potential dependent intracellular calcium increase, suggesting that circadian calcium rhythms in previous studies may be time-averaged signals of fast calcium events.

In the present study, we performed simultaneous imaging of circadian calcium rhythms and fast calcium activity. By using recombinant adeno-associated virus transfection and the neuron specific promoter, we expressed fluorescent calcium probe GCaMP6f into SCN neurons. For imaging of circadian rhythms, we took fluorescent images of the SCN slice every hour at an exposure time of 1 second. For imaging of fast activity, we took calcium events every hour with frame rates of 30-40 Hz and duration of 20 seconds, and monitored fast calcium activities with single cell resolution for more than three days. (COI:No)

3P-097

Rectal temperature, fluid intake, dehydration and biochemical and hematological data in the citizen half-marathon runners in the summer

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Marathon, a long distance running, is an aerobic exercise which promotes health and fitness, however it causes large heat stress and may induce heat illness especially in the summer. We investigated the influence of the summer marathon on the runner's body in Obuse Mini-Marathon 2015. 29 runners (24 men and 5 women) volunteered. Body weight was measured and blood samples were obtained before and after the race. Volume of fluid intake during the race was interviewed and rectal temperature (Tre) was measured after the race. The race started 6 AM at 22°C of WBGT and finished 11 AM at 28°C of WBGT. All subjects completed the race at 1h 22min - 3h 42min without any symptom of heat illness. Tre after the race was 38.5°C (range: 37.6 - 39.7°C, above 39°C in 5 subjects). Tympanic temperature showed a correlation between Tre, but did not detect any of 5 subjects who's Tre above 39°C. Dehydration rate was 1.6± 0.9% (0.6% increase - 3.0% loss, increases in 3, above 2% in 10 subjects). The blood analysis before and after the race were as followed; Alb:4.6 to 5.0 g/dL, AST:24.1 to 31.8 U/L, LDH:213 to 275 U/L, CK:145 to 251 U/L, BUN:16.8 to 18.3 mg/dL, Na:142 to 145 mEq/L, Cl:101 to 102 mEq/L, WBC:5514 to 9181 /μL, RBC:467 to 481 10^4/μL, Hb:14.2 to 14.6 g/dL, Hct:46.3 to 47.4 %. Hemoconcentration rate was 8.0± 0.9% calculated from the change of Alb and 5.1± 6.0% from Hb and Hct. The less increase in Na and Cl compared to hemoconcentration rate, indicates insufficient intake of NaCl. (COI:No)

3P-098

Developmental neurotoxicity of perfluorooctanesulfonate exsposure in different lactational periods in adult male mouse

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We examined the effects of exposure to PFOS during postnatal day (PND) 1-7 or during PND 8-14 on the performance of Rotarod test and visual discrimination learning task in the adult male mouse. Post pertum C57BL/6J mouse dams received 1 mg/kg b.w. of PFOS via gavage from PND 1 to 7 or from PND 8 to 14. Control dams received water as a vehicle. After male mice progeny reached adulthood, the Rotarod test and visual discrimination learning task was conducted. In the Rotarod test, the performance of PND8-14 PFOS-exposed group was significantly lower than that of control group. In the visual discrimination task, the performance of PND1-7 PFOS-exposed group was significantly lower than that of control group. These results suggest that neutoroxic effects of lactational PFOS exposure may be different depending on the period of exposure. (COI:No)

3P-099

Acute swimming exercise can accelerate the browning of skeletal muscle in interscapular region

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There has been a growing body of evidence that PR domain containing 16 (PRDM16), a zinc finger transcription factor, plays a central role in bidirectional switch to drive the differentiation of Myf-5-expressing mesenchymal stem cells into the skeletal muscle or the classical brown adipocytes (BA). However, little is known about the effect of physical exercise on PRDM16-mediated differentiation event of BA in skeletal muscle. Male C57BL/6J mice were randomly divided into 3 groups: the sedentary control group, the acute swimming exercise group and the cold exposed group, a positive control of BA function. The mice in the exercise group were subjected to acute swimming exercise at a water temperature 35°C for 60min. After exercise, several regions of skeletal muscle were removed and used for analysis of mRNA, protein and others. Levels of mRNA for UCP-1, a specific marker of BA, were detected only in interscapular muscles in each group. Of note, in interscapular muscles, levels of PRDM16 mRNA and protein were significantly increased in exercise compared with control. Under this condition, levels of PRDM16 / C/EBP- β complex, which is a pivotal key regulator for switching of PRDM16, were significantly higher in exercise than control. These results demonstrate that acute swimming exercise has effects on the browning of skeletal muscle in interscapular region. (COI:No)

3P-100

Treatment with Donepezil and Sigma Receptor ligand on H9c2 cells up-regulates the cell viability, the mRNA and protein levels of Sigma Receptor 1 and SR1 splicing variant.

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Donepezil is an inhibitor of Acetylcholinesterase (AchE) and is now used for the treatment of Alzheimer's disease. Recently, donepezil is reported to have an anti-apoptotic effect on heart failure. But the underlying mechanisms of Donepezil are largely unknown. Here we show that Sigma Receptor 1 (SR1) ligands increase the donepezil effects on the H9c2 cells.H9c2 cells were treated by 10μM MG132 (ubiquitin-proteasome inhibitor). After that, MG132 treated H9c2 cells were stimulated with donepezil and SR ligands (BD1042, PRE084, SM-21 respectively). WST-1 assay was performed to measure the cell viability. The cell viability of MG132 treated H9c2 cells were enhanced by treatment with donepezil and SR ligands in a dose-dependent manner. Furthermore, we measured the mRNA levels by Digital PCR (Bio-Rad) and the protein levels by Western Blotting. The mRNA and protein levels of SR1 and SR1 splicing variant (sv) were up-regulated by the treatment with donepezil and SR ligands. These results indicated that SR ligand increase the anti-apoptotic effects of donepezil using SR1 and SR1 sv. (COI:No)

3P-101

Pulsed electromagnetic field stimulation specifically modulates intracellular microenvironment of osteosarcoma cell line LM8 cells

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In this study, we report that pulsed electromagnetic field (PEMF) stimulation modulated the microenvironment of an osteosarcoma cell line (LM8 cells), with no influence on osteoblastic or bone marrow cells. Short stimulation times (1 h) led to upregulated metabolic activity of LM8 cells. Upon 9 or 12 h of PEMF, the cell number of LM8 cells treated with doxorubicin (DOX; an anti-cancer drug) was significantly reduced compared to the unstimulated group. In support of these results, flow cytometry also revealed that PEMF promoted DOX uptake in LM8 cells. Compared to the unstimulated group, a significant increase in intracellular Ca^{2+} concentration was seen upon 5 and 15 min of PEMF of LM8 cells in Ca^{2+}-containing medium and upon 5 min of PEMF of LM8 cells in Ca^{2+}-free medium. These results suggest that PEMF have effect on Ca^{2+} regulated-proteins in cytoplasm membrane and cytosol. The investigation of mitochondrial membrane potential during two stimulation durations (1 and 12 h) showed that mitochondrial membrane potential as well as intracellular Ca^{2+} concentration kinetics were changed, reflecting the reversible movement of Ca^{2+} between the mitochondria and cytosol. This study suggests that PEMF stimulation may be specifically applied in chemotherapy for osteosarcoma. (COI:No)

3P-102

Effect of Yokukansan or pentazocine on restriction of movement and hyperalgesia in an ankle-immobilization rat model

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To evaluate the effect of Yokukansan (YKS: Tsumura & Co) or pentazocine (PZ) treatment on range of motion (ROM) limitation and the anti-hyperalgesia in ankle-immobilization model. Wister male rats were used. YKS(3%) was contained in CE-2 cube diet (CLEA Japan Inc) and fed for 2 weeks: YKS 2.4 g/Kg/day. PZ (30 mg/kg) i.p. treatment was performed 30 min before behavior tests for pain threshold. ROM and pain threshold were measured in all rats once a week for the 2 weeks. ROM of ankle dorsiflexion (DF) was measured, and pain thresholds were evaluated by behavioral response with the von Frey test and Hargreaves Assay using a plantar test. All data were shown as % of right limb/left limb. Ankle DF in ankle-immobilization for 14 days (IM) group was significantly limited 2 week after immobilization (56%). And Ankle DF in IM+YKS group and IM+PZ group also significantly limited 70% and 63% after 2 weeks, respectively. The mechanical hyperalgesia threshold in IM was significantly decreased to 39% after 2 weeks compared to control group, however, in IM+YKS or in IM+PZ were not decreased. Thermal nociceptive thresholds were significantly decreased to 76% after 2 weeks in IM, but, in IM+YKS or in IM+PZ was not decreased. These results indicate that YKS or PZ treatment was not effect on ROM of ankle DF in this model. On the other, the decrease of mechanical hyperalgesia and thermal nociceptive thresholds in IM+YKS and IM+PZ compare to IM. It might act on central nerve system and/or spinal interneuron. (COI:Properly Declared)

3P-103

Investigation of ANO1 blocker and the effect on TRPV1-related pain

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Anoctamin 1 (ANO1) is activated by intracellular calcium and involved in pain sensation through TRPV1 activation in dorsal root ganglia (DRG) neurons. The intracellular chloride concentrations are maintained at a high level because of low (or no) expression of potassium-chloride co-transporter type 2 in DRG neurons. Therefore, the depolarization is evoked by chloride efflux through ANO1 activation followed by the action potential generation. We previously reported that the calcium entering the cells via TRPV1 induces the ANO1 activation and that capsaicin-evoked pain is reduced by an ANO1 blocker. In this study, we found that menthol and its analogues inhibited ANO1 currents in HEK293T cells expressing mouse or human ANO1. And the menthol reduced the pain-related behaviors evoked by capsaicin injection into the top of hind paw in TRPM8 KO mice. Moreover, the menthol inhibited the neuronal excitations in the spinal dorsal horn. It is well known that menthol reduces the pain and that TRPM8 is involved to some extent. However, the pain reduction mechanisms by menthol are still unclear. Our finding indicates that ANO1 inhibition is one of the mechanisms of menthol-induced analgesics. (COI:No)

3P-104

Effects of caffeine on histamine-induced rhinitis symptoms in mice

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Recently, we reported that administration of caffeine decreases allergic rhinitis symptoms induced by repeated ovalbumin treatment in mice. To clarify the putative mechanism of caffeine action on rhinitis symptoms, the effects of caffeine treatment on histamine-induced rhinitis symptoms and on corticosterone and catecholamine levels in plasma were investigated. Female BALB/c mice, aged 6 weeks, were used for the experiments. One hour after caffeine injection (30-100 mg/kg, i.p.), 2 μL of histamine solution (0.3-3 M) was administered into the nasal cavities using a micropipette, and the frequency of sneezing and nasal rubbing was monitored for 10 min. About 400 μL of whole trunk blood was collected into a heparinized tube following decapitation 1 h after caffeine injection, and was then centrifuged for 10 min to collect plasma. The levels of corticosterone and catecholamine in the plasma were measured by enzyme immunoassay and HPLC-ECD system, respectively. Nasal administration of histamine dose-dependently induced sneezing and nasal rubbing, but pretreatment with caffeine decreased these symptoms. Levels of corticosterone and catecholamine in plasma were increased by caffeine injection. The elevation of these hormones may be associated with the attenuating effect of caffeine on rhinitis symptoms. (COI:No)

3P-105

Research of antitumor effects of the Celastrus orbiculatus extract

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Celastrus orbiculatus is one of the important folk medicinal plants used in the anti - inflammatory and analgesic treatment of various diseases. Recentl y, study about Celastrus orbiculatus extract (COE) has shown COE has signifi cant antitumor effects. However, study of the effect of COE on suppressing t he epithelial-mesenchymal transition (EMT) have not been reported at home an d abroad so far. This experiment mainly study that COE inhibit EMT by regula ting cell cytoskeleton rearrangement. Activity of AGS cells was detected by MTT assay. Cell cytoskeleton staining and laser scanning confocal microscopy were used to detect the morphological changes in cell morphology and micros tructure. Invasion and migration assay were used to observe the metastatic a bility of AGS cells in vitro. Expressions of EMT biomarkers, matrix metallop roteinases and Cofilin 1 were examined by western blotting. The correlation between Cofilin 1 and EMT was tested by immunofluorescence technique and cyt oskeleton staining combining method. The results showed that COE could signi ficantly down-regulated the expression of Cofilin 1 and inhibit the EMT proc ess of cancer. In conclusion, Cofilin 1 was directly involved in the EMT pro cess, and played an important role. COE could significantly inhibit the EMT process by mediating Cofilin1 signalling pathway. This study may provide a b asis for the development of new anticancer drugs and a novel therapeutic str ategies targeting Cofilin 1 protein. (COI:No)

3P-106

Effect of triptolide on inflammatory cytokine expression in synovial fibroblasts of collagen-induced arthritis mice

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Triptolide, a compound originally isolated from *Tripterygium wilfordii Hook F* (TWHF), a vine used in China for autoimmune diseases such as rheumatoid arthritis (RA), is known to have immunosuppressive and anti-inflammatory effects. However, its mechanism of action is not well understood. In this study, we examined the effect of triptolide on inflammatory cytokines using synovial fibroblasts obtained from collagen-induced arthritis (CIA) mice, a commonly used model in RA studies.CIA was induced in 7-week-old BALB/c mice using a mouse monoclonal anti-type II collagen 5-clone antibody cocktail. On day 10, we collected synovial fibroblasts from the articular cartilage of both knees and used cells at passage 4-9. We replaced the culture media of cells with triptolide and lipopolysaccharide (LPS). We then measured the inflammatory cytokines level and transcription factors level.LPS significantly induced the production of inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-6, as well as that of inflammatory cytokine-related transcription factors, including nuclear factor (NF)-κB and phosphorylated IκB, in synovial fibroblasts. Triptolide significantly suppressed both LPS-induced cytokine and transcription factor expression in a dose-dependent manner. These data suggest that triptolide directly affects fibroblasts and exerts anti-inflammatory effects by suppressing the expression of inflammatory cytokines in a paracrine manner. (COI:No)

3P-107

A rarely existing natural sugar, D-allulose, prevents the development of obesity and diabetes in OLETF rats

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The present world is consequently at a critical point in obesity and diabetic issue. It was mentioned that while physical activity is a key part staving off diabetes, heart disease and dementia, where unhealthy eating with excess sugar and carbohydrate is the key point of being obese. We cannot avoid taking sugar but it is possible to cut down sugar- related energy intake keeping the taste and sweetness of foodstuff unaltered by taking rare sugars D-allulose. We found excellent outcome of D-allulose in controlling body fat and maintaining blood sugar levels. We fed 5% D-allulose to OLETF rats for 60 weeks and body weight, food intake, water intake, blood glucose, serum insulin, body fat were measured. Oral glucose tolerance test was performed. On sacrifice liver, pancreas and other organs were preserved and stained. D-allulose controlled abdominal fat accumulation and thus prevented excess body weight. D-allulose increased insulin resistance through maintenance of fasting, random and OGTT blood sugar and serum insulin levels. Immunostaining of the pancreas showed attenuation of progressive fibrosis with the preservation of insulin producing β-cells. Serum levels of proinflammatory and antiinflammatory cytokines were also controlled well by D-allulose. Rare sugar D-allulose might be a promising strategy for the prevention of obesity and the commencement and prevention of type 2 diabetes. (COI:No)

3P-108

Anti-stress effect of Kampo medicine Yokukansan via the control of orexin secretion

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Introduction: It is reported that the secretion of orexin, a neuropeptide, in the hypothalamus is increased in rats exposed to social isolation stress for one week. In this study, the anti-stress effect of Kampo medicine Yokukansan (YKS) via the control of orexin secretion was examined.

Methods and results: Initially, YKS (300 mg/kg or 1,000 mg/kg; p.o.) was administered to intact rats for one week, and then the plasma orexin level was significantly decreased by the administration of YKS (300 mg/kg). Next, rats were divided into three groups: the group-housed control group (Con), the single-housed stress group (St) and the single-housed and YKS(300mg/kg)-administered group (St+YKS). After one week, the resident-intruder aggression test was performed and the plasma levels of orexin and corticosterone were measured. In the St group, aggressive behavior and the levels of orexin and corticosterone were significantly increased; however, these increases were inhibited in the St+YKS group. Furthermore, an orexin receptor antagonist (TCS 1102; 10 mg/kg i.p.) was administered to rats exposed to isolation stress to examine whether orexin is involved in the stress responses. As a result, aggressive behavior and the level of corticosterone were significantly decreased.

Conclusion: These results suggest that orexin is involved in the control of the stress responses and YKS shows an anti-stress effect via the control of orexin secretion. (COI:No)

3P-109

Angiotensin II type 2 receptor attenuates the type 1 receptor signaling via protein kinase C

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Angiotensin II (AII) plays crucial roles in the development of cardiovascular diseases, in which the AII type 1 receptor (AT1R) preferentially mediates AII-induced actions. In contrast, the AT2R is reported to counteract such AT1R-mediated actions; however, the precise molecular mechanism of how AT2R inhibits AT1R signaling remains unclear. In this study, we explored in detail the molecular mechanism involved in the inhibitory effect of AT2R on AT1R signaling, by using live cell imaging, including the Forster resonance energy transfer (FRET) technique. In HeLa cells co-expressing YFP-tagged AT1R, CFP-tagged AT2R, and RFP-tagged extracellular signal-regulated kinase (ERK), it was demonstrated that AT2R suppressed AT1R-mediated ERK activation. FRET analysis also revealed that AT2R, even in the absence of AII, bound to AT1R at the plasma membrane, and AII stimulation modulated their spatial arrangement, followed by receptor internalization. The AT1R-specific and AT2R-specific inhibitors more or less inhibited both the conformational change and the internalization of the complex. In addition, protein kinase C (PKC) inhibitor treatment inhibited the AII-dependent accumulation of AT2R but not that of AT1R in the endosomes. Mutations in the putative phosphorylation sites for PKC in AT2R failed to induce conformational change and inhibit AT1R signaling, indicating that AT2R inhibits AT1R function in a PKC-dependent manner. (COI:No)

3P-110

Cardiac glycosides inhibit glucose uptake by modulation of the GLUT1 trafficking in human hepatocellular carcinoma cells

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Facilitative glucose transporter GLUT1 has been reported to be over-expressed in many types of human cancer cells. Up-regulation of glucose uptake exhibits a pivotal role in tumor growth. Here, we examined effects of cardiac glycosides, inhibitors of sodium pump (Na,K-ATPase), on the expression level of GLUT1 and the activity of glucose uptake in human hepatocellular carcinoma HepG2 cells. Ouabain, oleandrin and digoxin (300 nM and 1 μM) drastically decreased the expression of GLUT1 in the plasma membrane. Expression level of GLUT1 in the total cell lysate was also reduced by these cardiac glycosides (1 μM), suggesting that intracellular GLUT1 was degraded in the cells. Concomitantly, uptake of 2-deoxy-D-glucose was markedly decreased in the cardiac glycosides-treated cells. The ouabain (1 μM)-induced effects on GLUT1 was not affected by interfering endocytosis using nystatin (50 μg/ml) and cytocharasin B (20 μM). Interestingly, the ouabain-induced effects were blocked by the calcium chelator BAPTA-AM (25 μM) and CaMKII inhibitor KN-93 (20 μM). Methyl-β-cyclodextrin (10 mM), a disrupting agent of membrane microdomains, did not block the ouabain-induced effects. These results suggest that cardiac glycosides bind to Na,K-ATPase in the non-membrane microdomains, and that they suppress glucose uptake by interruption of the CaMKII-dependent transport of GLUT1 to the plasma membrane in human hepatocellular carcinoma cells. (COI:No)

3P-111

Effects of viniferin, a dehydrodimer of resveratrol, on transepithelial ion transport and epithelial permeability in the rat intestine

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Viniferin is a dehydrodimer of resveratrol, and synthesized in many plants including grapevine. The present study investigated the effects of viniferin and resveratrol on epithelial secretory and barrier functions in the rat intestine. Mucosa-submucosa tissue preparations of the terminal ileum, cecum, and proximal, middle and distal colon isolated from rats were mounted on Ussing chambers, and short-circuit current (Isc), tissue conductance (Gt) and FITC-dextran (4kD; FD4)-permeability were measured. In the cecum, which was the most sensitive region, mucosal, but not serosal, addition of viniferin (10^{-4} M) evoked an increase in Isc and FD4-permeability, and a rapid decrease followed by a sustained increase in Gt. The viniferin-evoked responses of Isc and Gt were shown in concentration-dependent manners (>10^{-5} M), and reduced by COX-1 inhibitors and EP4 receptor antagonists. In immunohistochemistry, COX-1 immunoreactive cells were found in the cecal epithelium with a variety of cells in the lamina propria. In conclusion, the present study have suggested that the luminal viniferin might induce a fluid secretion and modify the epithelial ion and macromolecule permeability mediated via the production of prostaglandins in the COX-1-expressed epithelial cells and EP4 receptor activation. (COI:No)

3P-112

Accessory cholera enterotoxin activates anoctamin 6 (TMEM16F) Cl^- channels

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Introduction: Vibrio cholera accessory enterotoxin (Ace), as well as cholera toxin and zonula occludens toxin, causes the endemic disease cholera. Ace has been shown to contribute to diarrhea by stimulating Cl^- secretion. However, the specific type of Cl^- channel activated by Ace has not been extensively investigated. **Objectives:** The present study aimed to identify Cl^- channel involved in the stimulation of Cl^- secretion by the action of Ace. **Methods:** We transfected anoctamin 1 and/or 6 (TMEM16A and/or F) protein into HEK293 cells and measured the whole-cell currents using patch-clamp techniques. We measured the intracellular calcium concentration in these cells. The experiments were also performed in human colonic cells (Caco-2). **Results:** Application of Ace stimulated Cl^- current in HEK293 cells, which expressed anoctamin 6 (from 0.48 ± 0.09 to 0.62 ± 0.09 nA at +59 mV) but not anoctamin 1. Co-expression with anoctamin 1 slightly augmented the activation of whole-cell Cl^- current through Ace stimulation in cells that expressed anoctamin 6 by 10%. Ace induced whole-cell Cl^- conductance and Ba^{2+}-sensitive K^+ conductance in Caco-2 cells. Interestingly, Ace was not able to induce rise of intracellular calcium concentration in HEK293 cells, which expressed anoctamin 6, and Caco-2 cells. **Conclusion:** These results indicate that anoctamin 6 contributes to Ace stimulated Cl^- secretion in intestinal cells. (COI:No).

3P-113

Pharmacological evaluation of the role of the Ca^{2+}-dependent BK and IK channels in fluid secretion in rat submandibular gland

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Two types of Ca^{2+}-activated K^+ channels, BK (or Maxi-K^+) and IK, but not SK, are expressed in mammalian salivary acinar cells and are believed to play critical roles in neurohumoral agonists-induced Ca^{2+}-dependent fluid secretion. Here, we investigated the role of BK and IK channels in fluid secretion in isolated perfused intact rat submandibular gland. We found that fluid secretion induced by acetylcholine was largely resistant to inhibitors of BK (TEA or paxilline) and IK channels (clotrimazole or TRAM34), but was inhibited by non-specific K+ channel inhibitors (Ba^{2+} or quinine). Similar observations were made for substance P-induced fluid secretion. We also found using the standard whole-cell patch-clamp technique that freshly isolated rat submandibular acinar cells displayed three types of Ca^{2+}-dependent K^+ currents, one of which was distinguished from BK and IK currents. These observations raise the possibility that a novel K^+ conductance might be involved, at least in part, in Ca^{2+}-mobilizing agonists-induced fluid secretion in these cells. Its molecular identity remains to be established. (COI:No)

3P-114

Electrophysiological characterization of ion transport in native porcine tracheal epithelium

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Porcine airway epithelia have been used as excellent models for the normal human airway physiology and for abnormalities in human diseases including cystic fibrosis (CF). In this study, we investigated electrogenic ion transport in unstimulated native porcine tracheal epithelium by measuring short-circuit current (I_{sc}) in Ussing-type chambers. Porcine tracheae were obtained from a local slaughterhouse. Ion substitution experiments showed that baseline Isc was mainly mediated by electrogenic transport of Na^+, but not Cl^-. Baseline Isc was saturated with increased luminal Na^+ concentrations and was strongly inhibited by the luminal application of amiloride or benzamil. Removal of glucose from the luminal side only had a minor effect on I_{sc}. Nystatin added to the luminal side (in the presence of amiloride) increased I_{sc}, which was inhibited by addition of ouabain to the basolateral side, suggesting that the rate-limiting step of the transepithelial Na^+ transport was most likely the apical Na^+ entry step. Apparent affinity for amiloride in inhibition of I_{sc} appeared to be affected by luminal Na^+ concentration. The luminal amiloride-sensitive conductance, which was measured in the tracheal epithelium permeabilized with nystatin on the basolateral side bathed in a high K^+ solution, was highly selective for Na^+ over K^+. Our data provide the characterization of the bioelectric properties and luminal Na^+ conductance of native porcine tracheal epithelium under basal conditions. (COI:No)

3P-115

High-salt intake failed to decrease intestinal Na^+/K^+-ATPase activity in hypertensive Dahl salt-sensitive rats

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It has been clarifying how high-salt intake causes salt-sensitive hypertension. In hypertensive Dahl salt-sensitive (DSS) rats, high-salt intake increases Na^+/K^+-ATPase (NKA) activity in the brain to enhance the production of endogenous ouabain. Then the secretion of cardiotonic steroid (CTS) from adrenals is chronically enhanced and effects on target organs by both inhibiting NKA pump activity and activating the intracellular signaling pathway via NKA. In the proximal renal tubules, the trafficking of basolateral NKA and luminal Na^+/H^+ exchanger is enhanced to stimulate natriuresis which is suppressed in hypertensive DSS rats. Furthermore, high-salt intake increases the intestinal secretion of Na^+, Cl^- and water in Sprague-Dawley (SD) rats but not in hypertensive DSS rats. The aim of this study is to elucidate the relationship between the inconsistent transepithelial ion transport and NKA activity in hypertensive DSS rats. DSS and SD rats were divided into two groups; one fed on a high-salt diet (DSSH and SDH) and the other fed on a regular diet (DSSR and 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, with or without bufalin (CTS). Bufalin-sensitive I_{sc} was significantly decreases in SDH compared with SDR, while no difference was found in bufalin-sensitive I_{sc} between DSSH and DSSR. These results indicate that high-salt intake decreases basolateral NKA activity in SDH but not in DSSH. (COI:No)

3P-116

Electrophysiological properties of chick embrio chorioallantonic membrane in ovo

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Chick embrio 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.

Relationships between tumor invasion and electrical potentials are sometime reported, but only few studies have been reported about trans-CAM potentials.

In this study we investigate electrophysiological properties of CAM in ovo, using microelectrode method. CAM generated endoderm negative electrical potentials and the mean of 14 CAMs was -16.7± 6.57 mV. Adding histamine (final 1 mM) made the potential more negative and vasodilation of CAM. These results add new insights into CAM assay. (COI:No)

3P-117

fMRI study on neural and cognitive aspects of urban landscape evaluation of human

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Urbanization has been hypothesised to cause various mental disorders and has necessitated the evaluation of the medical impact of cityscape appearances. We show that two kinds of landscape pictures, Japanese traditional architectures/natures (JTANs) and modern cities (MCs), have distinct impacts on human brain activation. Activation in the right precuneus was evident during viewing pictures of JTANs. This suggests that different neural mechanisms determine how people recognise, and evaluate various landscapes. (COI:No)

3P-118

Differential learning-related changes in climbing fiber inputs to cerebellar stripes

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Parasagittal alternate expression of aldolase C/zebrin II in the cerebellar cortex coincides with its longitudinal zones. Our recent study confirmed that fine scale structures with highly synchronous complex spike activities are identical to the cerebellar microzones. However, motor and cognitive functions of the microzones are not fully understood. To address this, two-photon calcium imaging was performed in Aldoc-tdTomato mice, transgenic mice in which aldolase C expression can be visualized in vivo, during auditory lick/no-lick task. The mice efficiently learned this task and reached an expert level (d prime > 3) within 3-7 sessions. Licking behavior was also refined so as to reduce redundant licks. Climbing fiber inputs to aldolase C-positive Purkinje cells were selectively enhanced after go-cue along the course of learning, while no-go cue-related inputs to both aldolase C-positive and -negative Purkinje cells were attenuated. Representation of climbing fiber inputs to the aldolase C-positive Purkinje cells were gradually shifted from lick-related to target behavior-related along with learning. These results suggest that motor and cognitive learning is differentially processed in aldolase C-positive and -negative zones. (COI:No)

3P-119

Neuronal activity in the monkey medial premotor areas during a duration classification task

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The medial premotor areas are proposed to play an important role in temporal information processing. In this study we investigated neuronal activity in the monkey medial premotor areas during a duration classification task. In the task, a visual cue was presented on the center of the monitor from 0.8 to 4.8 sec. Following a 1sec delay period, the monkey was required to press the proper key according to the classification of cue duration; the right, center and left keys for long (3.2-4.8 sec), middle (1.6-2.4 sec), and short (0.8-1.2 sec) categories, respectively. For the spatial control of key selection and movements, the subject also performed a spatially cued delayed response task, in which a visual stimulus cued the proper key spatially. Of 364 neurons we recorded, 103 were related to the task. We found three interesting types of task-related activity. The first one was phasic activity during the cue period with constant peak time after the cue onset. This phasic activity might function to filter current cue duration with the peak time. The second one was the build-up activity starting after the cue onset and ending around the cue offset. The build-up activity might function as an accumulator. The third one was phasic activity during the delay period, which changed according to the cue duration categories. This delay activity might represent temporal classification results of cue duration. These results suggest that the medial premotor areas are involved in the classification process of stimulus duration. (COI:No)

3P-120

Physiological effects of CNS and autonomic nervous system after drinking green tea or coffee

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In order to investigate physiological effects after drinking green tea or coffee, we measured the contingent negative variation (CNV) of EEG and a reaction time to push button against a target sound. Activities of the autonomic nervous system were analyzed by measuring heart rate and heart rate variability. Psychological conditions were monitored by describing two psychological tests, i.e. the multiple mood scale (MMS) and the General Arousal Checklist (GACL). Subjects were young healthy 10 students. Areas of the late CNV in central scalp positions, i.e. Fz, Cz and Pz, were decreased after drinking hot or cold tea, suggesting that tea might induce relaxation. However, after drinking coffee areas of CNV were increased, suggesting awakening effect. Reaction time for pushing button to target signal during CNV was shorten in 8.7% by cold green tea, 8.8% by hot tea and 12.4% by coffee comparing to the control. These results suggested that drinking green tea and coffee stimulated responsibility of nervous function from sensory to motor system. However, effects of coffee on CNV were little on favorite coffee drinker but arousal effect was obviously observed on non-daily coffee drinker. HF in heart rate variability was increased after drinking green tea but was reduced after drinking coffee, suggesting that tea induced relaxation but coffee reduced parasympathetic activity. Concentration of mind in MMS was increased after tea and coffee experiments, consisting with the results of reaction time to push button. However, difference in GACL between tea and coffee was not observed. (COI:No)

3P-121

Theta and gamma oscillation of amygdala linked with hippocampal high frequency oscillation and fear memory

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Direct pathway from CA1 region of ventral hippocampus (vHPC) to basolateral amygdala (BLA) is thought to contribute to form fear memory. The memory consolidation process occurs during rest stage including slow-wave sleep by sharp wave-ripple complex (SWRs) that observed in CA1 region of the hippocampus. However, the SWRs interaction of vHPC and BLA to fear conditioning is still unknown. We investigated the relationship between the freezing behavior correlated with fear memory and the high frequency oscillations including SWRs between vHPC and BLA during rest time after foot shock. In results, synchronous high frequency oscillations (100-250 Hz) between vHPC and BLA were observed during rest time at the home cage after foot shock. The synchronous rate was correlated with freezing behavior. In addition, phase locked theta band oscillation was observed in BLA activities that showed synchronous high frequency oscillation to vHPC. Power of the theta oscillation showed inverse correlation with freezing behavior after 24 hour after oot shock. Furthermore, power of gamma oscillation around the high frequency oscillation showed correlation with freezing behavior after 24 hour after oot shock. Thus, it can be presumed that the intensity of the BLA theta band oscillation linked with vHPC high frequency oscillation could enhance fear memory extinction. (COI:No)

3P-122

Chemogenetic Control of Neuronal Activity in the Primate Subthalamic Nucleus

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Since the development of DREADD, or Designer Receptors Exclusively Activated by a Designer Drug, by a group of Bryan Roth in 2007, the chemogenetic method has been applied to control neuronal activity with a series of modified muscarinic receptors. In the DREADD system, the administration of clozapine N-oxide (CNO), an otherwise biologically inert drug, changes the membrane excitability through the receptor activation. Although the neural activity modulation in target brain areas by a drug is suitable to treat neurological disorders, the efficiency and efficacy of the system in primates have yet to be examined. Here, we applied the DREADD system to suppress neuronal activity of the primate subthalamic nucleus (STN), a target nucleus of deep brain stimulation to alleviate parkinsonian symptoms.
The modified muscarinic M4 receptor was expressed in the STN of a Japanese monkey (Macaca fuscata) with AAV, and in response to the systemic administration of CNO, STN neurons showed a decrease in the spontaneous activity and loss of excitatory response to the motor cortical stimulation. In addition, a local microinfusion of CNO into the globus pallidus internal segment (GPi), resulted in a decrease in the spontaneous activity of GPi neurons, suggesting the neurotransmission block from the synaptic terminals of STN neurons. Our results indicate that the DREADD system is feasible not only in the treatment of neurological disorders but in the pathway specific blockage of brain circuits. (COI:No)

3P-123

Effect of mother-infant interaction on the development of prefrontal dopaminergic control

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We previously found that early-weaned rats exhibited higher anxiety accompanied with increase of the basal level of extracellular dopamine in the amygdala (about 265 % vs. the control, at the 2014 meetings). We here planned to determine whether the increase occurred in the local system within the amygdala or the whole limbic system of dopamine originated in the midbrain. The early-weaned rodents reportedly showed precocious myelin formation of the anterior basolateral amygdala (Ono et al, 2008), which is innervated by the medial prefrontal efferents. Therefore, we comparatively measured the prefrontal dopamine levels of the early- and late-weaned rats by in vivo microdialysis with concurrent recording of open-field behaviors on two successive days. While the numbers of the subjects were limited, the early-weaned group showed slightly higher basal release of dopamine (about 105 % vs. the control, n = 6 in each). The evoked response in the prefrontal cortex following open-field exploration seems to regain faster than the amygdalar one that we previously reported. As a tentative result at this moment, these imply that the limbic dopamine system is regulated in each brain area. We will present these statistical results at the 2016 meeting.(JSPS KAKENHI Grant No. 15K04202 and 15H02479; COI:No)

3P-124

Inhibitory neural response predicting visual information in monkey amygdala

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Visual stimuli can serve as positive or negative reinforcer for nonhuman primates even though those stimuli are not accompanied with fluid reward or aversive punishment. Especially, social stimuli that are visually presented attract much interest of nonhuman primates. One of candidates for neural substrate underlying prediction of social value is the amygdala that is known to respond to social stimuli such as facial expressions. To elucidate the involvement of the amygdala in prediction of sociality-related visual information, we recorded the activity of the monkey amygdalar neurons while an arbitrary geometric pattern was associated with one of the subsequent visual images of conspecifics or fluid reward. Sixty seven of 130 neurons in the amygdala of a macaque monkey responded to at least one of geometric patterns that were predictors of the subsequent visual stimuli, and sixty nine responded to at least one of predictors of the subsequent fluid reward. About half (33/67, 49%) of neurons predicting visual stimuli showed inhibitory response although the majority (58/69, 84%) of neurons predicting fluid reward showed excitatory response. We examined how the amygdalar neurons code social value and fluid value. Preference of social value positively correlated with preference of fluid value in the neurons showing inhibitory response, whereas there was no correlation between them in the neurons showing excitatory response. These results indicate that the inhibitory neural response in the monkey amygdala plays an important role in prediction of visual images of preferable social information. (COI:No)

3P-125

Reward value coding by single neuron in the monkey orbitofrontal cortex during decision-making

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When we choose from the alternatives, we consider their values and workloads to obtain them. To study the neuronal mechanism of decision-making, we developed a decision-making schedule task and recorded single unit activity from monkey orbitofrontal cortex (OFC) a cortical region that where the neurons carry information about rewards and their values.Two monkeys were trained to perform a decision-making schedule task in which two kinds of choice targets (CT) were presented sequentially (1st and 2nd CT, respectively). The CT brightness and length indicated reward amount and required number of trials (i.e. schedule), respectively. After the 2nd CT disappeared, both CTs reappeared simultaneously on the left and right. When the monkey chose one, the schedule represented by the target started. We recorded from 253 neurons in the OFC. In the second CT period, 60.1% (152/253) neurons showed correlation between the neuronal firing and 1st and/or 2nd CT values. For 30.0% (76/253) neurons, the monkey's choice could be estimated from the spike counts during CT presentation periods. Following bilateral injection of muscimol into the OFC, choice accuracy and speed were degraded. These results suggest that OFC not only encodes information about choice value, but is critical for normal choices to occur. (COI:No)

3P-126

Social defeat stress induces a cognitive deficit in 5-choice serial reaction time task in rats

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Social stress can induce anxiety- and depressive-like states in rodents and human. However, it is not clear whether social stress affects cognitive function such as sustained attention. In the present study, we investigated the effects of social defeat stress on cognitive performance of rats using a 5-choice serial reaction time task (5-CSRTT). Rats subjected to chronic social defeat stress for 7 days exhibited social avoidance, anxiety, and hypercortisolism, consistent with depressive-like symptoms. On 5-CSRTT, these defeated animals showed reduction of accuracy and made more omission errors than control rats did. In addition, social defeat stress induced decreases in correct choice-related neuronal activity in the anterior cingulate cortex (ACC). Suppression of incorrect choice-related neuronal activity during correct trials was inhibited by social defeat stress. Thus, chronic social defeat stress induces depressive-like state with attention deficits and enhanced behavioral inhibition. Our findings further suggest that these cognitive disturbances may be partly due to dysregulation of decision-related neuronal activity in the ACC. (COI:No)

3P-128

Salicylate-induced changes of the neural activity to repetitive sounds in the primary auditory cortex of guinea pigs observed by optical recording.

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The influence of salicylate on neural activities to repetitive sounds with different repetition rates in the primary auditory cortex (AI) of the guinea pig was investigated using optical imaging with a voltage-sensitive dye (RH795). Eight guinea pigs were used under anesthesia with ketamine (80 mg/kg) and xylazine (40 mg/kg). Activity patterns to repetitive sounds (8 kHz tone of 25-ms duration or click, 4-20Hz repetition rate , 75 dB SPL) were recorded from the AI on both sides before (control) and 8 hours after the intraperitoneal injection of 200 mg/kg salicylate. In control condition, the repetition-rate transfer functions (RRTF) of the 2nd, 3rd and 4th peaks measured with tones in AI had band-pass characteristics with a peak at 6 Hz but RRTFs measured with clicks had band-pass characteristics with a peak at 8 or 10 Hz showing a sharp drop-off. At 8 hours after the salicylate injection, the RRTF of the 4th peak to the tone in the right cortex frequently showed the large peak at the repetitive rate of 6-8 Hz and the small peak at 16 Hz. We discuss the salicylate-induced changes of the responses to repetitive sounds in AI of guinea pigs. (COI:No)

3P-129

The effect of landmark presentation on the response time in the oculomotor delayed response time task.

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Extensive studies have been examined the neural basis of working memory using oculomotor delayed response time (ODR) task. Our previous skill learning study with similar oculomotor task showed that the monkey subjects encoded the target position in relation to the visible candidates of target (landmarks, LMs) or other than the LMs, that is, they encoded the target in allocentric or egocentric coordinate system. Further, the response time was faster when LMs are present. Depending on the research groups, the LMs are/are not used in the ODR task, however, the presence of LMs might lead the subjects to take different strategy to encode the target position in the ODR task and thus show the difference in oculomotor parameter. In the present study, we used human subjects to perform the ODR task and visually-guided oculomotor task (VGO task, control) with/without LMs and analyzed the response times using 3way ANOVA with the factors of " Task type" , " presence of LMs" and " Target direction" . Our results showed significant main effect on the factor of " Task type" , suggesting that the longer response time in the ODR task. Further, we found significant interaction between the factors of " Task type" and " presence of LMs" , suggesting that faster response time in the ODR task with LMs but slower response time in the VGO task with LMs, and vise versa. Our results suggest that the subjects encoded the target position in different manner depending on the presence of LMs and the ODR task can examine the working memory of target position encoded in the different coordinate systems. (COI:No)

3P-130

The effect of transcranial direct current stimulus application over the parietal cortex on the response time in number comparison task.

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Human competence of numerical manipulation has been examined with the use of number comparison task, in which subjects are required to choose larger or smaller number according to the instruction as soon as possible between two numbers appeared on a computer monitor. Previous studies have shown the distance effect, the smaller the difference between two numbers, the longer the response time. Those studies using neuroimaging and non-invasive cortical stimulation techniques have also shown that the parietal cortex is involved in the numerical processing, that is, parietal cortex shows stronger activity when the difference between two number were small. Recently, extensive studies have applied a non-invasive cortical stimulation, called transcranial direct current stimulus (tDCS), over cortical areas in order to examine their functions. Those studies have shown that anodal stimulus over a cortical area leads excitation on the area, whereas cathodal stimulus leads depression. The present study examined the effect of tDCS over parietal cortex on the human numerical competence. We placed anodal electrode on either side of the cortex and cathodal electrode on the conrtralateral shoulder, and vice versa. The behavioral results showed the significant distance effect. Further, tDCS result showed that the anodal stimulus over the parietal cortex led faster distance effect, suggesting that the numerical processing was enhanced by the anodal stimulus of tDCS. (COI:No)

3P-131

Evaluation of prenatal treatments of bisphenol A using the pre-weaning rats and the predator odor

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Bisphenol A (BPA) is known as an environmental endocrine disrupter. Our previous studies showed that prenatal administration of BPA impaired the gender differences of the open-field behaviors and enhanced the response to predator odor after maturity. In this study, we focused on the behaviors in the pre-weaning period. We administered BPA (15μg/kg/day; low-dose and 1500μg/kg/day; high-dose) to prenatal rats, and offspring were examined at 8 days of age. In this, we designed the methods using the predator odor (fox odor), and examined a twitching, a pivoting, a head-movement, a crawling and an immobility. By the odor, decreases in head-movement and increases in immobility were seen in control and low-dose rats in both male and female. These alterations were seen only in females in high-dose rats. The scores of pivoting were decreased by the odor only in male rats in control and low-dose groups. The number of animals that displayed the crawling response was decreased by odor in male rats in both control and low-dose groups. In high-dose, both male and female rats displayed the decreasing of the crawling response. In the comparisons of spontaneous behaviors without odor, both BPA treatments did not induce any significant effects, and gender differences were not seen in all parameters and in all groups except for the immobility in low-dose group. High-dose BPA attenuated the basal responses to the predator odor in the head-movement, the pivoting and the immobility, however, the crawling response showed the opposite trend. (COI:No)

3P-132

Associations between salivary oxytocin concentration and subjective feeling during rubber hand illusion

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Oxytocin is a posterior pituitary hormone, which is known to promote lactation, maternal bonding and birth (Stoop, 2012). Our social recognition is partly modulated by oxytocin, and this hormone also associate with empathy (Kosfeld et al., 2005). Meanwhile, brain regions related to social recognition/empathy (e.g., insular cortex) are activated in the rubber hand illusion (RHI) (Tsakiris, 2010); illusory ownership sensation of a rubber hand induced by synchronous brush stroking to a rubber hand and participant's hidden hand (Botvinick and Cohen, 1998). However, it is not examined whether oxytocin affects the extension of body schema. In this study, we investigated relationship between salivary oxytocin concentration and RHI. We synchronously or asynchronously delivered brush stroking to participant's hand and a rubber hand over 4 sessions in separate days (N = 10). Following each session, they were asked to answer questions to evaluate their subjective feeling of RHI. Salivary oxytocin was measured before the behavioral tasks. We found that participants who had higher concentration of salivary oxytocin tended to feel stronger ownership that was caused by the rubber hand illusion ($r = 0.75$, $t\,(9) = 3.4$, $p < 0.01$). This result suggests that individuals' salivary oxytocin concentration can predict the degree of RHI. (COI:No)

3P-133

C-Fos and Arc expression during a rubber tail task in mice: an initial study

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Feeling of body ownership is sometimes extended out of our own body, as in the rubber hand illusion. We have developed a rubber tail task in mice, aiming to evaluate their body ownership. In the former study, the C57BL/6N mice (n=13) performed daily tests under two conditions using brushes: 1) synchronous stroking of a real tail and a rubber tail and 2) asynchronous stroking of the tails. After the stroking, an experimenter firmly grasped the rubber tail, and the response rate was evaluated. We reported that the response rates were significantly larger in the synchronous stroking condition, compared to the asynchronous stroking condition (Wada et al., 2014). This study examined the expression of c-Fos and Arc (neural activation markers) in the 3 out of 13 mice, which performed the rubber tail task. The mice were sacrificed on the day that experienced the synchronous stroking. Immunostained cell densities of these mice were evaluated in coronal brain sections.In the behavioral task, their response rates were larger in the synchronous stroking condition (mean, 0.43), compared to the asynchronous stroking condition (0.34). In the histology, strong expression of c-Fos and Arc in the superficial layers of the parietal association cortex was observed.Our preliminary results suggest that visual and tactile signals are integrated in the multimodal sensory area, and the area may have a role to generate the illusory experience. (COI:No)

3P-134

Respiratory synchronized activities in medulla and limbic system during resting state and olfactory stimuli: fMRI study

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Respiratory center in the medulla generates the basic respiratory rhythm, however the respiratory rhythm is modulated by inputs from higher centers involving sensory information and emotions. In this study, we performed simultaneous recordings of respiration and brain activities using functional Magnetic Resonance Imaging (fMRI) (Siemens Healthcare, Erlangen, Germany) during resting state and olfactory stimuli to identify synchronization of the inspiration onsets and bold signals in medulla, pons, parabrachial nucleus as well as higher centers such as amygdala and hippocampus. Respiratory flow and cardiac signal were simultaneously recorded during the scan for removing physiological noises from fMRI-BOLD signal. During resting state, dorsal and ventral medulla were activated with synchronization of inspiration onsets. Olfactory stimuli activated the amygdala, hippocampus and parabrachial nucleus in addition to dorsal medulla, and while activated less in ventral medulla. Olfactory stimuli caused emotional change with activations of amygdala and hippocampus which modulated respiratory frequency, and these respiratory change may relayed through activation of parabrachial nucleus and -dorsal medulla connections. (COI:No)

3P-135

Odor induced autographical memory associated with activity in the posterior parts of the brain as well as limbic olfactory areas.

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Memories induced by odors enable individuals to mentally travel back into their personal past. In this study, we used functional Magnetic Resonance Imaging (fMRI) to analyze brain areas related to odor-induced Autobiographical Memory (AM) and emotions in healthy subjects. Odor stimuli were used one of three choices, Tatami, Baby Powder or Osmanthust, which elicited a specific, pleasant and AM. Subjects were instructed to breathe normally with nose mask for delivering odors and for measurement of respiratory flow. AM odor increase tidal volume and decrease respiratory frequency with activations of the olfactory limbic areas including amygdala, hippocampus and orbitofrontal cortex. AM odor also activated posterior parts of the brain including retrosplenial cortex and medial area of the superior parietal cortex of the precuneus. Those areas had been reported roles of episodic memory retrieval and visuospatial processing, respectively. AM odor induced specific emotions with strong feeling back to the past and with retrieving of the vividness of the spatial and episodic memories and these feelings may link to retrosplenial cortex and precuneus activations. (COI:No)

3P-136

Real-time change of neural activity in the hippocampal CA1 before, during, and after the exposure to emotional episodes: spontaneous high frequent firing and following ripple diversity

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The emotional episode forms strong memory. To monitor the process of episodic memory, we recorded neuronal activity in the hippocampal CA1 in freely moving male rats before, during, and after an episode. Rats were experienced either of four episodes for 10 min: 1. a stressful episode (legs restraint on wooden board), 2. an episode with a young female rats in home cage, 3. an episode with a young male rats in home cage, 4. observation of novel object in home cage. After the initiation of episodes, CA1 neurons fired with high frequency (~100Hz) for seconds several times. In addition, the diverse ripple-like events were increased after the termination of episodes. These neural events were clearly observed in animals experienced with the stressful episode or the episode with female.
To further analyze synaptic plasticity, we made acute brain slices at 30 min after the episode. Although we found significant increase in the amplitude of miniature EPSCs or miniature IPSCs, the distribution and the extent of miniature EPSC vs miniature IPSC amplitude were different among the episodes. Our results suggest that episodes accompanied with intense emotion such as fear and love strongly induce memory related neural events and synaptic plasticity. (COI:No)

3P-137

Physiological responses induced by combination exposure to sound stimulation and scenery image in the human participants

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Perception of environmental sensory stimuli through sounds, odors, foods or air conditions induces several kinds of emotions, despite the underlying biological mechanism is still unclear. To explore the mechanisms of human emotion, experimental data from subjective methods provide a lot of useful data. But these data always contain risks of incorrectness when outputting participants evaluation on answer materials. In this study, we tried to find physiological features when participants fit their motional images to an answer material. Hearing experience actually evokes emotional response on the human. Then, we examined participant brain and autonomic nerve response during exposure to sound stimulation and scenery image. Briefly, participants were exposed several kinds of nature sound or music and scenery picture of landscape projection. During the exposure term, several physiological response of the participant were recorded. Then, we analyzed a correlation of the effect of physiological response and each coupling of sound stimulation and scenery image. (COI:No)

3P-138

Differences in cerebrocortical oxygenation during overground and treadmill walking in humans: a portable functional NIRS study

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Walking is one of the most common and important activity in our daily living, while the underlying central mechanism is still not fully understood. With portable functional near-infrared spectroscopy (NIRS, 22 channels), we examined whether and how the human sensorimotor cortices contribute to the ongoing walking, and whether the neural activity differs between overground and treadmill walking. The concentration of oxygenated hemoglobin (Oxy-Hb) along with surface electromyogram (EMG) of shoulder and lower leg muscles were assessed by telemetry during a 1-min overground or treadmill walking in eight healthy volunteers. The anatomical location of/between the NIRS optodes was estimated by a 3D digitizer system and, particularly, the motor cortices corresponding to the upper and lower limb were mapped by using the transcranial magnetic stimulation. EMGs of the muscles were almost similar between overground and treadmill walking. The Oxy-Hb concentration of the sensorimotor cortices increased with both walking modalities. Importantly, the Oxy-Hb responses were larger during overground walking compared to treadmill walking, especially in the sensorimotor hand area. These findings suggest the higher neural activities in the human sensorimotor cortex during overground walking than those during treadmill walking, which may contribute to adjusting the off-balance body in association with the forward movement. (COI:No)

3P-139

Butyrate activates barium-resistant potassium secretion in a dose-dependent manner 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 in regulating ion transport. Recently, I have shown that the effects of 30 mM butyrate application on rat colon with short-circuit current (I_{SC}) measurements. In this study, I revealed that butyrate induces I_{SC} shift after amiloride application (amiloride-resistant I_{SC} shift, referred as $I_{r\text{-}amil}$ shift) toward negative direction in a dose dependent manner. Formate, which has a chemical structure similar to SCFAs, but is not included in SCFAs, showed only a little $I_{r\text{-}amil}$ shift, if any. In addition, after formate or low dose butyrate application 30 mM butyrate showed the same amount of $I_{r\text{-}amil}$ shift in total as control experiments. In other words, $I_{r\text{-}amil}$ shift induced by butyrate application didn't show any desensitization. Bumetanide, a Na^+-K^+-$2Cl^-$ cotransporter inhibitor, recovered $I_{r\text{-}amil}$ shift to zero, thus butyrate might activate electrogenic transport. The direction of $I_{r\text{-}amil}$ shift was matched with cation secretion, but in the preliminary experiments bafilomycin A1, a vacuolar type H^+-ATPase inhibitor, didn't show any effect on $I_{r\text{-}amil}$ shift induced by butyrate as well as 5 mM $BaCl_2$. Thus, $I_{r\text{-}amil}$ shift induced by butyrate might activate not H^+ secretion but K^+ channel resistant to 5 mM $BaCl_2$. (COI:No)

3P-140

Rat in situ-experimental method for evaluating lipid absorption and gut immunology

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Recently, the clinical and experimental studies demonstrated that long-chain fatty acids such as olive oil stimulate regulatory T cells located in the mucosal tissues of small intestine and then control the innate immunology. To elucidate the food-intake dependent relationship between lipid absorption and gut immunology, we developed the in situ-experimental method with rats. In the conscious state, the lipid contained long-chain unsaturated fatty acid (olive oil or cocoa butter and so on) was applied orally to the rat. One hour after the administration of lipid, the tracheal tube was inserted through a tracheotomy under ether anesthesia. Next, continuous isoflurane anesthesia was carried out by a respirator. Physiological saline solution was also perfused constantly (1.0ml/hr) through the cannulated femoral vein. Efferent lymph vessel of the mesenteric lymph node was cannulated under a stereomicroscope, and then, the lymph was collected continuously via the cannula. The lymph was kept into tube every one hour. Measurement of the lymph volume, the number of lymphocytes in the lymph, fatty acid analysis of the collected lymph and flow cytometric analysis of lymphocytes were performed. Fat absorption increased gradually from starting collection of lymph from the rat intestine. The lipid absorption produced simultaneously an increase of B cell subsets in the lymph. (COI:Properly Declared)

3P-141

Anti-stress effects of electro-acupuncture via central oxytocin on gastric emptying in rats

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Electro-acupuncture (EA) has been used to treat gastric disorder. Animal studies have already shown that EA at the ST-36, located on tibialis anterior muscle, have improved stress induced-delayed gastric emptying (GE). On the other hand, central oxytocin attenuates in response of the digestive function to stress. However, it is unknown that central oxytocin is involved in improvement by EA to stress induced-delayed GE. The purpose of this study was to investigate whether EA at ST-36 improves restraint stress-induced alteration in the gastric responses of conscious rats via central oxytocin. Rats were given of solid food after 24-h fasting. Immediately after the ingestion, rats were loaded to restraint stress. Ninety minutes after the feeding, rats were euthanized and gastric content was removed to calculate GE. EA was performed at the bilateral ST-36 throughout the stress loading. To investigate whether central oxytocin was involved in mediating the stress-induced alterations of GE by EA, oxytocin antagonist was administered (i.c.v.) just after the start of restraint stress. GE in the 90 min study period was significantly delayed by restraint stress. This delayed GE was significantly accelerated by EA, which had been disappeared by oxytocin antagonist. In conclusion, central oxytocin is involved in mediating the stimulatory effects of EA on restraint stress-induced delayed gastric emptying. (COI:No)

3P-142

Postnatal development of slow waves in the rat ileocecum

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Motor function of the intestine should fully function after birth to drink milk, mix it with digestive juices and send to anal side. However, mammals at neonatal stages have a much higher incidence of intussusception than the adult. The most frequent site of intussusception is the ileocecum, while this reason remains unknown. Smooth muscle of the small intestine shows slow wave, and this is the electrical basis for contraction. Although the rat is most popular animal model for various diseases, the postnatal development of the slow wave in the ileum near and at the ileocolonic junction has not been studied. We examined this using rats at postnatal days (P) 0-2, 6-8, 13-15, 20-22. The electrical activities were recorded from these regions using glass suction electrodes. At P0-2, the obtained electrical activities were small and irregular, and were often difficult to discriminate from their noise level. In the ileum at about 15-30 mm apart from the ileocecal junction, the slow wave with stable cycle period have been observed at and after P6-8, and was ranged from 4 to 6 s/cycle. On the other hand, the amplitude of the slow wave in the ileum within 10 mm apart from the ileocecal junction was small, and was often difficult to discriminate especially in the rats at younger group. In some preparations at these regions, the large slow waves occurred synchronous with the movement of the cecum. These results suggest that the ileum near the ileocecal junction has not functioned at P0-2 and starts its motor function at and after P6-8. (COI:No).

3P-143

Effects of mulberry tea on starch digestion and glucose absorption in isolated small intestine of mice

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It is well-known that mulberry leaves include 1-deoxynojirimycin (DNJ) which inhibits α-glucosidase activity. So, it is generally said that increase in postprandial blood glucose level is suppressed by taking mulberry products (like mulberry tea (MT)) at the same time because DNJ in MT might inhibit starch digestion and glucose absorption. We are studying about processing methods for various kinds of MT, functionalities of processed MT, and analysis of contents including DNJ in MT. The present study investigated effects of MT on starch digestion and glucose absorption by using everted sacs of isolated small intestine of mice. After incubation of everted sac specimens in Ringer solution (without glucose) including 0.5% soluble starch for 1 hr, glucose concentration at mucosal and serosal sides were measured. From the measured concentrations, amount of generated glucose by digesting from the applied starch per 1g intestinal specimen was calculated. Generated glucose quantity was decreased by application of extracted solution of MT. That is, contents in MT (maybe DNJ and others) inhibited starch digestion. This inhibition could be obtained when DNJ was over some level (about 2μg in test solution) . However, glucose absorption was not inhibited in spite of application of MT. Now, further study for clarifying effects of MT application on glucose transport and absorption are continued by electrophysiological methods like transmural potential measurement and Ussing method. (COI:No)

3P-144

Gut pacemaker activity in the cross-link between IBD and IBS

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During the last several decades the number of patients in inflammatory bowel diseases (IBD) increased dramatically in Japan. This could be considered as a social problem, in relation to the recent westernized dietary habits and severe competitions in business and school lives.
Gastrointestinal motions are caused by spatially organized smooth contractions and relaxations. Formerly, the underlying mechanisms were accounted merely by the cooperated excitatory and inhibitory nervous activity and resultant smooth muscle activity. Lines of recent studies have revealed that interstitial cells of Cajal (ICC) existing in the myenteric plexus act as pacemaker cells. Also, we have previously shown that ICC networks produce reversibly propagating electric activity, parallelly operated with the nervous activity.
In this study, we further communicate the electric features of ICC which are likely to contribute to pathophysiology in IBD, and explain the cross-link between IBD and irritable bowel syndrome (IBS). Our recent microelectrode array measurements using gut muscular preparations fixed below dialysis membranes enabled stable recordings under numerous conditions. For example, the reduced form of glutatione (GSH) suppressed ICC pacemaking electric activity accompanied with reduction of spatial coordination. Also, additional applications of serotonin increased the amplitude of pacemaker potentials, restored the spatial cooperativity. The propagating rate was greater than that even in control conditions. In the light of our previous results, we discuss how ionic channels in the plasma membrane and intracellular stores are modulated. (COI:No)

3P-145

Fermented milk containing *Lactobacillus casei* Shirota prevents onset of physical symptoms under stress condition

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Probiotics are consumed in worldwide and good for our health. However, there is less evidence for a role of probiotics under stress conditions. To investigate effects of a probiotic *Lactobacillus casei* strain Shirota (LcS) on the stress-related dysfunction, a double-blind, placebo-controlled trial was conducted. Of the 47 healthy volunteers enrolled from medical students undertaking a nationwide examination for promotion, 23 and 24 subjects consumed an LcS fermented milk and a placebo milk daily, respectively, for 8 weeks until the day before the examination. Questionnaires revealed that the stress-induced increases in a visual analog scale of stress feeling, total score of abdominal dysfunction were suppressed in the LcS group compared with the placebo group. In addition, number of genes with changes in expression of more than 2-fold in leukocytes was suppressed in the LcS group, and pathway analysis revealed that the administration of LcS suppressed the stress-induced dopamine receptor signaling. Moreover, 16S rRNA metagenomics demonstrated that the LcS group had significantly higher numbers of species in their gut microbiota and a significantly lower percentage of Bacteroidaceae, compared with the placebo group. Our findings indicate that the daily consumption of LcS-fermented milk contributes to relieve the stress-associated physical responses such as abdominal dysfunction in healthy subjects. (COI:Properly Declared)

3P-146

Does Metabolic Heterogeneity in Liver Optimize the Efficiency of Ammonia Elimination? : a Simulation Study

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The liver metabolizes wide variety of chemical compounds. Human liver consists of half a million hepatic lobule in which 5 billion hepatocytes are assembled. In the liver lobule, hepatocytes line along a fenestrated capillary vessel termed sinusoid. The blood from the portral vein and hepatic artery flows into the sinusoid and out to the central vein. This construct is referred to as port-central axis(PCA), which is the smallest repetitive unit of liver metabolism. It is known that there is many heterogeneities of various metabolic aspects in PCA. It is referred to as "metabolic zonation". While many histological facts phenomena of metabolic zonation were reported, their biological significances and/or functions are still unclear. Even the present gene engineering technologies cannot such as conditional gene knockout and/or knockdown are insufficient to regulate metabolic zonation at will. In this study, we tried to elucidate what drives metabolic zonation of liver ammonia metabolism using mathematical model and computer simulation. Mathematical model allows to change arbitrary variable in the model. Various "possible" PCA models were created through virtual evolution using genetic algorithm (GA). We compared the models and estimated which elements play a major role to adapt various selective pressures in PCA. The simulation results suggested that gradual expression of solo or a few enzymes along PCA enhanced the efficiency of ammonia elimination. (COI:No)

3P-147

wx/ae brown rice reduced the fat accumulation and improved dyslipidemia

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A double mutant wx/ae mutant is generated by crossing amylose-free waxy (wx) mutant and amylose-extender (ae) mutant in Kinmaze rice. wx/ae rice includes more resistant starch and γ-Oryzanol than Koshihikari, which have effects of reducing the lipid in the blood. In this study, male C57BL/6J mice aged 8 weeks were fed of chow diet (10% kcal), high-fat diet (HFD:45%kcal), HFD+ Kinmazemai brown rice which is original breed of wx/ae, HFD+ wx/ae brown rice, HFD+starch derived wx/ae for 12 weeks. The powder of brown rice was contained 30%. In the HFD+ wx/ae brown rice group, feces amount is increased to 1.5 times against others. The HFD+ wx/ae brown rice group, cholesterol in the blood is reduced to 50%. The size of fat cells in mice fed HFD+wx/ae was smaller than others. In addition, after feeding HFD for 8 weeks, these mice divided 4 groups and switched to chow diet,HFD+ Kinmazemai brown rice, HFD+ wx/ae brown rice, HFD+ starch derived wx/ae for 4 weeks. The large size of fat cells by feeding HFD became smaller in mice fed HFD+wx/ae, not HFD+ Kinmazemai brown rice and HFD+starch derived wx/ae. These results suggested that wx/ae brown rice has greater effects of the reduction of the fat accumulation and improvement dyslipidemia by the excretion of lipid into the feces. This work was supported by the Promotion Project of Knowledge-based Industrial Clustering of Okinawa prefecture. (COI:No)

3P-148

EID1 suppresses fat accumulation of adipocytes through expression of UCP-1

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White and brown adipocytes both are derived from mesenchymal stem cells.Molecular mechanisms underlying the differentiation of the two kinds of adipocyte remain unknown. A recent study has shown that EP300-interacting inhibitor of differentiation 1 (EID1) reduces the accumulation of triglycerides in pre-adipocyte 3T3-L1 cells. However, little is known about the mechanism of inhibitory effect on fat accumulation of EID1. Here we report that EID1 suppresses fat accumulation of 3T3-L1 cells though the increase of uncoupling protein-1 (UCP-1) expression, a marker of brown adipocytes. DNA of mouse EID1 was cloned by PCR using appropriate primers and mouse genomic DNA library. An expression vector was constructed by cloning the EID1 cDNA into pcDNA3. Cultured pre-adipocytes were stimulated with IBMX, Dexamethasone, Insulin, and Rosiglitazone for induction to differentiated adipocytes. Then, EID1 expression vector was transfected after 3 days of hormonal stimulation. After 9 days of stimulation, differentiation was evaluated by noting the extent of accumulation of lipid droplets. Almost all 3T3-L1 cells induced by hormonal stimulation were observed the accumulation of lipid droplets. In contrast, 70% of 3T3-L1 cells transfected with EID1 expression vector did not differentiate and expression of UCP-1 was elevated. These findings indicate that the EID1 has an important role in regulating adipocyte differentiation through expression of UCP-1. (COI:No)

3P-149

EID1 inhibits adipogenesis by increasing expression of the brown fat-defining marker, PGC-1

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Obesity is a medical condition characterized by increased body weight, specifically adipose tissue, of sufficient magnitude to produce adverse health problems. As one of the two types of adipocytes, a reduction in the amount and/or the ability of triglycerides accumulation of white adipocytes are effective for improvement of obesity. A recent study has shown that EP300-interacting inhibitor of differentiation 1 (EID1) reduces the accumulation of triglycerides in pre-adipocyte 3T3-L1 cells. Here we report that over-expressed EID1 suppresses fat accumulation of 3T3-L1 cells through the increase of peroxisome proliferator-activated receptor-γ coactivators-1 (PGC-1) expression, a marker of brown adipocytes. Cultured pre-adipocytes were stimulated with IBMX, dexamethasone, insulin, and rosiglitazone for induction to differentiated adipocytes. Then, EID1 expression vector was transfected after 3 days of hormonal stimulation. After 9 days of stimulation, differentiation was evaluated by noting the extent of accumulation of lipid droplets, as determined by oil red-O staining. Almost all 3T3-L1 cells induced by hormonal stimulation were observed the accumulation of lipid droplets. In contrast, 70% of 3T3-L1 cells transfected with EID1 expression vector did not differentiate and the expression of PGC-1 was elevated. These findings indicate that the EID1 has an important function in regulating adipocyte differentiation through expression of brown fat genes. (COI:No)

3P-150

UCP2-regulated mitochondrial fission in the ventromedial hypothalamus controls whole body glucose homeostasis

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The ventromedial nucleus of the hypothalamus (VMH) plays a critical role in regulating glucose homeostasis. Uncoupling protein 2 (UCP2) is robustly expressed in the VMH, however its physiological role is ill-defined. Here we report that glucose administration induced mitochondrial fission and reduced reactive oxygen species (ROS) production in the VMH neurons. These changes were UCP2-mediated, since glucose administration to UCP2 knockout mice (Ucp2KO) did not induce mitochondrial fission and increased ROS levels in the VMH, while selective re-expression of UCP2 in SF1-expressing VMH neurons ($Ucp2KOKI^{Sf1}$) restored these processes. Ucp2KO mice displayed impaired glucose metabolism, that was restored in $Ucp2KOKI^{Sf1}$ mice. In support of these roles for VMH UCP2 in glucose metabolism, selective UCP2 overexpression in SF1 neurons ($Ucp2KI^{Sf1}$) significantly increased the number of glucose-excited neurons resulting in improved peripheral glucose metabolism. Finally, silencing of VMH neurons via inhibitory DREADD (AAV-hM4Di-mCherry) reversed the effect of UCP2 overexpression on peripheral glucose metabolism. Taken together, our data unmasked a critical role of UCP2 in mitochondrial dynamics and neuronal activation in the VMH and thus regulates systemic glucose homeostasis. (COI:No)

3P-151

Effects of stress-load on the preference for taste and the locomotor activity of juvenile mice

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Early exposure to isolation-stress has been proposed as a major contributor to physical and/or psychosocial disadvantages in adulthood. We studied whether early stage-stress influences the preference for taste and the locomotor activity of juvenile mice (4 weeks old) or not, using two-bottle preference tests and locomotor activity. We adopted singly-breeding with isolation-stress as Stress(+), and sucrose solution (S) and green tea beverage (T) as two-bottle preference tests. The findings were compared with those obtained from group-breeding mice as Stress(-). Namely, we used the following 4 groups: 1. Stress(-)+S, 2. Stress(-)+T, 3. Stress(+)+S, 4. Stress(+)+T. Locomotor activity was measured as both spontaneous locomotor activity and behavior in elevated cross-maze. Further, fasting blood glucose, oxidative stress and antioxidant capacity also were measured. The isolation-stress induced a significant increase in blood glucose of T groups, but not in S groups. The findings that the stress led to decrease of body weight in both T and S groups suggest that early-life stress influences the growth of animals. However, 2 weeks after the isolation-stress, the increases in body weight were observed in both T and S groups, although there was no significant difference in the food intake volume in both groups, suggesting that this may be due to isolation-stress and sucrose intake. On the other hand, 2 weeks after the isolation, the locomotive distance in closed arm of cross-maze tended to decrease in T group, suggesting the decrease in anxiety, which may mean the effects of green tea. (COI:No)

3P-152

The Effects on Voluntary Exercise of Metabolism-Related Signal Molecules, Ghrelin

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There is an increasing interest to elucidate the molecular mechanisms by which voluntary exercise is regulated. Therefore we examined how exercise motivation regulates via central nervous system. We used SPORTS rats which established in our laboratory as high voluntary exercise model. SPORTS rats showed lower level of serum ghrelin compared with Wister rats, same line as SPORTS rats. Intracerebroventricular (icv) injection of ghrelin decreased wheel running (WR) activity, on the other hand, did not affect the oxygen consumption. In addition, daily injection of ghrelin inhibitor JMV3002 into the lateral ventricle of Wistar rats increased WR activity. Co-administration of ghrelin and obestatin did not show an antagonistic effect on the decreased WR activity caused by ghrelin. These data indicated that ghrelin suppressed voluntary exercise to the same degree as feeding behavior, but that this effect was not inhibited by obestatin, despite the fact that ghrelin-induced food intake were decreased by obestatin. To elucidate exercise regulation site of ghrelin, we made arcuate nucleus destruction model rat using monosodium glutamate (MSG). MSG-treated SPORTS rats did not cancel effect of ghrelin. In conclusion, ghrelin regulates to exercise motivation via central nerve system which is other than hypothalamus. Ghrelin might be the new key molecule for the biological regulation of voluntary exercise. (COI:No)

3P-153

Combined pre-cooling application of fanning and hands and feet water immersion attenuates dehydration and thirst in the heat

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Pre-cooling (i.e., removal of heat from the body immediately prior to exercise) is a popular strategy for alleviating thermal strain. Although whole body water immersion is most commonly used to pre-cool in experimental studies, this method is not always possible in all field settings, or practical. In the present study, we examined the effectiveness of a novel combination of fanning and hands and feet water immersion as practical pre-cooling method on heat strain. Nine males engaged in 60 min of walking at a moderate speed (2.5 km/h) in a hot environment (37°C, 50%RH) while wearing protective clothing. Before walking, they were exposed to fanning (4 m/s) and spraying over the body and to hands and feet water immersion (18°C) for 30 min (FAN+WI). Rectal temperature and heart rate at the end of walking were lower in WI+FAN trial than in the control trial (without the pre-cooling, $p < 0.05$). The attenuations in FAN+WI trial did not differ to that in WI only trial (we have previously reported). Sweat rate in the chest and reduction in body weight were inhibited by WI+FAN, and the inhibitions were greater than in WI only trial ($p < 0.05$). Perceptions by ratings of visual analogue scale (thermal sensation and pleasantness, fatigue, thirst, and damp sensation) were lower in WI+FAN trial than in the control trial ($p < 0.05$). The attenuation in thirst has not been seen in WI only trial. These results suggest that combined pre-cooling of fanning and hands and feet water immersion can inhibit dehydration and thirst more compared to each cooling conducted alone. (COI:No)

3P-154

Novel anthropometry-based calculation of the body heat capacity in the Korean population

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This study compared the previously known body heat capacity (HC) estimating equations and sought how to define HC using simple anthropometric indices. Six hundred participants were randomly selected from a pool of 902 healthy volunteers aged 20 to 70 years for the training set. The remaining 302 participants were used for the test set. Body composition analysis using multi-frequency bioelectrical impedance analysis was used to access body components including body fat, water, protein, and mineral mass. Four different HCs were calculated and compared using a weight-based HC (HC_Eq1), two HCs estimated from fat and fat-free mass (HC_Eq2 and HC_Eq3), and an HC calculated from fat, protein, water, and mineral mass (HC_Eq4). HC_Eq1 generally produced a larger HC than the other HC equations and had a poorer correlation with the other HC equations. HC equations using body composition data were well-correlated to each other. If HC estimated with HC_Eq4 was regarded as a standard, interestingly, the BSA and weight independently contributed to the variation of HC. The model composed of weight, BSA, and gender was able to predict more than a 99% variation of HC_Eq4. Validation analysis on the test set showed a very high satisfactory level of the predictive model. The results suggest that gender, BSA, and weight are the independent factors for calculating HC. Our anthropometry-based equation could be useful for estimating HC in the general Korean population without body-composition measurement (No. 2014M3A9D7034366). (COI:No)

3P-155

Development of GFP-based fluorescent thermosensors to visualize thermogenesis *in vivo*

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Temperature is an essential parameter to maintain the homeostasis of living organism. Subcellular organelles such as mitochondria or ER are suggested to play important roles in thermoregulation. However, the detailed mechanism remains unclear due to the lack of the methods to directly measure the intracellular temperature accurately.
We have previously developed genetically encoded, GFP-based thermosensors (tsGFPs) that can visualize thermogenesis in discrete organelles in living cells. In tsGFPs, a tandem formation of coiled-coil structures of the thermosensing protein TlpA transmits conformational changes to GFP, converting temperature changes into visible and quantifiable fluorescence changes. The length or the stability of the TlpA region is important for the temperature range that the sensor can sensitively detect. Using this sensor, we succeeded to visualize subcellular thermal changes in mitochondria and ER of live cells. Next, we tried to utilize this sensor *in vivo*. To date, a variety of model organisms such as *D.melanogaster* and *C.elegans* has been utilized, most of which grow at around 25°C. Sensors which can detect this temperature region sensitively would be useful for clarifying the mechanism of phenomena in organelles. In this study, we have designed and constructed tsGFP mutants which is most sensitive around 25°C by partially destabilizing the coiled-coil formation. The attempt to visualize thermogenesis *in vivo* will be discussed. (COI:No)

3P-156

Localization of monoacylglycerol lipase in mice brain during fever

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It is well established that fever is evoked by prostaglandin E_2 (PGE_2) that is produced in brain endothelial cells through the cyclooxygenase-2 (COX-2) and microsomal PGE synthase-1 pathway. However, little is known how arachidonic acid (AA), a substrate of COX-2, is supplied to endothelial cells during fever. Recent studies have suggested that 2-arachidonoyl glycerol (2-AG), an endocannabinoid, is the major source of AA leading to fever. This is evidenced by the finding that mice deficient for monoacylglycerol lipase (MGL), the enzyme that hydrolyzes 2-AG into AA and glycerol, do not develop fever with little elevation of brain PGE_2 after intraperitoneal injection of lipopolysaccharide (LPS). In this study, we examined a hypothesis that MGL is colocalized with COX-2 in brain endothelial cells after the LPS challenge. C57Bl6 mice were intraperitoneally injected with LPS or saline, and killed three hours later by CO_2 inhalation. Their brains were freshly frozen and processed for immunohistochemistry for MGL, COX-2 and PECAM, an endothelial marker. COX-2 was expressed in endothelial cells in LPS-injected mice but not saline-injected ones. MGL was expressed in non-endothelial cells juxtaposing to endothelial cells both in LPS- and saline-injected mice. This result does not support the hypothesis that MGL is colocalized with COX-2 in endothelial cells during fever. AA might be supplied to endothelial cells from neighboring cells in a transcellular manner. (COI:No)

3P-157

Role of microsomal prostaglandin E synthase in fever following intracranial hemorrhage in mice

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Fever is common after intracerebral hemorrhage (ICH) though its molecular mechanism is unknown. We previously reported that brain prostaglandin E_2 (PGE_2) level elevated after ICH, and that nonselective cyclooxygenase inhibitors, such as diclofenac and ketoprofen, suppressed ICH-fever, suggesting the involvement of PGE_2 in ICH-fever. In this study, we examined if ICH-fever is mediated by microsomal prostaglandin E synthase-1 (mPGES-1), an enzyme essential for fever under infectious/inflammatory state. ICH was induced in wild-type mice and mPGES-1 deficient mice by injecting collagenase (Type VII) into the preoptic area unilaterally under isoflurane anesthesia. After recovery from anesthesia, their abdominal temperature was measured by a telemetry system. Three hours after the injection of collagenase,they were killed by inhalation of carbon dioxide. Their brains were freshly frozen and sectioned by a cryostat. Brain sections were used for the determinations of ICH area by diaminobenzidine staining, and of PGE_2 content using an enzyme immunoassay kit. Rises in both abdominal temperature and PGE_2 were significantly small in mPGES-1 deficient mice, indicating the involvement of mPGES-1 in PGE_2 synthesis and fever in ICH. However, some of the mPGES-1 deficient mice showed a significant rise in body temperature with less significant rise in PGE_2. In these mice, ICH was large and extended to the other side of the preoptic area. These results suggest that ICH-fever is mediated through both mPGES-1-PGE_2-dependent pathway and PGE_2-independent pathway. (COI:No)

3P-158

Postnatal White to Brown Conversion of Interscapular Adipose Tissue in Syrian Hamster

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Brown adipose tissue (BAT) is a tissue specified for non-shivering thermogenesis. Most mammals possess functional BAT at birth, and it contributes to the maintenance of body temperature. In this study, we investigated the postnatal development of BAT in newborn Syrian hamster, which is known to have low thermoregulatory ability during neonatal period.
Adipose tissue of interscapular depot, where BAT exists in most species, was sampled from 5 to 20 days-old hamsters, and histologically examined. At Day5, tissue was occupied mainly with white adipocytes with unilocular lipid droplets. Clusters of small cells without lipid droplet were scattered at the edge of the tissue. The small cells filled approximately 50% of the tissue at Day 10, and became predominant cellularity at Day14. In contrast, white adipocyte was scarcely observed at Day14. The small cells stored lipid droplet thereafter, and differentiated into brown adipocytes with multilocular lipid droplets. At Day20, BAT formation seemed to be completed in terms of tissue morphology. In accordance with the histological change, mRNA expression of uncoupling protein 1, a specific marker of BAT, was not observed at Day7, and increased with a peak at Day16.
These results demonstrate that BAT develops postnatally in Syrian hamster, possibly accounting for their low ability of thermoregulation. (COI:No)

3P-159

Effects of intranasal insulin administration on thermoregulatory responses during passive heating in humans

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Thermoregulatory responses during passive heating were enhanced with glucose ingestion prior to heating compared with fructose ingestion. We hypothesized that insulin increased by glucose ingestion might enhance thermoregulation responses via central mechanisms. In this study, we assessed the effects of insulin delivery to the brain by intranasal insulin administration on thermoregulatory responses during passive heating in humans.**Methods**: Five healthy male subjects were participated. After fructose solution (75g of fructose with 300 mL of water) was ingested, insulin (Insulin trial, 160 IU/1.6mL in total) or normal saline (Control trial, 1.6 mL in total) was administrated intranasally during 15 min in a random order. Then, the subjects were passively heated by lower legs immersion (42°C of water) for 60 min. Esophageal (T_{es}) and skin temperatures, cardiorespiratory variables, cutaneous blood flow and sweat rate at the forearm and chest were continuously measured, and blood samples were taken.**Results**: There were no effects of insulin on cardiorespiratory variables, blood constituents, and mean skin temperature. T_{es}) was lower, chest sweat rate and sweat loss were higher in Insulin trial than Control trial.**Conclusion**: Intranasal insulin administration enhances thermoregulatory responses in passively heated humans. (COI:No)

3P-160

Evidence for systemic pre-hibernation remodeling in a mammalian hibernator, Syrian golden hamster

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Hibernation is a strategy to conserve energy amongst the harsh conditions of winter. Small mammalian hibernators undergo dramatic changes in body temperature, heart rate, and basal metabolic rate, during the approximately six months of hibernation season. In order to avoid tissue injuries and cell death due to stresses experienced during hibernation, it is assumed that hibernators remodel their bodies and become adapted to hibernation prior to hibernation season. However, little is known about the nature and mechanisms of such adaptive remodeling at the systemic, molecular and cellular levels. To elucidate this point, we utilized Syrian golden hamsters (*Mesocricetus auratus*), whose hibernation can be induced season-independently after prolonged (approximately two to three months) exposure to cold and short photoperiod. We conducted exhaustive gene expression analyses on several organs including liver, white adipose tissues, skeletal muscle and kidney, and identified many genes that were up-regulated or down-regulated prior to hibernation season. These include genes involved in cholesterol biosynthesis and metabolism in livers or in lipid metabolism and synthesis in white adipose tissues. These results indicate that Syrian hamsters indeed undergo adaptive remodeling for hibernation in the pre-hibernation season at the molecular and cellular level. (COI:No)

3P-161

Neuronal phenotype and responsiveness to heat of newborn neurons in the hypothalamus of heat-acclimated rats

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We have reported that constant exposure to moderate heat facilitates progenitor cell proliferation and neuronal differentiation in the hypothalamus of rats. We investigated neural phenotype and responsiveness to heat of hypothalamic newborn cells. Male Wistar rats, 5 weeks old, were subjected to an ambient temperature (Ta) of 32 ° C for 40 days (HE), while control rats (CN) were constantly kept at a Ta of 24 ° C. Bromodeoxyuridine (BrdU) was intraperitoneally injected daily for 5 consecutive days (50 mg/kg/day) after commencing heat exposure. After the end of heat exposure period, all rats were kept at a Ta of 24 ° C for 48 h, and subjected to a heat exposure test to estimate heat tolerance level. After the test, brain was removed for immunohistochemical analyses. Heat tolerance in HE were improved. In HE, approximately 15% of newborn cells in the hypothalamus were stained by GAD67, a GABAergic neuron marker, or EAAC1, a glutamatergic neuron marker. Also, most of newborn cells in the hypothalamus were immunolabeled with c-Fos antibody, a neural activation marker. Moreover, administration of mitosis inhibitor, cytosine-arabinoside, interfered with improvement of the intensity of heat tolerance in HE. Changes of thermoregulatory function in long-term heat-acclimated rats may be in part attributable to generations of functional neurons in the hypothalamus. (COI:No)

3P-162

Effects of high-fat diet and perinatal dioxin exposure on development of body size and expression of PDGF receptor β in the rat brain

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High fat (HF) diets, dioxin exposure and platelet-derived growth factor (PDGF) receptor β (PDGFR-β) in the brain affect feeding behavior, which is an important determinant of body growth. In the present study, the effects of prenatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and HF food after weaning on body growth and expression of PDGFR-β in the brain were investigated in rat pups. TCDD (1.0 μg/kg) was orally administrated to dams on the 15th gestational day. Body size parameters were measured daily, and expression of PDGFR-β was assessed by western blotting using samples from brain areas. Results indicated that consumption of the HF diet decreased PDGFR-β levels in the amygdala and hippocampus in both sexes compared to the control group, while TCDD decreased PDGFR-β levels in the amygdala and striatum only in females receiving a HF diet. Furthermore, PDGFR-β levels in the hippocampus and striatum were negatively correlated with increases in body length, while those in the amygdala and NAc were related to body weight gain or body mass index. Together with findings from previous studies, the results suggest that the changes in body growth and brain functions due to dioxin and HF diets may be partially mediated by changes in PDGFR-β levels. (COI:No)

3P-163

Phosphorylation of signal transduction and activator of transcription 5 (STAT5) in the mouse liver involves high-fat diet (HFD)-induced obesity and glucose intolerance via platelet-derived growth factor receptor alpha (PDGFRα)

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Previously, we reported the possible involvement of PDGFR α -mediated signals in the regulation of feeding status. On the other hands, it is widely used HFD feeding to induce the obesity since this feeding is similar to western life style. In the present study, we examined whether HFD (45 kcal% fat, D12451 Research diets) affected the PDGFR α expression or not in male mice liver. C57BL/6 mice were fed chow or HFD ad libitum. After 4 weeks of diet feeding, mice were treated with imatinib, an inhibitor of PDGFR and used for treating certain types of cancer, for additional 4 weeks. Control mice were treated with saline. We confirmed that HFD resulted in an increasing body weight and developed glucose resistance. HFD increased the expression of PDGF-A mRNA without effect of imatinib. PDGF-C mRNA was not changed by diet. Imatinib treatment did not decreased body weight in chow fed groups. However, imatinib treatment partially but significantly prevented HFD-induced obesity and worsened glucose tolerance. These changes were well correlated with changes in STAT5 phosphorylation in the liver: HFD significantly and markedly increased the phosphorylation of STAT5 and this increase was prevented by imatinib treatment. We suggest from the present study that HFD induces an inflammation reaction as a result of STAT5 phosphorylation via PDGFR α signals. (COI:No)

3P-164

Estradiol administration modulates thermoregulatory responses induced by application of menthol to the skin of whole trunk in ovariectomized rats

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INTRODUCTION Rats place their tails underneath their body trunks in the cold (tail-hiding behavior), which may be a thermoregulatory behavior. Transient receptor potential melastatin-8 (TRPM8) is identified in sensory neurons as low-temperature and menthol-activated cation channel. The aim of the present study was to test the effect of estradiol (E_2) on thermoregulatory responses mediated TRPM8 in the cold. **METHODS** Ovariectomized rats were implanted a silastic tube with or without E_2 (22.3mg) underneath the dorsal skin (E_2(-)) and E_2(+) groups), and applied 10% menthol or ethanol (menthol and vehicle subgroups) to skin of whole trunk, and exposed to 27°C or 16°C for 2-h with continuous body temperature (T_b), tail skin temperature (T_{tail}), and tail-hiding behavior measurements. **RESULTS** T_b in the menthol subgroup was higher than in the vehicle. T_{tail} in the menthol subgroup was higher at 27°C and lower at 16°C than in the vehicle. Only in the menthol subgroup at 27°C, T_b and T_{tail} in the E_2(-) were greater than in the E_2(+). The duration of tail-hiding behavior in the menthol subgroup was lower than in the vehicle in the E_2(+) at 27°C. **CONCLUSION** E_2 might suppress the elevation of T_b and T_{tail} induced by application of menthol in ovariectomized rats at 27°C. (COI:No)

3P-165

The ubiquitin E3 ligase TRIM23 regulates adipocyte differentiation via stabilization of the adipogenic activator PPARγ

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Adipocyte differentiation is a strictly controlled process regulated by a series of transcriptional activators. Adipogenic signals activate early adipogenic activators and facilitate the transient formation of early enhanceosomes at target genes. These enhancer regions are subsequently inherited by the late enhanceosomes. PPARγ is one of late adipogenic activators and is known as a master regulator of adipogenesis. However, the factors that regulate PPARγ expression remain to be elucidated. Here, we show that a novel ubiquitin E3 ligase, tripartite motif protein 23 (TRIM23), stabilizes PPARγ protein and mediates M1 and K27-linked atypical polyubiquitin conjugation. TRIM23 knockdown caused a marked decrease in PPARγ protein abundance during preadipocyte differentiation, resulting in a severe defect in late adipogenic differentiation, whereas it did not affect the formation of early enhanceosomes. Our results suggest that TRIM23 plays a critical role in the switching from early to late adipogenic enhanceosomes by stabilizing PPARγ protein via M1 and K27-linked atypical polyubiquitin conjugation. (COI:No)

3P-167

Stimulation to the oral cavity increases diet-induced thermogenesis

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The present study examined the effect of stimulation to the oral cavity on diet-induced thermogenesis (DIT). Eleven healthy normal-weight males (mean±SD: age, 23±1 years; height, 176±4 cm; body mass, 68±5 kg; BMI, 22±1 kg/m2) participated in three trials with a randomized crossover design. A 200-mL cocoa-flavored drink (200 kcal) was divided into ten 20-mL cups. After the baseline measurement for 20 min in the overnight fasting state, subjects swallowed the ten 20-mL test drinks for 5 min in three manners on different days. In the control trial (C trial), subjects swallowed one 20-mL test drink immediately every 30 s. In the long-duration stimulation trial (D trial), subjects kept the 20-mL test drink in their mouth for 30 s without mastication and then swallowed it. In the masticatory stimulation trial (M trial), subjects masticated the 20-mL test drink for 30 s (at a masticatory frequency of once per second) and then swallowed. Oxygen uptake was measured until 90 min after swallowing the test drink and converted into energy expenditure. DIT was calculated from the body mass and increments of energy expenditure after swallowing the test drink above the baseline obtained before each trial. DIT accumulated over 90 min after the test drink was the greatest in the M trial than in the C and D trials (mean±SEM: C trial, 43±5 cal/kg; D trial, 75±9 cal/kg; M trial, 107±12 cal/kg). The result indicated that stimulation to the oral cavity enhanced DIT, implying that the stimulation can be helpful for weight management by increasing DIT (COI:No).

3P-168

Development of stable induction method of torpor in mice

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Some mammals leverage active hypometabolism to survive severe environmental stress such as cold climate or food shortage. The reduced basal metabolic rate during active hypometabolism is very attractive for implementing hypometabolic medicine such as patient transportation or organ stock, although the mechanism of torpor/hibernation is totally unknown. We, therefore, would like to understand the mechanism of active hypometabolism to realize hypometabolic medicine. To investigate the mechanism of torpor, we are using mouse, which can enter daily torpor when exposed to a certain ambient temperature and food restriction. Although past studies report several methods to induce torpor in mice, they differ in strains, ambient temperature, light condition, and in the timing of food restriction. To carry torpor research forward, we are trying to find the optimal method for stable and efficient induction of torpor. When torpor is induced by food restriction from the beginning of light phase, in C57BL/6NJcl, the minimal metabolic rate (oxygen consumption) during active phase can be reduced from 3.06 ± 0.30 ml/g/h to 0.96 ± 0.28 ml/g/h while body temperature changed from 35.42 ± 0.33 °C to 25.65 ± 1.11 °C. (COI:No)

3P-169

Association of the gustatory salivation with body mass index and Epworth sleepiness scale (ESS) score

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We aimed to evaluate the association between gustatory salivation (GS) and body mass index (BMI) among healthy young individuals. We also aimed to evaluate the relationship between GS and Epworth sleepiness scale (ESS) score. The BMI and ESS score of 26 healthy young individuals (mean age: 26.0 years; age range: 20-38) were measured using a stadiometer, weight scale, and self-completed questionnaire. GS with 3 prototypical tastants (PTs: sucrose, acetic acid, and NaCl) was used as the control. GS with fat stimulation was also evaluated using oleic acid (OA) in non-fat milk (0-160 mM). The rates of salivation during resting and gum-chewing states were also measured. All participants showed normal rates of salivation. No association was observed between the BMI and the amount of PT-induced salivation. However, the amount of OA-induced salivation was associated with the BMI. Moreover, the amount of salivation induced by NaCl, but not the other PTs or OA, was associated with the ESS score. These results suggest that a BMI-dependent mechanism regulates fatty-acid-induced salivation. In addition, a part of the regulatory mechanism for GS reflex might be closely related to the impairment of the respiratory control mechanism during sleep. (COI:No)

3P-170

Antinociceptive effect of transcutaneous electrical nerve stimulation via an opioid mechanism in rats with adjuvant arthritis (part II)

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We previously reported that the antinociceptive effect of low frequency (4 Hz) transcutaneous electrical nerve stimulation (TENS) on chronic inflammatory pain is inhibited by the administration of the μ-opioid receptor antagonist naloxone in rats with adjuvant arthritis (AA). The present study was performed to investigate whether TENS may promote the release of β-endorphin, which is the endogenous ligand of the μ-opioid receptor.
Intact rats were randomly divided into three groups: a control group, a low frequency (4 Hz) TENS group and a high frequency (100 Hz) TENS group. TENS (4 Hz or 100 Hz, 30 min) was applied to both hindpaws of the rat in a single session. The plasma level of β-endorphin was measured immediately after the TENS treatment. A significant increase was observed in the level of β-endorphin in the low frequency TENS group.
Next, rats were divided into four groups: a control group, an AA group, an AA+TENS group, and an AA+TENS+naloxone group. Arthritis was induced by an intraplantar injection of complete Freund's adjuvant into the right hindpaw. TENS (4 Hz, 30 min, three times a week, for two weeks) was applied. The levels of β-endorphins in the plasma samples were analyzed on day 14. There was a significant decrease in the AA group; however, the decrease was not observed in the AA+TENS and the AA+TENS+naloxone groups.
These results suggest that the secretion of β-endorphin was involved in the analgesic effect of low frequency TENS. (COI:No)

3P-171

Effects of noradrenaline and adrenergic agonists on microglial cells in culture

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Rat microglial cells express adrenergic &aipha;1, α2, and β2 receptors, and noradrenaline (NA) has been said to exert immunosuppressive effects on microglial cells mainly through β2 receptor that elevated intracellular cAMP level. However, we have also showed that an α1 agonist phenylephrine (Phe) also exert suppressive effects on activated microglial cells treated with Toll-like receptor (TLR) 4 ligand LPS as strong as a β2 agonist terbutaline (Ter). The suppressive effects of NA, Phe and Ter may be correlated with the suppression of LPS-induced NFkB translocation into nuclei. In this study, we further investigated the effects of adrenergic agonists on microglial cells incubated with pIC, a ligand for TLR3. pIC did not cause NFkB translocation into nuclei and significant nitric oxide release in contrast to LPS. However, pIC induced phosphorylation of STAT1 and upregulated expression of IRF1 at 70 ~ 90 min after addition of pIC into microglial culture. When incubated with adrenergic agonists and pIC, the agonists induced STAT3 phosphorylation at 30 min after addition of the agents. The agonists suppressed pIC-induced IRF1 expression and STAT1 phosphorylation. There were no significant distinctions in the effects of NA, Phe and Ter on the pIC-treated microglial cells. Furthermore, NA, Phe and Ter similarly induced phosphorylation of p38 and ERK. These results suggest a possibility that NA may act on microglial cells independently of adrenergic receptors. (COI:No)

3P-172

Analysis of ADAMTS9/GON-1 function in insulin secretory cells and peripheral tissues

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ADAMTS9 is a metalloprotease that cleaves components of the extracellular matrix and is also implicated in transport from the ER to the Golgi. It has been reported that an ADAMTS9 gene variant is associated with type 2 diabetes. However, the molecular mechanisms underlying ADAMTS9 function in beta cells and peripheral tissues are unknown. First, We investigated whether GON-1, the *C.elegans* homolog of ADAMTS9, is involved in the insulin secretion and insulin signaling in *C.elegans*. We found that both insulin secretion and the insulin-signaling pathway are compromised by GON-1 depletion. These defects in *gon-1* mutants were restored by GON domain expression. The GON domain is present in the most C-terminal region in the ADAMTS9/GON-1. These data suggest that the GON domain alone, but not the protease domain, is required for insulin secretion and signaling. Next, we investigated whether ADAMTS9 is involved in the insulin secretion and insulin-stimulated glucose uptake by using mammalian cell lines. Glucose-stimulated insulin secretion was compromised after depletion of ADAMTS9 in the INS-1 cells, a glucose-sensitive pancreatic beta cell line. Depletion of ADAMTS9 decreased insulin-stimulated glucose uptake in differentiated 3T3-L1-derived adipocytes. Our data suggest that ADAMTS9/GON-1 is involved in both mechanisms; insulin secretion from insulin secretory cells and insulin signaling at the peripheral tissues. (COI:No)

3P-173

Nitric oxide-induced calcium release: *S*-nitrosylation of the type 1 ryanodine receptor and neuronal cell death

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Nitric oxide (NO) is an important signaling molecule and is implicated with neurodegenerative diseases. Protein post-translational modification by NO, *S*-nitrosylation, regulates signal transduction pathways. We recently identified that NO induces Ca^{2+} release from the endoplasmic reticulum through an *S*-nitrosylation of Cys-3636 in the type 1 ryanodine receptor (RyR1) channel in central neurons. To investigate the pathophysiological significance of NO-induced Ca^{2+} release (NICR) in the brain, we generated a Cys-3636 to Ala knock-in mutation in the mouse *Ryr1* locus ($Ryr1^{C3636A}$). We found that NICR was abolished in primary cultured neurons from the $Ryr1^{C3636A}$ mice. NO-induced neuronal cell death was milder in the $Ryr1^{C3636A}$ neurons compared with wild-type neurons. Furthermore, kainate-induced neuronal cell death in hippocampus was significantly reduced in the $Ryr1^{C3636A}$ mice. Thus, RyR1 may be a candidate for the treatment of neurodegeneration in epilepsy. (COI:No)

3P-174

Progressive abnormalities in short- and long-term synaptic plasticity and dendritic Ca^{2+} signals in spinocerebellar ataxia type 1 (SCA1) model mice

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Spinocerebellar ataxia type 1 (SCA1) is an inherited progressive neurodegenerative disease and exhibits cerebellar ataxia and atrophy of Purkinje cells (PCs). Previous studies indicated that SCA1 is related to downregulation of the metabotropic glutamate receptor type 1 (mGluR1), which is indispensable for synaptic plasticity and motor coordination. However, it remains unknown which synaptic defect contributes to SCA1 pathology in the cerebellum. In this study, we report that SCA1 model mice develop progressive synaptic abnormalities at cerebellar parallel fiber (PF)-PC synapses. SCA1 mice older but not younger than 5 weeks, showed loss of endocannabinoid-mediated retrograde short-term synaptic plasticity and lack of long-term synaptic depression (LTD). These types of plasticity are known to be dependent on mGluR1-mediated Ca signals in PC dendrites. Our fast confocal Ca imaging revealed that mGluR-mediated dendritic Ca signals are progressively impaired in age-matched SCA1 PCs. Moreover, reduction in the Ca signals were correlated with small but substantial progressive PC atrophy detected by measuring the PC membrane capacitance. These results suggest that reduced dendritic mGluR1-mediated Ca signals and consequent loss of synaptic plasticity may underlie cerebellar ataxia in SCA1 and that progressive impairment of dendritic mGluR1-mediated Ca signals might accompany slight progressive PC atrophy in SCA1. (COI:No)

3P-175

Chronic restraint stress triggers the dopaminergic and noradrenergic neurodegeneration-possible role of chronic stress in the onset of Parkinson's disease

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Department of Physiology, Nippon Medical School, Tokyo, JapanParkinson disease (PD) is a neurodegenerative disease characterized by loss of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc) and, to a lesser extent, in the noradrenergic neurons of the locus coeruleus (LC). The majority of PD cases are idiopathic and sporadic and are believed to be the result of both environmental and genetic factors. Here, we report the first evidence that chronic restraint stress (8 hr/day, 5 days/week) in the rat substantially reduces the nigral dopaminergic and locus coeruleus noradrenergic neuronal cells. Loss of DA SNpc neurons was evident after 2 weeks of stress and progressed in a time dependent manner reaching 61% at 16 weeks. This reduction was accompanied by robust microglial activation and oxidative stress, marked by nitrotyrosine in the SNpc and LC in the midbrain. These results indicate that chronic stress may trigger the dopaminergic and noradrenergic neurodegeneration by virtue of increased oxidative stress, and that activated microglia, detected in the SN and LC, may play an important role in modulating the neurotoxic effects of oxidative stress. Thus, our findings suggest that exposure to chronic stress itself may trigger the dopaminergic and noradrenergic neurodegeneration, a cause of PD. (COI:No)

3P-176

The dynamics of blood circulation and high energy phosphates in ischemia/reperfusion injury in mouse brain

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Objectives To observe the dynamics of high energy phosphates, mice were exposed to high +Gz acceleration to induce cerebral ischemia/reperfusion injury, using the centrifugal acceleration device.Methods Nineteen mice (C57BL/6NCrSlc, BW 24.4 ± 1.1 g) were randomly assigned to the three groups; control (Con, n = 6), cerebral ischemia (CI, n = 9) and cerebral ischemia/reperfusion (CIR, n = 4). The brain was in-situ frozen before (Con), right after (CI) or 5 min after (CIR) the +8 Gz acceleration for 30 s. Serial coronal sections (8-μm) were made with a cryomicrotome and thaw-mounted on ITO-coated glass slides. Imaging mass spectrometric analysis was performed by a MALDI TOF/MS (AXIMA, Shimadzu, Kyoto). Energy charge (EC) was calculated as indices of energy states of brain.Results and Conclusion High +Gz acceleration decreased cerebral blood flow (CBF) and heart rate (HR) to 15% and 35% of the baseline, respectively. CBF and HR recovered to the baseline levels soon after the device stopped. But EC did not recovered 5 min after the reperfusion. No recovery was observed in high energy phosphates 5 min after the cessation of +Gz acceleration when CBF had already recovered to the baseline level. These might indicate possible delayed recovery of energy metabolism of brain tissue after cerebral ischemia. (COI:No)

3P-177

Role of tissue plasminogen activator/ plasmin system on the recovery of neurological dysfunction and histological repair in ischemic stroke in mice.

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The fibrinolysis system is an extracellular protease cascade to degrade clots and extracellular proteins.Plasminogen (Plg), a fibrinolysis system component, is converted to plasmin by plasminogen activators, such as tissue-type plasminogen activator (tPA). Recently, we reported the importance of tPA on the recovery of the neurological dysfunction and the repair of the brain injury after the ischemic stroke by using tPA gene deficient mice. Science it is known that Plg and tPA are participated in the plasticity of the neural network, we hypothesis that tPA/Plg system might promote the functional recovery and histological repair after the ischemic stroke. Therefore, we studied the neurogical recovery on Plg gene deficient (KO) mice and the wild type (WT) mice on three behavioral assessment with histological analysis. It was found that the functional recovery after the ischemic stroke was significantly delayed in Plg KO mice than Plg WT mice. In addition, the reduction of damage size was significantly delayed in Plg KO mice. Relating, accumulation of microglia at injury periphery was significantly less in Plg KO mice. These findings suggested that Plg participated in the recovery of neuronal dysfunction and histological repair via accumulation of microglia. Considering with the importance of tPA, on the recovery of neurological dysfunction and histological repair, tPA is likely via activation of Plg on the process. COI:Properly Declared

3P-178

Mechanisms of amiodarone-induced schwannopathy and myelinopathy

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Amiodarone hydrochloride (AMD), an anti-arrhythmic agent, has been shown to cause peripheral neuropathy; however, its pathogenesis remains unknown. We examined the toxic effects of AMD on an immortalized adult Fischer rat Schwann cell line, IFRS1, and cocultures of IFRS1 cells and adult rat dorsal root ganglion neurons or nerve growth factor-primed PC12 cells. Treatment with AMD (1, 5, and 10 μM) induced time- and dose-dependent cell death, accumulation of phospholipids and neutral lipids, upregulation of the expression of gangliosides and lectin molecules, and oxidative stress (increased Nrf2 in nuclear extracts, reduced GSH/GSSG ratios, and upregulated 4-hydroxynonenal expression) in IFRS1 cells. It also induced the upregulation of LC3-II and p62 expression, with phosphorylation of P62, suggesting that deficient autolysosomal degradation is involved in AMD-induced IFRS1 cell death. Furthermore, treatment of the cocultures with AMD induced detachment of IFRS1 cells from neurite networks in a time- and dose-dependent manner. These findings suggest that AMD-induced lysosomal storage accompanied by enhanced oxidative stress and impaired lysosomal degradation in Schwann cells might be a cause of demyelination in the peripheral nervous system. (COI:No)

3P-179

Expression of oxytocin receptor in ischemic lesions of rat brain: protection of oxytocin for injured neurons

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Oxytocin is a nonapeptide produced in the paraventricular and supraoptic nuclei of the hypothalamus, which is released during physical contact. Exogenous oxytocin administration alleviates tissue damage in a variety of animal models of injury. However, the action mechanism of oxytocin in the cerebral infarctions remains unclear. In the present study, we evaluated the spatial and time-dependent changes of oxytocin receptor expression on a transient middle cerebral artery occlusion (MCAO) model of rats. Rats subjected to MCAO were deeply anesthetized with carbon dioxide at 1, 2, 3, 5, 7, and 14 days postreperfusion (dpr). Tissues from the cerebral cortex representing the three regions: the ischemic core, the peri-infarct tissue and the contralateral cortex were dissected out. Oxytocin receptor mRNA expression peaked at 1 dpr in the peri-infarct tissue, where many neurons undergo degeneration. These degenerated cells including neurons were found to express oxytocin receptor. Recent reports show that oxytocin promotes recovery of injured neurons. It is critical for better prognosis following stroke to rescue damaged neurons in this region during the subacute phase. Thus, these findings suggest it may be the most effective to treat oxytocin within 1 day of stroke onset. (COI:No)

3P-180

Identification of oxLDL blocking protein and its anti-atherogenic function

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Background: Oxidized LDL (oxLDL) is crucial factor to promote atherosclerosis. Through binding to scavenger receptors, oxLDL induces endothelial dysfunction, causing inflammation and lipid accumulation, and subsequent foam cell formation.Aim: To identify and characterize protective factors against oxLDL.Methods and Results: We found that endothelium-derived secreting protein Del-1 (developmental endothelial locus-1) bound to oxLDL but not to native LDL in a cell-free assay. Del-1 inhibited the uptake of DiI-labeled oxLDL (DiI-oxLDL) by oxLDL receptors, such as LOX-1, SR-A, CD36, and SR-BI. We also found that Del-1 inhibited DiI-oxLDL uptake by cultured human endothelial cells (HCAEC) and THP-1-deribed macrophages. Furthermore, Del-1 suppressed oxLDL-dependent signal transduction. To examine in vivo effects of Del-1 on atherogenesis, we established Del-1 transgenic mice (Del-1Tg), and fed high-fat diet to male Del-1Tg and wild-type mice (WT) for 20 weeks from the age of 24 weeks. Plasma oxLDL activity was significantly decreased in Del-1Tg compared with WT mice ($P<0.05$), while other lipid parameters except triglycerides were not different. Futhermore, Oil red O-positive atheromatous area at aortic roots dramatically decreased in Del-l Tg compared with WT ($P<0.001$).Conclusion: Disrupted balance among modified LDL, scavenger receptors, and protective molecules might lead to the progression of atherosclerosis. (COI:No)

3P-181

Possible role of the endocannabinoid 2-arachidonoylglycerol in autism-like behavior in mice

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The endocannabinoid system is important for the modulatory of neural circuit activity and has recently been implicated in the pathophysiology of ASD. However, the mechanisms of how the endocannabinoid system is involved in ASD are largely unknown. To address this issue, we analyzed the mice deficient in diacylglycerol lipase (DGLα), the enzyme which synthesizes the endocannabinoid 2-arachidonoylglycerol (2-AG) in the brain. We found that DGLα KO mice showed impaired sociality, lower behavioral flexibility, abnormal pup communication and susceptibility to audiogenic seizures. Moreover, we found change in 5-HT release and re-uptake as well as hypofunction of 5-HT 2A receptor in DGLα KO mice. Impaired sociality of DGLα KO mice was partly rescued by administration of 5-HT 2A receptor agonist, suggesting that 2-AG influences social behavior through the 5-HT system. Next we tested the effect of JZL184, an inhibitor of monoacylglycerol lipase (MGL) that hydrolizes 2-AG. Acute treatment of JZL184 increased sociality, reduced 5-HT release in the prefrontal cortex, but did not modify 5-HT 2A receptor function. On the other hand, chronic treatment of JZL184 increased sociality and enhanced 5-HT 2A receptor function. In line with the effects of JZL184, we found that MGL heterozygous KO mice exhibited enhanced sociality and 5-HT 2A receptor function. Taken together, these results suggest that the 2-AG system could be involved in the pathophysiology of ASD through modulation of the 5-HT system. (COI:No)

3P-182

Renal involvement in the pathogenesis of mineral and bone disorder in dystrophin-deficient mdx mice

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Duchenne muscular dystrophy (DMD) is a most severe muscular disorder, which often complicated with osteoporosis with advancement of the disease. Recently, renal dysfunction is reported as a common complication in the advanced stage of DMD patients. We aimed to clarify involvement of renal function in the pathogenesis of mineral and bone disorder (MBD) in mdx mice, a murine model of DMD. We revealed impaired renal function in mdx mice. Elevated serum calcium and deceased activity levels in mdx mice suggest that involvement of immobilization plays a role in pathogenesis of hypercalcemia. Urinary volume and hematocrit were significantly increased, suggesting that hypercalcemia and dehydration partly triggered renal dysfunction in mdx mice. We also examined the effects of high phosphorus (HP) diet on MBD of the model animal. Bone mineral density and bone integrities of the tibia in mdx mice were even worsen by dietary phosphorus in a dose-dependent manner. Blood levels of phosphate and parathyroid hormone (PTH) were significantly increased in mdx mice by dietary phosphorus in a dose-dependent manner, which were not observed in wild-type mice. Despite of significantly increase in PTH levels, bone alkaline phosphatase levels were significantly lower in HP-fed mdx mice, indicating that renal-PTH resistance induced uncoupling of bone formation and resorption in mdx mice. These results designate that renal dysfunction is a potential factor of MBD in DMD. (COI:No)

3P-183

Accumulation of adventitial adipocytes develops abdominal aortic aneurysm

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Abdominal aortic aneurysm (AAA) is a common cardiovascular disease among the elderly. The molecular mechanisms that underlie the pathogenesis of AAA remain unknown. We utilized comprehensive matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) of intraoperative dissected human AAA samples to visualize compositional changes in cellular metabolites related to AAA. We studied 30 patients who underwent elective open surgery to repair infrarenal AAAs. The localization of each lipid molecule in the aortic wall was assessed by MALDI-IMS. MALDI-IMS revealed a characteristic distribution of triglyceride (TG) specifically in the aneurismal adventitia of distended aorta. Pathological analysis revealed that characteristic TG distribution was derived from ectopic appearance of adipocytes in adventitia. Accumulated adipocytes were found to locally produce proinflammatory cytokines and matrix metalloproteinases, with subsequent disruption of the local collagen network. PPAR&gamma2 was expressed in not only these adipocytes, but also in fibroblast-like cell marker positive cells. We propose that ectopic adventitial PPAR&gamma2 expression in fibroblast-like cells contributes to mechanical weakening of the AAA wall via two pathways. One is transformation from fibroblast-like cells into adipocytes. The other is the decrease of collagen production. (COI:No)

3P-184

Influence of Periostin on Synovial Fibroblasts in Knee Osteoarthritis

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Osteoarthritis (OA) is a slowly progressive degenerative joint disease characterized by joint space narrowing, osteophyte formation, and subchondral sclerosis. Despite extensive efforts, actual breakthroughs in the identification of biochemical biomarkers of OA have been limited. Therefore, we investigated in vivo periostin production in knee synovial fluid of OA patients and assessed its influence on the extracellular matrix using synovial fibroblasts in vitro. The study population included 40 OA patients (mean age, 75.3 ± 6.6 years) who were classified according to the Kellgren-Lawrence system. Our results showed that periostin and IL-13 levels were upregulated along with progression of OA. A second round of in vitro experiments using human fibroblast-like synoviocytes suggested that elevated periostin mediated an increase in matrix metalloproteinase-9, which is an important molecule in bone turnover. Taken together, these observations indicate that periostin may be a useful diagnostic and/or prognostic marker of OA. (COI:No)

3P-185

An evaluation of neutrophil activity against MRSA infection in temporally neutropenic lys-EGFP mice

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In this study, we evaluated the activity of neutrophils against methicillin resistant Staphylococcus aureus(MRSA) using an animal model.To monitor the activities of neutrophils and MRSA colonization, we used lys-EGFP C57BL/6 mice and bioluminescent MRSA(Xen31,PerkinElmer).The mice were at first rendered to a temporally neutropenic state using cyclophosphamide(CPM) treatment (300mg/kg or 375mg/kg).We subcutaneously injected bioluminescent MRSA into the dorsal skin of the lys-EGFP mice.Then, the mice were divided into a group of treated saline for 3 days(control group) and vancomycin(66mg/kg,treated group) for 3 days.To visualize the infectious lesion, we used an in vivo imaging system(LAS-4000,GE).The results showed that vancomycin has the capacity to kill bacterial cells and promoting inflammation.In addition, the regeneration of neutrophils in CPM treated mice were dependent on the dose of CPM.We could conclude that vancomycin induces inflammation but this effect is only seen when the immune system is substantially suppressed. (COI:No)

3P-186

Dynamism of the hypothalamic transcriptome uncovers "stages" during the development of diet-induced obesity in mice

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Hypothalamus plays a central role in energy homeostasis. Although high-fat diet (HFD)-provoked hypothalamic injury is implicated in obesity, precise molecular mechanisms remain unveiled.

arcuate(ARC), paraventricular(PVN), and lateral(LH) nuclei were micro-dissected from mice fed either HFD or control(CD) for 3days, 2, 6 and 16weeks, and transcripts analyzed by RNAseq (n=3). Transcripts statistically altered 1.5 fold by HFD were defined as "altered transcripts(ATs)". (1)Numbers of ATs, (2)Clustering of ATs, (3)Gene ontology (GO) assigned to each cluster, (4)Upstream regulators (UR) by IPA are shown.

(1)The number of ATs peaked at 6weeks in ARC and LH, whereas at 3days in PVN. (2)ATs in every nucleus was clustered into 5 groups: one with commonly downregulated transcripts throughout four time points, and the other four groups each consisting of transcripts specifically upregulated at only one time point. (3)GO terms assigned to each cluster were unique to the nucleus/time point combination. (4)3 nuclei shared URs associated with proliferation on the 3rd day, but shared URs associated with inflammation in 16weeks.

We here propose a concept of "stages" of obesity defined by a specific group of upregulated hypothalamic transcripts corresponding to a specific set of cellular locations or functions. Although responses to HFD differed among the nuclei, proliferation and inflammation signified common early and late stages of obesity. (COI:Properly Declared)

3P-187

Neural network remodeling underlying motor map reorganization induced by rehabilitative therapy after stroke

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Back groundAfter brain injury, survived neuronal cells complement impaired functions by remodeling the neural network. Previous study has shown that motor map reorganization is a form of neural network remodeling, which plays important role for functional recovery. Despite many studies have demonstrated rehabilitative therapy enhances functional recovery and motor map reorganization, the neural network remodeling underlying rehabilitative therapy induced recovery is unknown.MethodsRats were assigned to control, photothrombosis (PT) or PT + rehabilitative therapy group. After 4weeks recovery period, anterograde tracer, biotinylated dextran amine (BDA) was injected into rostral forelimb area (RFA). The spinal cord sections ranging C2 - T1 were prepared and the number of CST fiber in dorsal column (DC fiber) and the length of CST collateral fiber were quantified.ResultsIn comparison between control rats and PT rats, any significant differences were not observed both in DC fiber and collateral fiber. However, PT + rehabilitative therapy group showed significantly increased number of DC fiber and the length of collateral fiber than control and PT groups. ConclusionOur results indicate that rehabilitation therapy enhance sprouting of CST axon in spinal cord. These anatomical changes in spinal cord may contribute to functional recovery and motor map reorganization induced by rehabilitative therapy after stroke. (COI:No)

3P-188

Effect of exercise on the motor recovery and neurogenesis in rats with motor cortex infarct

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Although exercise therapy in rehabilitation promotes motor functional recovery after cerebral infarction, little is known about the neurological mechanisms. We hypothesize that the exercise may induce neurogenesis to reconstruct the neuronal circuit damaged by infarction. To address this, we established rat model of motor cortex infarction using photochemically-induced thrombosis (PIT) method and have examined the effect of exercise on neurogenesis and functional recovery after the localized infarction. Previously, we showed that the newly born cells after the infarction were still observed periinfarct area in 4 weeks by BrdU labeling experiments. Although treadmill exercise seemed to promote the growth, functional recovery was difficult to detect because of mild severity. Thus, we have tried to find optimal methods to evaluate motor function. We found that beam-walk test was useful to detect motor recovery during rehabilitation and the exercise likely promote the functional recovery. We also performed fluorescent immunohistochemistry to characterize new born cells. Most of BrdU (+) cells were GFAP (-), suggesting that these cells should not be caused by growth of astrocytes. These results suggest the potential of physical exercise to promote the motor recovery and the neurogenesis after infarction. (COI:No)

3P-189

Treadmill walking in water induces greater respiratory muscle fatigue than treadmill walking on land at the same exercise intensity in healthy young men

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The purpose of the present study was to investigate the effect of walking in water on respiratory muscle fatigue compared with that of walking on land at the same exercise intensity.
Methods: Ten healthy males participated in 40-min treadmill walking trials on land and in water at an intensity of 60% of peak oxygen consumption in a randomized order. The maximum inspiratory (PImax) and expiratory (PEmax) pressures in the oral cavity as indices of inspiratory and expiratory muscle strength, respectively, were evaluated before and after walking trials.
Results: PImax was significantly decreased immediately after walking in water compared with the baseline, and the decrease was significantly greater compared with that after walking on land. PEmax was significantly decreased immediately and 5 min after walking in water compared with the baseline, and the decrease was significantly greater compared with that after walking on land.
Conclusions:In conclusion, greater inspiratory and expiratory muscle fatigue was induced by walking in water than by walking on land at the same exercise intensity in healthy young men. Our results indicate that walking in water may be an effective and preferable option for strengthening respiratory muscles in the settings of respiratory rehabilitation. (COI:No)

3P-190

Features of the body compositions of university-level, front-row rugby football players

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[Introduction] Most studies on the body composition of rugby football players categorize players into 2 groups: forwards and backs. None have focused on front-row players. This study aimed to clarify the body composition features of university-level, front-row rugby football players. [Subjects and Methods] The subjects were 47 university students (mean age±SE: 20.2 ± 0.2 years) who were members of rugby football clubs. Subjects were placed into 3 groups for comparison: front-row group (F) (n = 11), second/back-row group (S) (n = 15), and backs group (B) (n = 21). Using bioelectrical impedance analysis, we calculated their somatic fat, skeletal muscle, and extracellular fluid rates. [Results] The somatic fat rates were 26.4 ± 1.9, 17.1 ± 0.9, and 14.9 ± 1.0 for F, S, and B, respectively, with F having the highest rate ($p < 0.01$). The skeletal muscle rates were 42.3 ± 1.2, 47.7 ± 0.6, and 48.9 ± 0.6 in F, S, and B, respectively, and the corresponding extracellular fluid rates were 0.199 ± 0.005, 0.222 ± 0.003, and 0.227 ± 0.003, respectively; F had the lowest values for both rates ($p < 0.01$). [Conclusions] Compared to second/back-row players, front-row university-level rugby football players had a higher somatic fat rate but lower skeletal muscle and extracellular fluid rates. (COI:No)

3P-191

Transforming growth factor - β1 increase after 12-min maximal running in young men having the body mass index above 25 kg/m2

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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)

3P-192

Functional and structural damages after eccentric contraction in mouse skeletal muscle

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Purpose: Eccentric contraction (ECC) of modest intensity is known to increase muscle mass, but damage muscle if strenuous. To optimize ECC protocol for muscle growth, we examined contractility and structures of skeletal muscle after ECC. Methods: Tibialis anterior muscle in adult male mice underwent a protocol of isometric contraction (ISO), ECC, or passive stretch. Contraction was elicited by supra-maximal electrical stimulation via sciatic nerve *in situ*. Each protocol comprised 3 sets of 30 tetani and/or stretch every 10 sec with 5-min intervals between the sets. Contractility at optimal muscle length (Lo) and electron microscopic structure was examined after the protocol. Several initial muscle lengths (1 and 0.9 Lo) and stretch extents (0.05, 0.1 and 0.2 Lo) were tested. Result: Passive stretch caused no functional changes. ISO reduced maximum force and contraction time of twitch more prominently at longer muscle length. ECC prominently reduced maximum twitch/tetanus force and twitch contraction time, and prolonged tetanus contraction. Extra force developed during ECC beyond Lo seemed crucial for the functional deteriorating effects. Microscopic observation suggested that ECC beyond Lo caused failures in excitation coupling, cross-bridge formation, and the integrity of sarcomere to induce the functional deteriorations. Conclusion: ECC beyond Lo should be avoided in training protocols for muscle growth. (COI:No)

3P-193

Acute exercise preserves cardiac function against ischemia-reperfusion injury

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Ischemic preconditioning is well known to preserve cardiac function against ischemia/reperfusion (I/R) injury. Exercise-induced myocardial ischemia also may have cardioprotective effect on I/R injury. However, the precise mechanisms remain unclear. In the present study, we examined the effect of acute exercise on I/R-induced depression of cardiac function using Langendorff-perfused rat heart. Male SD rats were divided into two groups; sedentary control (CON) and exercise (EX) group. In EX group, rats received one session of exercise, which consisted of running on a treadmill at 20-25 m/min for 30 min. At ten minutes after the completion of exercise session, the hearts were excised from the rats for Langendorff perfusion, and exposed to global ischemia (20 min) followed by reperfusion (45 min), and left ventricular developed pressure (LVDP) and heart rate (HR) during I/R were measured. Cardiac contractile activity expressed as pressure rate product (PRP = LVDP × HR), was significantly higher in EX group than in CON group during 5-20 min after reperfusion ($p<0.05$). At the end of ischemia, the glycogen content in the heart of EX group was significantly higher than that in CON group ($p<0.05$). There was no difference in cardiac ATP content between groups. In Western blotting analysis, the Akt phosphorylation involved in glucose uptake in the heart of EX group was significantly higher than that in CON group ($p<0.05$). Our data suggest that exercise-induced cardioprotection against I/R injury might be due to the suppression of glycogen utilization during ischemia. (COI:No)

3P-194

Exercise motivation is enhanced by peripheral PPAR-α stimulation

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Exercise habituation has a benefit for the health of body and mind although it is difficult to begin the exercise for many people, who have to exercise for their health. We and others found that transient intake of high fat diet increased spontaneous wheel running activity in rodents. Previous papers indicated that some kind of fatty acid activate peroxisome proliferator-activated receptor alpha (PPAR-α) in the gut, thereby inhibiting food intake partly through the vagal afferent-dopaminergic neuronal pathway. In this study, we tried to clarify the mechanism which high fat diet-induced increase of wheel running activity in mice focusing on PPAR-α in the gut. Wheel running activity following high fat diet feeding for 2 hours was higher in wild type mice than that of PPAR-α knock out mice. Oral administration of PPAR-α agonist (Wy-14643) increased wheel running activity in male C57BL/6J mice. This effect was reduced by intracerebroventricular injection of dopamine receptor antagonist, olanzapine. Moreover, oral PPAR-α agonist-induced enhancement of wheel running activity was not inhibited by the intracerebroventricular injection of PPAR-α antagonist, GW6471. These results suggest that peripheral PPAR-α, probably in gut, is an important molecule for the regulation of exercise motivation with activation of dopaminergic pathway. (COI:No)

3P-195

Effects of artificial high concentration CO_2-water bath on quiet standing posture control

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This study aimed to investigate the effect of CO_2-water immersion on postural sway in healthy young adults (n=5) of the upright position. Body sway was evaluated by recording horizontal ground reaction force for 1 min continuously with a force platform equipped with a data processor. The recording was performed at pre- and post-bathing under the conditions of eyes-opened (EO) and then eyes-closed (EC). Spectrum analysis by fast Fourier transform (FFT) method was applied to the waveform of body sway in the mediolateral and anteroposterior axes. Power spectra of body sway were evaluated by comparing powers of two frequency bands: 0.02-1.0 Hz (BAND 1), 1.0-10.0 Hz (BAND 2). Each subject was immersed up to the nipples for 15 min in tap- and CO_2-water (35°C). Cutaneous blood flow (BF) was measured on the upper chest and abdomen by laser-Doppler flow meter. BF was significantly larger in CO_2-water immersion compared to tap-water immersion throughout the recording period. The path length of the center of pressure (COP) was significantly decreased at post CO_2-water immersion in EO (66.1±11.8 vs 55.5±15.7 cm, $p<0.05$) and EC (88.9±21.8 vs 76.9±17.7 cm, $p<0.05$). Body sway of BAND 1 was significantly decreased at post CO_2-water immersion in the mediolateral axis of EO (1.41±0.15 vs 1.13±0.26 cm^2, $p<0.05$) and the anteroposterior axis of EC (1.72±0.19 vs 1.49±0.33 cm^2, $p<0.05$), while that of BAND 2 was not affected. Present results indicate that CO_2-water bath can stabilize the upright posture after the bath. (COI:No)

3P-196

Effects of oxamate on spinal seizure-like activity and medullary respiratory neurons in the brainstem-spinal cord preparation from newborn rats

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L-lactate is produced by neurons and astrocytes, and reversibly converted to and from pyruvate by L(+)-lactate dehydrogenase. Oxamate, a structural analog of pyruvate, has been known as a LDH inhibitor, preferentially acting on astrocytes. L-lactate plasma concentration rises dramatically in people with epilepsy or ischemia. Several studies have suggested that oxamate attenuated the eptiform activity on hippocampus. In the present study, we examined the effects of oxamate on spinal seizure-like activity and medullary respiratory neurons. Medulla-spinal cord preparations from postnatal day 0-3 Wistar rats were isolated under deep isoflurane anesthesia and were superfused with artificial cerebrospinal fluid (95% O2 and 5% CO2, pH 7.4, at 25-26°C). Inspiratory activity was monitored from the fourth cervical ventral root (C4).The seizure-like activity was consistently induced by two methods: bath application of 20 μM DL-threo-β-benzyloxyasparatate (TBOA, a glutamate uptake blocker) or coadministration of GABAA antagonist bicuculline (10 μM) and glycine antagonist stychinine (10 μM). Oxamate (10 mM) did not block the seizure-like activity induced by TBOA or bicuculline/strychinine, but decreased the amplitude. We also found that 10 mM oxamate consistently decreased the C4 burst rate. Oxamate depolarized 60% of pre-inspiratory neurons and hyperpolarized 60 % of inspiratory neurons. We proposed that the different responses to oxamate could reflect unique metabolic features of these neurons. (COI:No)

3P-197

Development of inhibitory inspiratory neurons in the pre-Botzinger complex of neonatal mice

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There is an ongoing debate regarding the contribution of inhibitory and excitatory neurons for the generation and modulation of the neuronal control of breathing. The respiratory rhythmic activity can be preserved in medullary transverse slices containing the pre-Botzinger complex (preBotC). During embryonic development, the ratios of GlyT2-positive neurons (glycinergic neurons), GABA-positive neurons (GABAergic neurons) and double-positive neurons to the total number of inhibitory neurons were shown to change in the ventrolateral medulla (Rahman et al., 2014). However, it is still not known whether the results reflect changes in the number of rhythmic or non-rhythmic inhibitory neurons and what developmental changes occur after birth. Here, we analyzed the contribution of glycinergic and/or GABAergic neurons in the preBotC from P3 to P8 using transgenic mice expressing EGFP in GlyT2-positive neurons and tdTomato in GAD65-positive neurons. During this period, 30-40% of inspiratory neurons expressed only EGFP, less than 3% only TdTomato and approximately 4-5% both fluorescent proteins. These results suggested that glycinergic neurons represent the majority of inhibitory inspiratory neurons in the preBotC in the first week after birth. (COI:No)

3P-198

Analysis of neuronal network of medullary respiratory center in transgenic rats expressing Archaerhodopsin in Phox2b expressing cells

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Preinspiratory (Pre-I) neurons in the parafacial respiratory group (pFRG) compose one of the respiratory rhythm generators in the medulla of the newborn rat. A subgroup of pFRG/Pre-I neurons express the transcription factor Phox2b. To analyze detailed neuronal mechanisms of respiratory rhythm generation using optogenetics, we made transgenic (Tg) newborn rats in which Phox2b positive cells expressed Archaerhodopsin (Arch). Brainstem-spinal cord preparations were isolated from 0-4 day old Tg newborn rats and were superfused at a rate of 3.0 ml/min with the artificial cerebrospinal fluid, equilibrated with 95% O2 and 5% CO2, pH 7.4, at 25-26°C. Inspiratory C4 ventral root activity was monitored and membrane potentials of Pre-I or inspiratory neurons were recorded in the pFRG. Continuous photostimulation up to 60 s of the rostral ventral medulla covering the pFRG by green laser light (532 nm) induced decrease of respiratory rate or complete cessation of respiratory rhythm accompanied with membrane hyperpolarization of pFRG-Pre-I neurons. We confirmed that this method is useful for analysis of respiratory rhythm generation in the en bloc newborn rat preparation. Supported by KAKENHI on Innovative Areas (Comprehensive Brain Science Network) from MEXT. (COI:No)

3P-199

Effects of hypercapnia on respiratory motor activity in nerves innervating the infrahyoid and laryngeal muscles in situ

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"Can't intubate can't ventilate" (CICV) situation is one major cause of death associated with general anesthesia, since CICV situation caused hypercapnic acidosis, severe brain disorder. In the present study, we examined the respiratory motor activity in the efferent nerves innervating muscles during respiration under normocapnic condition and hypercapnic acidosis. Experiments were performed on arterially-perfused decerebrate rats aged between postnatal days 21-33. We recorded efferent nerve activities in the recurrent laryngeal nerve (RLN) innervating the laryngeal muscles and a branch of the cervical spinal nerve innervating the infrahyoid muscles (CN) with the phrenic nerve (PN). Inspiratory discharge was observed in all these nerves, and expiratory discharge observed in RLN. When CO_2 concentration in the perfusate was increased from 5% to 8% to make preparations hypercapnic acidosis (pH = 7.2), the onset of the inspiratory discharge in the RLN and CN was advanced with the reference of the onset of the inspiratory discharge in the PN (RLN: 0.14 ± 0.02s in control, 0.39 ± 0.06s in hypercapnia, CN: 0.28 ± 0.03 s in control, 0.83 ± 0.04 s in hypercapnia, n = 5). These results suggest that the phase of activation of infrahyoid and laryngeal muscles maybe modulated by blood CO_2 concentration. (COI:No)

3P-200

Inhibition of hydrogen sulfide synthesis induces gasping in the *in situ* arterially perfused preparation of rats.

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Hydrogen sulfide is well known as toxic gas. However, it is endogenously synthesized in a body and has various physiological effects, which are cytoprotection, vasodilation, synaptic modulation and so on. However, it is still unclear that roles of hydrogen sulfide to the respiratory pattern generation in the brain. In this study, in order to examine roles of the hydrogen sulfide production to the respiratory pattern generation, we evaluated effects of pharmacological modulation of the hydrogen sulfide production to respiratory pattern generation in the *in situ* arterially perfused preparation of rats. We administrated cystathionin β-synthase (CBS) inhibitor, hydroxylamine, into the perfusate, and analyzed the effect on respiratory activities of the phrenic nerve (PNA) and the central vagus nerve (VNA). In the absence of CBS inhibitor, eupnea, which is characterized by incrementing inspiratory PNA and inspiratory and expiratory VNA, was observed. On the other hand, administration of CBS inhibitor switched the respiratory pattern from eupnea into gasping, which is short decrementing inspiratory PNA and VNA. The gasping was also suppressed by administration of persistent sodium channel blocker, riluzole. The data may indicate that eupnea is sustained by endogenous hydrogen sulfide production, and inhibition of the production switches the network-based respiratory pattern generation into pacemaker-based generation. (COI:No)

3P-201

Development of a model mouse of Sudden Unexpected Death in Epilepsy (SUDEP) by loading hypoxic stress

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Sudden Unexpected Death in Epilepsy (SUDEP) is defined as the sudden, unexpected, witnessed or unwitnessed, non-traumatic, and non-drowning death in patients with epilepsy with or without evidence for a seizure, and excluding documented status epilepticus, in which postmortem examination does not reveal a structural or toxicologic cause for death. SUDEP is increasingly recognized as a common and devastating problem, however the mechanisms underlying SUDEP remain unclear. To investigate the pathophysiological mechanisms of SUDEP, various animal models have been developed. However, most of the reported models use genetically-engineered or pharmacologically-induced seizure-prone mice. To study the pathophysiology in wild type animals without using pharmacological agents, we intended to develop an animal model without genetic engineering or pharmacological intervention. We monitored ventilation by whole body plethysmography and EEG in unanesthetized C57BL6 mice, and loaded hypoxia. Continuous loading of severe hypoxia (6% O_2 in N_2) augmented ventilation. However, then, a number of mice suddenly exhibited seizure with epileptic waves in EEG, followed by ventilatory fall-off and respiratory arrest. We conclude that loading of severe hypoxia could mimic SUDEP without genetic engineering or pharmacological seizure-induction. (COI:No).

3P-202

Effects of vocalization to physical activities and circulatory states during *kendo* exercises

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We have reported that vocalizations during *kendo* exercise affect breathing patterns and cause a reduction in VCO_2 and an increase in $FetCO_2$. In addition to these, we report physical activities, rate of perceived exertion (RPE) and circulatory states. Eight university *kendo* athletes were measured physical activities using a calorie counter during *kendo* exercise, and RPE using Borg scale and blood pressure in pre and post exercise with (Voc) and without vocalization (non-Voc). Physical activities in non-Voc calculated by steps tended to be more than that in Voc. RPE in non-Voc tended to be less than that in Voc. A rate of increase of diastolic blood pressure in Voc tended to be less than that in non-Voc. We assume that the low value of RPE in non-Voc is led to the low value of $PaCO_2$ during *kendo* exercise in non-Voc, and the low value of RPE lead an increase physical activities in non-Voc. Thus, we conclude that *kendo* practice without vocalization may be effective for the purpose of more increase physical activities. On the other hands, we conjecture that peripheral vascular resistance decrease because of a rate of increase of diastolic blood pressure in Voc. Nitric oxide (NO) have been reported to produce in the paranasal sinuses by humming, we consider the possibility that vocalization during *kendo* exercise may cause the same effect. (COI:No)

3P-203

Imaging of human fecal enteric bacteria by fluorescently labeled glucose derivatives.

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D-glucose is an essential energy source for most organisms. Uptake of D-glucose into single living mammalian cells through a major glucose transporter GLUT have been visualized by a fluorescently labeled D-glucose derivative 2-NBDG (Yamada *et al*, ***Nature Protocols***, **2007**), which is widely used in a variety of fields (Rouach *et al*, ***Science***, **2008**; Viale *et al*, ***Nature***, **2014**; Ait-Ali *et al*, ***Cell***, **2015**). However, only a limited number of studies were conducted for prokaryotes. In this study, we show an application of 2-NBDG as well as its antipode 2-NBDLG, a fluorescently labeled L-glucose derivative, to Escherichia coli DH5α and human fecal enteric bacteria. We discuss some unresolved issues for conducting the live cell imaging of bacteria and human fecal samples by fluorescently labeled glucose derivatives. (COI:Properly Declared)

3P-204

Novel multiscale in vivo brain functional imaging

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For revelation of the brain function, the activity of multiple neurons or regions should be measured. In this regards, imaging is a feasible method, thus, we have developed and applied two novel imaging methods for whole and deep brain imaging.
One is the activation-induced manganese-enhanced MRI (AIM-MRI). This method is based on the use of Mn^{2+} as a surrogate marker of Ca^{2+} influx. We have applied quantitative AIM-MRI (qAIM-MRI) to the whole brain activity imaging in Parkinson's disease (PD). A highly active region was observed in the caudate-putamen, sensorimotor cortex, and parafascicular nucleus of thalamus in PD-model mice, that correlated to the severity of PD. Our results suggest qAIM-MRI can be used for quantitative activity mapping in the entire brain for the study of various neurological disorders.
Another is the fluorescence micro-endoscope. This endoscope is consisted with a GRIN lens and an image fiber. This endoscope has a smaller diameter and a higher spatial resolution, thus, can visualize the individual cells in a local circuit of deep brain region less-invasively. We successfully visualize the neurons expressing GFP in a mouse and the activity dependent fluorescence changes of flavoprotein in a monkey. This imaging system would facilitate the in vivo imaging studies with less invasive manners. (COI:No)

3P-205

Near-infared (NIR) up-conversion optogenetics for neural manipulation

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Non-invasive remote control technologies designed for manipulation of neural functions are long-awaited for understanding of neuronal network in the brain as well as for the therapy of neurological disorders. Recently the neuronal activity is enabled to be optically manipulated using biological photo-reactive molecules such as channelrhodopsin-2 (ChR2). However, ChR2 and its relatives are mostly reactive to visible light which does not effectively penetrate through biological tissues. In contrast, near-infrared (NIR) light (650-1450 nm) penetrates deep into the tissues. Here we used lanthanide nanoparticles (LNPs) as luminous bodies to activate channelrhodopsins (ChRs) since LNPs absorb low-energy NIR light to emit high-energy visible light. Under whole-cell patch clamp, NIR laser light (976 nm) was irradiated to LNP ($NaYF_4$:Sc/Yb/Er, peak emission 543 nm) particles under the culturing rat cortical neurons expressed chimeric ChRs (C1V1 or mVChR1), the membrane potential was depolarized to evoke action potentials. However, the NIR laser light of the same power evoked negligible current without C1V1/mVChR1 or LNP particles. It is suggested that the green luminescent light emitted from LNPs activated C1V1 or mVChR1 to generate enough photocurrent to depolarize the membrane potential above threshold of action potentials. This system would be applied to actuate neurons deep in the brain non-invasively. (COI:No)

3P-206

Sub-chronic real-time measurement of dopamine in the brain with novel dopamine-selective biosensor

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To measure real-time dopamine (DA) level in the brain, biosensor specific to DA was developed with three layer membranes: iron-exchange membrane, enzyme-containing membrane and antioxidant-containing membrane. Fast-scan cyclic voltammetry that detects oxidative current of membrane permeable substances electrochemically was used monitoring the current to a triangular waveform (-0.4 to +1.0 V, 300 V/s). Currents were recorded with a newly-developed voltammetric and amperometric Amplifier IMEC-701 (inter medical co., ltd, Nagoya, Japan). In vitro experiments, oxidative current was measured in phosphate buffered saline solution added each 1 μM of DA, serotonin (5-HT) and noradrenaline (NA). It is confirmed that permeable DA was selectively detected using the three-layer biosensor. We next investigated whether DA-selective probe detect the changes of DA content in vivo. The probe was implanted to monitor the level of DA in the striatum. Administration of methamphetamine (MA, 3.0 mg/kg) or electrical stimulation of medial forebrain bundle (MFB, 300 μA, 2ms pulse, 60Hz, i.p.) was given and DA level was monitored. Oxidative current was gradually increased from 10 min after the administration of MA, and it was changed in a phasic manner in the case of MFB stimulation. Successful monitoring of DA level was confirmed at least one week after implantation. Data suggest that real-time change of DA was measurable for in vivo with a biosensor that has relative high-sensitivity to DA. (COI:No)

3P-207

Morphological analysis of dendritic spines using structured illumination microscopy with a clearing method for the fixed mouse brain

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Detailed morphological analyses of the dendritic spine have been required for evaluating synaptic functions, because spine morphology is thought to be involved in the efficiency of excitatory synaptic transmission. However, such finer structures have not been observed in the deeper region of the fixed brain samples because the high light scattering in the fixed tissue deteriorated the spatial resolution even by super-resolution fluorescence microscopy. Here, we applied a clearing method for super-resolution microscopy, structured illumination microscopy (SIM), and successfully visualized finer structures of dendritic spines in the fixed brain slice. This clearing method reduced such light scattering that might cause artifacts in the SIM image. We next evaluated dendritic spine forms including the roundness on the single dendrite, after chronic treatment with dexamethasone which induces structural changes of the neuron in the prefrontal cortex (PFC). These analyses showed that the spine form was altered and tended to become thinner in the PFC of dexamethasone-treated mice. Thus, the combination of the cleaning reagent and SIM might be useful for the morphological analyses of the dendritic spine. (COI:No)

3P-208

A new method for a practice of physiology to learn about the excitation-contraction coupling of muscles and volume conduction

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Electrophysiological techniques such as electromyography and electrocardiogram are widely used for medical diagnosis. Therefore, it is important for medical students to understand about the volume conduction and mechanisms underlying excitation-contraction coupling of muscles. For effective learning about them, we developed the following method for a practice of physiology using rat. Rats were anesthetized and sciatic nerves were exposed by dissection of skin and gluteus muscles near the femur. First, recording electrodes were set on rat's limb surface. As a result of electric stimulation of the ipsilateral sciatic nerve, students confirmed that the biphasic potential is generated by excitation of limb muscle. Next, rats were put on a filter paper soaked with Ringer's solution and recording electrodes were set on the paper. As a result of stimulation, students confirmed that the potentials could be detected through volume conductor (i.e. Ringer's solution). Furthermore, students identified the invisible potential distribution on the volume conductor by plotting the amplitude of potentials at multiple points on the paper. Finally, students confirmed that the potential and motion of limbs were eliminated by pharmacological disturbance of neuromuscular transmission and observed caffeine contracture by following injection of caffeine. Through this practice, it is expected that students promote understanding about the volume conduction and excitation-contraction coupling of muscles. (COI:No)

3P-209

E-learning materials on energy metabolism for beginners created with students' participation

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In 'Step-by-step study of human life sciences', numerous straight-forward illustrations that are easy for beginners to understand and simple quizzes are presented. Also, Moodle functions are expanded; spatial movement, chemical reactions, and facilitation are specifically indicated by various types of arrows, and information is presented in very small steps. The design was applied to energy metabolism, and original material was created which explains the basic points of nutrition education, such as degradation of nutrients in energy metabolism, chemical energy in atomic bonds, but not the atoms, in the nutrients being necessary for ATP synthesis, etc. Over an 8-year-period, revisions and additions have been made based on opinions from students and experts. A Moodle course with 31 steps, including 22 animations and 186 quizzes, was created. An anonymous survey (answer options: very high/high/moderate/low/very low) completed by students who have seen other materials on the topic, evaluated the following: easiness for the material to be understood, 11/20/9/1/1; helpfulness of the small steps, 16/22/3/1/0; helpfulness of the illustrations, 13/20/9/0/0; value of student participation in the creation, 14/19/8/0/0; academic level of the content, 1/1/26/14/0 (very easy/easy/moderate/difficult/very difficult). In conclusion, the core, yet complex, points of energy metabolism are presented in an easy-to-understand original material by including student participation. (COI:No)

3P-210

Automated emails linked to Moodle quizzes enhances self learning

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'Step-by-step study of human life sciences' is an innovative digital educational material with straight-forward illustration/animation and simple multi-choice questions presented in very small steps for beginners. The materials were installed on Moodle, which was customized to automatically send a celebratory email immediately after submission of a quiz which was successfully passed, and an encouragement email immediately after submission of a quiz which was a not passed, and also a reminder email on 6, 5, 4, 3, 2, 1 day(s), and at 12 hr and 1 hr before the deadline of non-submitted quizzes. An introductory 'step-by-step' material including 144 steps, 200 illustrations, 119 animations and 394 questions comprised the Moodle course with 27 quizzes. This was assigned to perspective students before entrance to a health care school to be completed by self learning in 15 weeks. Compared to the 2014 year students (n=124), when the email function was not used, the 2015 students (n=132) showed significantly higher quiz-submission rate, percentage of students passing each quiz, average score of each quiz, and score of the evaluation paper test given in class upon completion of the introductory course. The scores of a test given before the introductory course, however, were the same in both 2014 and 2015 students, respectively. In conclusion, automated emails linked to Moodle quizzes may enhance not only the amount of self online learning, but also the level achieved. (COI:No)

3P-211

A Support System for Tours in A Human Specimen Museum with Augmented Reality Technology

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Human anatomical specimens are mainly used by medical, nursing or paramedical students. Visitors are able to perceive the detailed shapes and textures of organs, which cannot be expressed in books. Visitors, particularly abecedarians or beginners, find it difficult to identify parts of organs without additional explanations offered by the docent.Many exhibits in museums and aquariums use augmented reality (AR) techniques. This enables visitors to get much information from the exhibited objects, generating more interest in them. In this study, we propose the application of AR techniques to anatomical specimens. AR technology enables the displaying of virtual objects in the video-captured real world. One of the AR methods involves using an image marker to realize the AR environment. In our system, since most of the specimens in the display cases are cross sections, an image of each specimen captured in advance works as the marker. The user (visitor) captures the marker with their tablet's camera, and then virtual elements, consisting of labels of parts and supplemental remarks, are displayed on the marker. A head-mounted display connected to the information terminal is also used, with an added function through which a virtual object can be operated naturally. The AR system should be further extended to human specimens other than those with cross sections, and be improved to display various types of virtual objects such as invisible structures. (COI:No)

3P-212

VEGFC mediated lymphangiogenesis is regulated by a G-protein activator, Activator of G-protein signaling 8

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Receptor independent regulatory proteins for heterotrimeric G-protein provide alternative inputs to signaling system and may influence cellular events through its interaction with G-proteins. We previously identified an ischemia-inducible Gβγ signal regulator, activator of G-protein signaling 8 (AGS8). The recent study indicated that AGS8 was involved in angiogenic events of vascular endothelial cells. AGS8-G βγ regulated the subcellular distribution of vascular endothelial growth factor receptor-2 (VEGFR-2) and influenced VEGF-induced signaling for angiogenesis. Because one of VEGF receptor, VEGFR-3 mediates VEGF signal in lymphatic endothelial cells, AGS8 may be involved in lymphangiogenesis. Here, we report an effect of AGS8 in VEGF signaling in human dermal lymphatic endothelial cells (HDLECs). Expression of AGS8 was suppressed by AGS8 siRNA to 19.5 ± 1.5% of control siRNA treated cells. VEGFC (100 ng/ml) stimulated tube formation by HDLECs (133.4 ± 8.4% of control, $p<0.02$, mean±SEM), which was inhibited by AGS8 siRNA (112.3 ± 14.9% of control, $p>0.05$, mean±SEM). VEGFC (50 ng/ml) also stimulated cell growth determined by MTT assay (123.0 ± 2.6% of control, $p<0.01$, mean±SEM). AGS8 siRNA again inhibited growth of HDLECs (106.6 ± 14.9% of control, $p>0.05$, mean±SEM). These data suggested the involvement of AGS8 in VEGFC-VEGFR3 signaling in HDLECs and a potential role of AGS8 in lymphangiogenesis. (COI:No)

3P-213

Effect of free radical scavenger in thrombolytic therapy.

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The intravenous application of alteplase, a recombinant tissue plasminogen activator, and edaravone, an antioxidant, reportedly enhances recanalization after acute ischemic stroke. Blood samples from healthy volunteers were exposed to edaravone, alteplase, a combination of alteplase with edaravone or hydrogen peroxide. Whole blood was perfused into a capillary device; capillary occlusion was monitored with a flow-pressure sensor. All capillaries were occluded by thrombi in the control and edaravone groups. The pressure after 30 minutes from perfusion was 69.9% lower in the alteplase-edaravone than alteplase group. The thrombolytic effect of alteplase was significantly attenuated in the presence of hydrogen peroxide, suggesting that oxidative stress might hinder thrombolysis. Edaravone may prevent free radical-related inhibition of alteplase activity on thrombi. (COI:Properly Declared)

3P-214

Transplantation of rat umbilical cord blood cells for hypoxic-ischemic brain injury in neonatal rats.

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Despite recent advances in the treatment of neonatal hypoxic-ischemic encephalopathy (HIE) using therapeutic hypothermia, considerable number of infants still has moderate/severe neurological disability. Stem cell therapy using umbilical cord blood (UCB) cells could be a promising option for the treatment of HIE. Although many animal studies showed that human UCBCs had a favorable effect in treating HI-induced damage, their outcomes appeared variable, which might be due to xenotransplantation. In order to investigate the effectiveness of UCB cells against hypoxic-ischemic (HI) brain injury in an allogeneic system, mononuclear cells from UCB of GFP- transgenic rats were expanded and intraperitoneally injected into rat pups harboring a neonatal HI injury 3 days after the insult. At 3 weeks after the transplantation, the infarct area of the cell-treated rats reduced compared to that of control rats. Motor function of the cell-treated rats improved more than that of the control group. While the GFP-positive cells were readily observed in the spleen, those were hardly detectable in the brain parenchyma. The number of activated microglia decreased in the ipsilateral hemisphere of cell-treated rats. Our data indicate that intraperitoneal injection of UCB cells could ameliorate HI injury, possibly by the secretion of certain trophic factors protective of damaged neurons, but not by supplying the brain with neurons that had differentiated from the injected stem cells. (COI:No)

3P-215

Neural mechanisms of memory retrieval during relearning

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We often recall remote memory by using the current information as a hint. This process appears to require activities of some brain regions, e.g. hippocampus (HPC) and prefrontal cortex (PFC). There are a lot of studies reporting that these two regions are involved in memory consolidation and retrieval. Hippocampal neurons correlate with PFC neurons when the rats retain memory and it is significant for the memory consolidation to send information from HPC to PFC. Moreover, inputs from PFC to HPC are hypothesized to be important in memory retrieval. However, it remains unclear how HPC and PFC are involved in retrieval of remote memory. Therefore we investigate neural activities of these two regions when rats are performing tasks in which the rats need to recall remote memory. We use two kinds of tasks (task-A and task- B) which are different but interfere to each other. At first, the rats perform task-A until they have learned the task completely. Next, they have learned task-B, and finally they perform task-A again. When they execute task-A for the second time, they presumably recall the information about task-A that they performed for the first time. We record neuronal activities and LFPs from HPC and PFC simultaneously when the rats are performing task-A for the second time in order to explore neural mechanisms underlying retrieval of remote memory. We will report preliminary data. (COI:No)

3P-216

Influence of Electroacupuncture Stimulation on Nitric Monoxide Production in Vascular Endothelial Cells in Rats

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In Chinese medicine, blood stasis termed as "Oketsu" means "preceding state" or "symptomatic of sickness". Traditional Chinese medicine may improve blood flow by vasodilation or blood clotting inhibition. Although acupuncture influences the blood circulatory system, its underlying mechanisms remain unclear. Here we evaluated changes in NO, as reflected by changes in NO_2^-, platelet aggregation, oxidative stress and endocrine responses after acupuncture stimulation in rats. Acupuncture stimulation was administered to rats randomly divided into five groups: control, L-NAME (N^G -nitro-L-arginine methyl ester hydrochloride) injection, restraint stress (RS), restraint plus acupuncture stimulation (RA), and restraint plus acupuncture with L-NAME (RLA). Compared with those in the RS group, levels of NO_2^-, eNOS (endothelial nitric oxide synthase) protein and its mRNA significantly increased and those of hydroperoxide and soluble P-selectin significantly decreased in the RA group.Acupuncture stimulation regulates vascular endothelium NOS function and affects vascular resistance and blood characteristics through NO. Additionally, NO produced may modulate excessive reactive oxygen development and blood platelet activation. (COI:No)

3P-217

The assessment of emotion evoked by passive change of facial expression

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The purpose of the present study was to examine whether the passive change of facial expression would affect emotion. Healthy volunteers performed passive facial expression by deforming the skin around eyes or corners of mouth by their fingers for 5 minutes. Psychological features before and after deformation of the skin were examined with the profile of mood states (POMS). This test reveals five factors of negative mood states [tension-anxiety (T-A), depression-dejection (D), anger-hostility (A-H), fatigue (F) and confusion (C)], and a factor of positive mood state [vigor (V)]. Passive opening of eyes wide (raising upper eyelids and lowering lower eyelids) varied the scores of POMS most strongly, increasing the positive scores V and decreasing all five negative scores significantly. In tests deforming the outer corner of eyes, lowering of the corner of both eyes decreased V and increased F, and outward pulling decreased V. In tests deforming the outer corner of mouth, raising of them decreased D and increased V, and lowering of them increased T-A, D and F and decreased V. The study demonstrates that the passive change of facial expression affects the mood states, and the effects are different among expressions. Passive opening of eyes wide and passive raising of the corners of mouth increased a positive mood score and decreased negative mood scores. On the other hand, passive lowering of the corners of mouth decreased a positive mood score and increased negative mood scores. The study suggests that negative moods can be changed to positive moods by passive facial expressions and vice versa. (COI:No)

3P-218

Change in the body composition of rugby players between before and after a camp

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[Introduction] Many competition athletes often participate in a camp to update their self-record and to win in a game. We investigated a summer camp of a rugby club and examined the change in body composition of competition athletes caused by camp participation.
[Methods] The subjects were 15 university students who were members of rugby football clubs. Using bioelectrical impedance analysis, we calculated their body composition before and after the camp.
[Results] The weight [pre (90.0 ± 2.5 kg), post (84.8 ± 2.6 kg)] (p<0.01), fat mass [pre (18.3 ± 1.7 kg), post (14.9 ± 1.5 kg)] (p<0.01), body fat percentage [pre (20.3 ± 1.5%), post (17.3 ± 1.4%)] (p<0.01), and body fluid volume [pre (51.6 ± 1.2 L), post (50.9 ± 1.3 L)] (p<0.05) showed a significantly low value after the camp in comparison with before the camp. Skeletal muscle mass [pre (40.6 ± 1.0 kg), post (40.4 ± 1.0 kg)] showed no significant difference. Skeletal muscle percentage [pre (45.8 ± 0.9%), post (47.8 ± 0.8%)] and extracellular water [pre (0.21 ± 0.003 L/kg), post (0.22 ± 0.003 L/kg)] (p<0.01) showed a significantly high value after the camp in comparison with before camp.
[Conclusions] This study demonstrates that decrease in weight and body fat percentage and increase in skeletal muscle percentage depends on the attenuation of extra fat mass and body fluid volume, not on the quantity of muscle increased. (COI:No)

3P-219

Effects of press tack needle treatment in rats subjected to chronic social isolation (part II)

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We have already reported that aggressive behavior and the level of corticosterone were significantly increased in rats that were exposed to social isolation stress, and that these stress responses were inhibited by press tack needle (PTN) treatment. Moreover, in this rat model, the secretion of orexin, a neuropeptide, was increased in the hypothalamus; however, this increase was also inhibited by PTN. This study was performed to investigate if the control of orexin secretion is involved in the anti-stress effects of PTN treatment.
Male rats were divided into a group-housed control group (Control), a single-housed stress group (Stress), and a single-housed plus PTN-treated group (PTN). In the PTN group, a PTN was fixed on the GV 20 acupuncture point (Hyakue; Baihui) on day 7. The levels of plasma corticosterone and orexin were measured on day 8. A statistical analysis revealed that the level of corticosterone was correlated with the level of orexin.Furthermore, an orexin receptor antagonist was administered to rats that were exposed to isolation stress to examine if orexin is involved in their stress responses. This resulted in significant decreases in aggressive behavior and in the level of corticosterone.
These results suggest that orexin is involved in the control of the stress responses in rats and that PTN treatment has an anti-stress effect via its control of orexin secretion. (COI:No)