1-O-01

Involvement of Ca^{2+}-sensing receptor in activation of nitric oxide synthase of human vascular endothelial cells.

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Ca^{2+}-sensing receptor (CaSR) is a seven-transmembrane G protein-coupled receptor (GPCR), and is activated by an increase in extracellular Ca^{2+} concentration. In vascular endothelial cells, stimulation of CaSR induces nitric oxide (NO) release via activation of endothelial nitric oxide synthase (eNOS) and membrane hyperpolarization via activation of intermediate Ca^{2+}-activated K^+ channels, contributing to vasodilation. In the present study, we have pharmacologically characterized eNOS activation in response to stimulation of CaSR in human endothelial EA.hy926 cells. In 2 mM Ca^{2+}-containing Krebs-HEPES solution, the phosphorylation level of eNOS at serine 1177 was markedly reduced by NPS 2143 (a CaSR antagonist) and YM-254890 (a $G_{q/11}$ protein inhibitor). In organ bath study with endothelium-removed ring preparations of rat thoracic aorta, addition of EA.hy926 cell suspension produced relaxation of the rings precontracted with phenylephrine. The endothelium-dependent relaxant response was inhibited by pretreatment of EA.hy926 cells with NPS 2143, YM -254890, and L-NAME (an eNOS inhibitor). These results suggest that stimulation of CaSR expressed in endothelial cells with extracellular Ca^{2+} induces NO-mediated vasorelaxation via $G_{q/11}$-protein-dependent activation of eNOS.

1-O-02

Blockade of gap junction increases endothelial cellular stiffness

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Endothelial cell dysfunction underlies the development of vascular inflammatory diseases. The cellular stiffness of endothelial cells has been shown to increase during chronic inflammation. Abnormalities of endothelial gap junctions facilitate endothelial dysfunction and participate in the progression of cardiovascular diseases; however, the impact of gap junction on endothelial cellular stiffness remains poorly understood.

To evaluate the cellular stiffness, we have measured the force curve of live human umbilical vein endothelial cells (HUVECs) by using atomic force microscopy. We have shown that stimulation of HUVECs with tumor necrosis factor-α (TNF-α) increased the endothelial cellular stiffness. We have also shown that the blockade of gap junctions not only increases endothelial cellular stiffness, but also enhances focal adhesion formation and cytoskeletal rearrangement. Inhibition of gap junctions appeared to prolong the effects of TNF-α, increasing endothelial stiffness.

Aberrant regulation of gap junctions may be involved in the vascular stiffening commonly observed in vascular inflammatory diseases. Our study provides a new insight into the potential pathogenic role of endothelial cellular stiffening driven by vascular inflammation.

1-O-03

The involvement of advanced glycation end products in angiogenesis

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Diabetes induces proangiogenic response which is characterized by fragile blood vessels. Advanced glycation end products (AGEs) accumulating under hyperglycemic conditions are acknowledged to play a causative role in the vascular complications of diabetes including retinopathy associated with excessive angiogenesis. However, the detailed mechanism of AGEs-mediated excessive angiogenesis is poorly understood. Therefore, we elucidate one part of this mechanism. Matrigel tube formation assay was performed using the mouse endothelial cell line, b.End5 cells. The areas and total lengths of the tubes were calculated as the degree of tube formation. Uptake of AGEs by b.End5 cells were measured using flow cytometry. pNFκB/NFκB ratio were analyzed by western blot. AGE2 and AGE3 concentration-dependently increased the area and length of tubular structures. Uptake of AGE2 and AGE3 by b.End5 cells concentration-dependently was increased. AGE2 and AGE3 activated NFκB. Induction of tube formation by AGE2/3 and AGE2/3 uptake by b.End5 cells were significantly suppressed by pinocytosis inhibitor, EIPA, or NFκB inhibitor, PDTC. Our results indicate that AGEs uptake by b.End5 cells may be mediated by pinocytosis. Moreover, pinocytosis of AGEs may induce phosphorylation of NFκB in endothelial cells, and then promote angiogenesis.

1-O-04

mPGES-1/PGE_2/EP4 axis induces blood flow recovery via accumulation of regulatory T cells to ischemic muscle

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Microsomal prostaglandin E synthase-1 (mPGES-1) /Prostaglandin E2 (PGE_2) induces angiogenesis. Immune cells, especially regulatory T cells (Treg) related to cancer growth and angiogenesis. Based on these previous reports, we hypothesized mPGES-1/PGE_2-EP signaling contribute to recovery from ischemic condition by accumulation of Treg.

Compared to wild type mice (WT), the blood recovery was significantly suppressed in mPGES-1 deficient mice (mPGES-1KO). The number of $FOXP3^+$cells in the ischemic muscle was decreased in mPGES-1KO compared to WT. Expression of TGF-beta was suppressed in mPGES-1KO. Those accumulated $FOXP3^+$cells and blood recovery was significantly suppressed by injecting folate receptor 4 (FR4) antibody in WT ($P<0.05$) but not in mPGES-1KO. Compared to other EP receptors, the expression of EP4 in the ischemic muscle was significantly enhanced . The blood recovery was significantly suppressed in EP4 receptor deficient mice (EP4KO) compared to WT ($P<0.05$). Furthermore, expression of mRNA level of FOXP3 and TGF-beta were significantly suppressed in EP4KO. Moreover, the numbers of FOXP3+ cells were diminished in EP4KO compared to WT. These results suggested that mPGES-1/PGE_2induces blood flow recovery from ischemia via EP4 by accumulating Tregs.

1-O-05

Foam cell formation is regulated by plasmin in a murine model of type IIa hypercholesterolemia

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Due to atherosclerosis is asymptomatic nature, it is difficult to investigate the progression of this disease in humans. Animals, especially mice with select genetic alterations, are very useful to investigate this disease. Mice with a double deficiency of LDLr and Apobec1 (*Ldlr*$^{-/-}$/*Apobec1*$^{-/-}$) show high levels of plasma total cholesterol on a normal chow diet, most of which is distributed in the LDL fraction. Consequently, spontaneous atherosclerotic plaques form in the aorta, a situation that is similar to human familial hypercholesterolemia.

We have revealed the important role of thecoagulation and fibrinolytic system in atherosclerosis. In this study, we examined the role of plasminogen in atherosclerosis with *Ldlr*$^{-/-}$/*Apobec1*$^{-/-}$mice.

We established *Ldlr*$^{-/-}$/*Apobec1*$^{-/-}$/*Plasminogen*$^{-/-}$(*Ldlr*$^{-/-}$/*Apobec1*$^{-/-}$/*Plg*$^{-/-}$) triple deficient mice from *Ldlr*$^{-/-}$/*Apobec1*$^{-/-}$mice and *Plg*$^{-/-}$mice. We have found that total cholesterol levels were significantly higher in *Ldlr*$^{-/-}$/*Apobec1*$^{-/-}$/*Plg*$^{-/-}$mice than in *Ldlr*$^{-/-}$/*Apobec1*$^{-/-}$mice. Almost all of the cholesterol accumulated in the LDL fraction, and the HDL cholesterol level was not different between both groups. However, *Ldlr*$^{-/-}$/*Apobec1*$^{-/-}$/*Plg*$^{-/-}$mice showed much smaller plaques in the aortic sinus. Furthermore, the migration of macrophages was suppressed by a plasminogen deficiency. Plasmin, the activator of plasminogen, promoted OxLDL uptake of macrophages. On the other hands, plasminogen didn't affect the expression of various scavenger receptors in macrophage. These results suggest that theactivation of plasminogen and the interaction plasminogen and OxLDL promote atherosclerosis in type lla familial hypercholesterolemia.

1-O-06

A splice switch in SIGIRR causes a defect of poly(I:C)-dependent anti-inflammatory IL-37b-SIGIRR negative feedback loop in cystic fibrosis airway epithelial cells

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Cystic fibrosis (CF) is typically characterized by infection-associated airway epithelial inflammation. Here, we showed that cell surface expression of SIGIRR/TIR8, an immunoglobulin-like membrane protein that is essential for negative regulation of toll-like receptors (TLRs)-associated inflammatory signals, is specifically and remarkably decreased in CF and CF-like airway epithelial cells. These CF-associated cells specifically and highly expressed a unique, alternative splice isoform of SIGIRR that lacks exon 8 (Δ8-SIGIRR),
which contributes to the suppression of functional SIGIRR plasma membrane expression. Consistently, a SIGIRR ligand IL-37b failed to suppress inflammatory signals induced by poly(I:C), a synthetic analog of viral RNA. Finally, poly(I:C)-dependent anti-inflammatory IL-37b secretion was also significantly decreased in CF cells, suggesting the dysregulation of anti-inflammatory receptor SIGIRR as well as its ligand IL-37b in CF cells. Thus, our studies demonstrate that poly(I:C)-dependent anti-inflammatory feedback loop, or IL-37b-SIGIRR negative feedback signal, is defective in CF airway epithelial cells due to unique splicing switch of SIGIRR gene.

1-O-07

Molecular mechanisms underlying dedifferentiation of pathogenic lung myofibroblasts.

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Idiopathic pulmonary fibrosis (IPF) is a chronic and progressive lung disease with a poor prognosis. Thus, to discover drugs providing new therapeutic options for patients with IPF is necessary. Myofibroblasts (MyoFs) that produce abundant extracellular matrix play key roles in the development of IPF. Although the pathogenic MyoFs mainly differentiated from resident fibroblasts have been considered as the irreversible phenotype, it has been recently reported that some agents dedifferentiating MyoFs into fibroblasts can attenuate bleomycin-induced lung fibrosis in mice. This finding tempts us to consider induction of MyoF dedifferentiation as a new beneficial strategy for patients with IPF. However, the detailed mechanisms of MyoF dedifferentiation are still unknown.
We have already established several strains of primary cultured MyoF-like cells (MyoLCs) from the fibrotic lungs of patients who underwent lung transplantation as a result of severe fibrosis. Employing the MyoLCs (S100A4^{-}α-SMA^{high}ED-A-fibronectin^{+}: > 90%) as an *in vitro* screening system, we found that JQ1, a bromodomain inhibitor could induce MyoF dedifferentiation associated with the downregulation in expression of α-SMA and ED-A fibronectin. Then, we comprehensively investigated the change in expression of microRNAs in JQ1-treted MyoLCs. Based on these results, we will discuss the molecular mechanisms of MyoF dedifferentiation.

1-O-08

RAMP1 signaling in hepatic macrophages plays a critical role in immune-mediated hepatitis

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Calcitonin gene-related peptide (CGRP) regulates inflammation through receptor activity-modifying protein 1 (RAMP1), a subunit of CGRP receptor complex in immune cells. In this study, we examined the role of RAMP1 in immune-mediated liver injury. RAMP1-knockout ($RAMP1^{-/-}$) mice or their wild-type counterparts (WT) were treated with Concanavalin A (Con A). Compared with WT, $RAMP1^{-/-}$ mice exhibited higher levels of ALT, necrotic area, and the mRNA for pro-inflammatory cytokines including TNF and IFN. The numbers of macrophages and T cells in $RAMP1^{-/-}$ mice were greater than those in WT mice. Deletion of hepatic macrophages with clodronate liposomes attenuated ALT and necrotic area in both genotypes as compared with vehicle, which was associated with reduction in TNF and IFN. By contrast, splenectomy and deletion of T cells with anti-CD4 antibody partly attenuated Con A hepatitis. Adoptive transfer of splenic T cells from RAMP1-deficient mice led to a modest increase in liver injury elicited by Con A. Co-culture of hepatic macrophages with splenic T cells led to increased cytokine expression by both cells in a RAMP1-dependent manner. Thus, immune-mediated hepatitis develops via crosstalk between immune cells. RAMP1 signaling in hepatic macrophages plays a critical role in immune-mediated hepatitis.

1-O-09

Involvement of histamine H_3 receptor in the development of chemotherapy-induced anorexia in mice

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Cancer patients often develop anorexia during the course of cancer chemotherapy. It is known that daytime sleepiness is also the major complaints made by cancer patients, and this sleep problem can worsen anorexia. Previous studies reported the histamine H_3 receptor as a target for treating sleep disorders, and selective H_3 receptor inverse agonists are effective at reducing daytime sleepiness. In this study, we investigated the involvement of the H_3 receptor in the development of chemotherapy-induced anorexia in mice. Cisplatin (7.5 mg/kg, i.p.) induced anorexia within 24 hours of its administration and it continued for 3 days, and daily administration of an H_3 receptor inverse agonist (ciproxifan, 1 mg/kg, s.c.) significantly inhibited the development of anorexia. Daily administration of an orexin 2 receptor agonist (YNT-185, 20 mg/kg, s.c.), which was reported to induce wakefulness in mice and to activate the histaminergic system, also inhibited the cisplatin-induced anorexia, and its inhibitory effects were antagonized by daily administration of an H_3 receptor silent antagonist (VUF5681, 5 mg/kg, s.c.). These results suggest that sleep problem may contribute to the development of cisplatin-induced anorexia in mice, and H_3 receptor inverse agonists have the potential to be candidates used as its treatment.

1-O-10

Properties of spontaneous electrical activity in the mouse proximal colon.

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Properties of spontaneous electrical activity in the mouse proximal colon were studied using intracellular recording. Spike complexes with a frequency of 1 ～ 3 cycles/min occurred in the longitudinal muscles. Each spike complex consisted of a burst of action potentials (APs) 15 ～ 35 mV in amplitude and 10 ～ 40 APs per complex. The resting membrane potentials were -40 ～ - 50 mV. Spike complexes were abolished by verapamil, a Ca^{2+} blocker, pinacidil, a K_{ATP} channel opener, NPPB, a Ca^{2+} - activated Cl^- channel (CACC) blocker, and bumetanide, a Na^+ - K^+ - $2Cl^-$ co-transporter (NKCC1) inhibitor. Spike complexes and spontaneous transient depolarizations (STDs) were recorded from myenteric interstitial cells of Cajal (ICC-MY). Spike complexes in ICC-MY were also abolished by verapamil, pinacidil, NPPB and bumetanide. The amplitude of STDs were inhibited by verapamil and NPPB, but increased by pinacidil. These results suggest that the activation of CACC is associated with the generation of spontaneous electrical activity in the mouse proximal colon. Since CACC and NKCC1 are expressed only in ICC-MY, spike complexes seem to be generated in ICC-MY and transferred to nearby smooth muscle layers electrotonically.

1-O-11

Significance of SGLT2 in glucagon secretion from α-TC cells

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SGLT2 inhibitors lower blood glucose levels by preventing renal glucose reuptake, but they often cause hyperglucagonemia. Recent studies suggested its expression and potential role as a glucagon suppressor in pancreatic alpha cells, though it has been under debate. Thus, we conducted functional analyses of SGLT2 in a typical model of pancreatic alpha cell, α-TC cells to unveil roles of SGLT2 in the glucagon secretion. Glucagon secretion as well as intracellular ATP level decreased in response to glucose deprivation. SGLT2 inhibitors reduced glucose uptake, but glucagon secretion nor ATP level was affected. An inhibitor of KATP channel increased glucagon secretion without changing ATP level. Therefore, glucose starvation should not facilitate but mitigate glucagon secretion in α-TC cells possibly by raising AMP/ATP ratio which mitigates membrane potential through KATP channel. We also found SGLT2-mediated glucose uptake in α-TC cells. Nevertheless, the glucose influx is supposed to be too small to take effects on ATP level, and SGLT2 inhibitors should not directly alter glucagon secretion. Glucose starvation-induced glucagon secretion may require interaction among different types of the cells in islets.

1-O-12

Thiamine supplementation modulates oxidative stress by inhibiting hepatic ADP-ribosylation in obese diabetic rats

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Overactivation of poly (ADP-ribose) polymerase-1 (PARP-1) has been implicated in the pathogenesis of oxidative stress-related diseases, including diabetes and its complications. Obesity is linked with type 2 diabetes. We previously reported that thiamine supplementation prevents obesity and diabetes-related liver disease. In this study, we focused on hepatic ADP-ribosylation, which reflects the activation of PARP-1. Obese diabetic Otsuka Long-Evans Tokushima fatty (OLETF) rats were randomly divided into two groups: a thiamine-supplemented group and an unsupplemented control group. ADP-ribosylated protein expression in the liver was determined by western blotting. Obese diabetic OLETF rats showed increased ADP-ribosylated protein expression in the liver. Hepatic ADP-ribosylated protein expression in thiamine-supplemented OLETF rats was lower than that in the unsupplemented control OLETF rats. These results suggest that thiamine supplementation modulates oxidative stress by inhibiting hepatic ADP-ribosylation in OLETF rats. The beneficial effect of high-dose thiamine on oxidative stress-related diseases may also be attributable to its inhibitory effect on PARP-1activation in addition to its role as a coenzyme.

1-O-13

PPARα agonist-induced nicotinic receptor α2 subunit gene expression in the rat liver

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In our previous study, we extracted many genes commonly up-regulated by PPARα agonists (fibrates) in the rat liver using the transcriptome database created by The Toxicogenomics Project (TG-GATEs). Of these, nicotinic receptor α2 subunit (Chrna2) and acetylcholine (Ach) esterase anchoring protein (Colq) were markedly up-regulated by fibrates without any changes in other Ach receptors. Their induction was confirmed by quantitative PCR in the rats received fenofibrate for 3 days. The expression of Chrna2 was largely increased with repeated administration but not observed in the primary cultured hepatocytes. In the meantime, it was reported that Chrna2 was up-regulated in PPARγ-activated beige adipocytes and Ach released from immune cells stimulated the receptor to induce thermogenic protein, UCP1（Nature Med. 24 814, 2018). We then checked UCPs and found that UCP3, not UCP1, was markedly up-regulated by fibrates. The present data suggest that hepatocytes have a specific energy consuming system, PPARα>Chrna2>UCP3, similar to but different from that in adipocytes.

1-O-14

The role of phosphatidylcholine transfer by STARD10 and synthesis by LPCAT1 in lipid droplet formation

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Lipid droplet (LD) is surrounded by phospholipid monolayer mainly composed of phosphatidylcholine (PC). Steroidogenic acute regulatory protein (StAR)-related lipid transfer domain containing 10 (STARD10) has been shown to transfer PC between membranes *in vitro*. Lysophosphatidylcholine acyltransferase1 (LPCAT1) catalyzes the conversion of lysoPC to PC. The purpose of this study was to elucidate the role of STARD10 and LPCAT1 in LD formation. We hypothesized that the LD size depends on the surface-to-volume ratio. STARD10 and LPCAT1 were partly co-localized at the surface of LD in mouse hepatoma cells (Hepa1-6). The number of small LDs was increased by LPCAT1 overexpression, while the number of large LDs was increased by the overexpression of both STARD10 and LPCAT1. The percentage of small LDs was significantly higher in *Stard10* knockout Hepa1-6 cells than that of normal Hepa1-6 cells. In the liver of *Stard10* knockout mice, the total LD area was smaller and the sphericity of LD was lower than those of wild type mice, indicating that surface-to-volume ratio was higher. These results indicate that STARD10 and LPCAT1 are involved in LD formation by regulating surface-to-volume ratio through its activity of PC transfer and synthesis.

1-O-15

Copper chelater cuprizone inhibited high-fat diet induced adiposity.

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[Introduction] With the increase of obese and diabetic patients, anti-obesity drugs which decrease fat content without muscle atrophy are expected. In obese or diabetic patients, serum copper concentration was reported to increase compared with that in healthy subjects. However, it is still unknown whether the lowering of serum copper levels has anti-obese effects. Therefore, we investigated the effects of copper chelator, cuprizone on high fat diet (HFD)-induced obesity in mice model.

[Method] We administered cuprizone (0.2%w) mixed in food pellet. Mice fed (1) normal chow (NC), (2) NC with cuprizone, (3) HFD or (4) HFD with cuprizone for 4 weeks, and then metabolic parameters were obtained.

[Results] Serum copper level was decreased in both cuprizone groups. Cuprizone significantly decreased the body weight extensively in HFD, but slightly in NC fed mice, without changes of food intake. Interestingly, cuprizone specifically decreased 60% of epididymal and inguinal fat weights, but not liver and muscle (soleus and gastrocnemius) weights, only in HFD. Furthermore, HFD-induced glucose intolerance (ipGTT) and insulin resistance (ITT) were significantly improved by cuprizone

[Conclusion] Cuprizone inhibited HFD-induced adiposity via the decreased amount of fat depot.

1-O-16

Induction of mPGES-1 in dopaminergic neurons contributes to neurodegeneration in Parkinson's disease

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Although increased production of prostaglandin E_2 (PGE_2) has been implicated in tissue damage in several pathological settings, the role of microsomal prostaglandin E synthase-1 (mPGES-1), an inducible terminal enzyme for PGE_2 synthesis, in dopaminergic neurodegeneration remains unclear. Here we show that mPGES-1 is up-regulated in the dopaminergic neurons of the substantia nigra of postmortem brain tissue from PD patients and in 6-hydroxydopamine (6-OHDA)-induced PD mice. The expression of mPGES-1 was also up-regulated in cultured dopaminergic neurons stimulated with 6-OHDA. The genetic deletion of mPGES-1 not only abolished 6-OHDA-induced PGE_2 production but also attenuated 6-OHDA-induced dopaminergic neurodegeneration both *in vitro* and *in vivo*, while it did not affect the productions of PGI_2, PGD_2 and TXA_2. Nigrostriatal projections, striatal dopamine content, and neurological functions were significantly impaired by 6-OHDA administration in wild-type mice, but not in mPGES-1 knockout mice. These results suggest that induction of mPGES-1 in dopaminergic neurons enhances 6-OHDA-induced dopaminergic neurodegeneration through excessive PGE_2 production. Thus, mPGES-1 may be a valuable therapeutic target for treatment of PD

1-O-17

Midnolin promotes neurite outgrowth and expression of parkin ubiquitin ligase, and is associated with Parkinson's Disease.

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Parkinson's Disease (PD) is one of the most common neurodegenerative diseases, resulting from degeneration of dopaminergic neurons in substantia nigra. Although more than twenty causative genes have been identified to date, the majority is sporadic and the detailed pathological mechanism remains unclear. We identified a novel PD-associated gene, *Midnolin* (*MIDN*) by Yamagata cohort study. The copy number of *MIDN* was reduced in 10.5% of patients with sPD, whereas no copy number variation was found in healthy people. Therefore, we examined the pathophysiological roles of MIDN in this study. NGF caused gene expression of *MIDN* in a time- and concentration-dependent manner in PC12 cells. NGF-induced neurite outgrowth was completely inhibited in PC12 cells where *MIDN* gene was knocked out by genome-editing. Furthermore, we found that mRNA and protein expression of parkin E3 ubiquitin ligase was inhibited by *MIDN* knockout or siRNA targeting *MIDN* in PC12 cells and SH-SY5Y cells. These results suggest that the loss of *MIDN* gene causes inhibition of neurite outgrowth and parkin expression, which may result in the onset of PD.

1-O-18

Facilitation of functional synaptic formation of grafted-iPSC-derived dopaminergic progenitors with host striatal neurons

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To realize cell transplantation therapy for Parkinson's disease (PD), the grafted neurons should be integrated into the host neuronal circuit in order to restore the lost neuronal function. Here, using wheat germ agglutinin-based trans-synaptic tracing, we show that integrin α5 is selectively expressed in striatal neurons that are innervated by midbrain dopaminergic (DA) neurons. Additionally, we found that integrin α5β1 was activated by the administration of estradiol-2-benzoate (E2B) in striatal neurons of adult female rats. Importantly, we observed that the systemic administration of E2B into hemi-parkinsonian rat models facilitates the functional integration of grafted DA neurons derived from human induced pluripotent stem cells into the host striatal neuronal circuit via the activation of integrin α5β1. Finally, methamphetamine-induced abnormal rotation was recovered earlier in E2B-administrated rats than in rats that received other regimens. Our results suggest that the simultaneous administration of E2B with stem cell-derived DA progenitors can enhance the efficacy of cell transplantation therapy for PD.

1-O-19

C9ORF72-linked proline-arginine dipeptide repeat protein inhibits RNA helicase-mediated ribosome biogenesis and causes neurotoxicity

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A GGGGCC repeat expansion in the *C9ORF72* gene has been identified as the most common genetic cause of amyotrophic lateral sclerosis and frontotemporal dementia. The repeat expansion undergoes unconventional translation to produce dipeptide repeat proteins. Although it has been reported that dipeptide repeat proteins cause neurotoxicity, the underlying mechanism has not been fully elucidated. In this study, we show that the expression of proline-arginine repeat protein (poly-PR) reduces levels of ribosomal RNA and causes neurotoxicity. The poly-PR-induced neurotoxicity is restored by the acceleration of ribosomal RNA synthesis. This result suggests that the poly-PR-induced inhibition of ribosome biogenesis contributes to the poly-PR-induced neurotoxicity. Furthermore, we show that poly-PR interacts with multiple DEAD-box RNA helicases and inhibits the function of at least one of them, and that the reduction in the levels of some RNA helicases results in both the decrease in ribosomal RNA levels and the increase in neuronal cell death. Altogether, these results suggest that poly-PR causes neuronal toxicity by inhibiting the DEAD-box RNA helicase-mediated ribosome biogenesis.

1-O-20

Sulfite protects neurons from oxidative stress.

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Hydrogen sulfide (H_2S) and polysulfides (H_2S_n) are signalling molecules that mediate various physiological responses including cytoprotection. Their oxidized metabolite sulfite (SO_3^{2-}) is found in blood and tissues. However, its physiological role remains unclear. In this study, we investigated the cytoprotective effect of sulfite on neurons exposed to oxidative stress caused by high concentrations of the neurotransmitter glutamate, known as oxytosis. Free sulfite, present at approximately 2 μM in the rat brain, converts cystine to cysteine more efficiently than H_2S and H_2S_n and facilitates transport of cysteine into cells. Physiological concentrations of sulfite protected neurons from oxytosis and were accompanied by increased intracellular concentrations of cysteine and GSH probably due to converting extracellular cystine to cysteine, more efficiently than H_2S and H_2S_n. In contrast, thiosulfate only slightly protected neurons from oxytosis. Our present data have shown sulfite to be a novel cytoprotective molecule against oxytosis, through maintaining cysteine levels in the extracellular milieu, leading to increased intracellular cysteine and GSH. Our results provide a new insight into the therapeutic application of sulfite to neuronal diseases caused by oxidative stress

1-O-21

Involvement of α-melanocyte-stimulating hormone-thromboxane A_2 system in spontaneous scratching in mice with atopy-like dermatitis

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α-Melanocyte-stimulating hormone (α-MSH) is an endogenous peptide hormone that is involved in cutaneous pigmentation. Recent our study has demonstrated that α-MSH elicits scratching, an itch-related response, in mice. In this study, we investigated whether α-MSH was involved in spontaneous scratching in mice with atopy-like dermatitis (AD-mice). α-MSH and the prohormone convertase 2, which is the key processing enzyme for the production of α-MSH, were distributed mainly in keratinocytes in AD-mice. In primary cultures of mouse keratinocytes and dorsal root ganglion (DRG) neurons, α-MSH receptors (MC1R and MC5R) mRNAs were expressed. MC1R antagonist agouti-signaling protein inhibited spontaneous scratching in mice with atopy-like dermatitis. In mice, α-MSH elicited itch-associated responses, which were inhibited by TP thromboxane (TX) receptor antagonist. In mouse keratinocytes, α-MSH increased the production of TXA_2, which was decreased in mouse keratinocytes treated with siRNA for MC1R and/or MC5R. α-MSH increased intracellular Ca^{2+} ion concentration in DRG neurons and keratinocytes. These results suggested that α-MSH-TXA_2 system is involved in spontaneous scratching in AD-mice.

1-O-22

Analysis of the model for food allergy sensitized via skin to use mice

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Food allergy is defined as "a phenomenon in which adverse reactions are caused through antigen-specific immunological mechanisms after exposure to a given food". The number of patients with food allergy is increasing in the last decades. Food allergy develops by failure of acquiring oral tolerance for the foods. However the reasons that acquiring oral tolerance is inhibited are unclear. Previously, we made the models of murine food allergy and oral tolerance for the food. In the models, we used ovalbumin (OVA) as a food antigen. OVA mixed with alum (OVA/alum) was injected into mice intraperitoneally for sensitizations, and the mice were challenged by oral treatment with OVA in the food allergy model. As indexes for food allergy, drop of body temperature, fecal condition, and the level of OVA-specific IgE in blood were estimated. In oral tolerance model, prior oral treatment with OVA prevented the development of food allergy. In recent years, it is reported that food allergy is associated with exposure of food antigens via skin. In this study, we made the model of food allergy sensitized via skin. Additionally, we researched whether prior oral treatment with OVA prevented the development of food allergy. As results, pre-treatment with OVA in the model of food allergy by skin exposure to OVA was able to suppress the indexes of food allergy partially.

1-O-23

Maackiain suppresses histamine H_1 receptor gene expression through the enhancement of dexamethasone-induced ERK dephosphorylation in HeLa cells

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As the expression level of a disease-sensitive gene is correlated with the symptom severity, suppression of its gene expression should be good therapeutics. We demonstrated that histamine H_1 receptor (H1R) gene is an allergic rhinitis (AR)-sensitive gene. We isolated maackiain (MCN) from Kujin that suppresses H1R gene up-regulation. We also showed that MCN bound to Hsp90 and disrupts the interaction between PKCδ and Hsp90, suggesting MCN modulates steroid signaling. Western blot analysis showed that MCN completely inhibited ERK phosphorylation although the inhibition of PKC dphosphorylation was partial, suggesting that MCN suppresses H1R gene expression by the additional mechanism. MCN enhanced dexamethasone-activated GRE promoter activity. MCN also enhanced dexamethasone-induced gene up-regulation for dual-specificity phosphatase 1 (DUSP1), that dephosphorylizes ERK. On the other hand, dexamethasone suppressed H1R gene up-regulation. These findings suggest that MCN suppresses H1R gene expression through not only the disruption of interaction between PKCδ and Hsp90 but also the activation of ERK dephosphorylation by the enhancement of dexamethasone-induced DUSP1 gene expression.

1-O-24

Modulation of histamine H_4 receptor signaling by transglutaminase and histamine transporter in mast cells

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Mast cells migrate toward histamine through H_4 receptor (H_4R), which involves the activation of Rac1 and Rac2. Transglutaminase catalyzes the incorporation of histamine into cellular proteins, the process called histaminylation. Although several reports suggest possible roles of histaminlylation in mast cell signaling, there is no direct evidence showing its significance in cellular function. To explore the functional significance of histaminylation, we first investigated the effect of cystamine, a transglutaminase inhibitor, on H_4R-Rac pathway in mast cell. Cystamine attenuated histamine-induced migration and Rac activation, suggesting that H_4R-Rac signaling requires transglutaminase. Organic cation transporter 3 (OCT3) transports histamine into cytoplasm from extracellular space. Given the histaminylation occurs and regulates signaling in mast cells, we next examined if OCT3-mediated transport of histamine plays any role in H_4R signaling. Decynium-22, an inhibitor of OCT3, suppressed histamine-induced migration and Rac activation, suggesting the involvement of OCT3 in H_4R-Rac signaling. Our findings support the idea that transported histamine and transglutaminase activity modulate H_4R-Rac pathway.

1-O-25

Disseminated intracellular coagulation in mice with cecal ligation and puncture-induced sepsis.

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Disseminated intracellular coagulation (DIC) is a serious, life-threatening disorder characterized by systemic activation of the blood coagulation pathway, which leads to generation and deposition of fibrin, resulting in microvascular thrombi in various organs and contributing to multiple organ dysfunction syndrome. Sepsis is frequently complicated by coagulopathy and, in about 35 % of severe cases, by DIC. The development and severity of *DIC* correlate with mortality in severe *sepsis.* We examined whether overt DIC is found in mice with cecal ligation and puncture (CLP)-induced sepsis, which serve as an animal model that has high clinical relevance to humans. We found that the number of blood platelets strikingly declined in CLP-induced septic mice as compared with sham-operated controls. Plasminogen activator inhibitor-1, a critical regulator of the fibrinolytic system, was markedly increased in all of major organs after CLP, and tissue factor, which has a pivotal role in initiating the extrinsic pathway of blood coagulation, was significantly elevated in lungs and kidneys after CLP. Finally, CLP mice exhibited an abnormal prothrombin time, suggesting that they represent an appropriate model for investigating sepsis-induced DIC.

1-O-26

Analyses of nanoscale motions in cochlear sensory epithelium

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The cochlea of the inner ear converts sounds into electrical signals. This process is triggered by sound-evoked nanoscale vibrations in the sensory epithelium inside the organ. The epithelium contains outer hair cells that have mechanosensory hair bundles at the apical surface. The deflection of the bundles enters cation through ion channels. The epithelial vibrations are modulated by cation-induced elastic motions in the cell bodies. How the vibrations are regulated *in vivo* has not yet fully elucidated. Here we develop an advanced laser interferometry that precisely detects the vibrations. When a live guinea pig was exposed to acoustic stimuli, the interferometer quantitatively recorded the vibration amplitude of the sensory epithelium as described elsewhere. Additionally, an upward baseline shift of several nanometers was also detected. This motion was observed with loud sounds of >70 dB, and it was negligible when the animal was dead or under pharmacological perturbation of hair-bundles or cell body motions. A theoretical approach further suggested that the shift protects the epithelium from injury induced by strong stimuli.

1-O-27

Disruption of gap junction-mediated intercellular communication in the spiral ligament causes outer hair cell loss in the cochlea.

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It is well-known that cochlear outer hair cell (OHC) loss concomitant with permanent hearing loss induced by intense noise. Our earlier studies demonstrated the production of hydroxynonenal and peroxynitrite, as well as the disruption of gap junction-mediated intercellular communication (GJIC), in the cochlear spiral ligament prior to noise-induced hearing loss. The purpose of this study was to evaluate the mechanism underlying cochlear OHC loss induced by intense noise exposure. In organ of Corti explant cultures from mice, no significant OHC loss was observed after exposure to 4-hydroxynonenal (4-HNE, a product of lipid peroxidation), SIN-1(peroxynitrite generator), and carbenoxolone (a GJ inhibitor). *In vivo* intracochlear carbenoxolone injection through the posterior semicircular canal caused marked OHC and hearing loss, as well as the disruption of GJIC in the cochlear spiral ligament. However, no significant OHC loss was observed *in vivo* in mice treated with 4-HNE and SIN-1. In conclusion, our data suggest that disruption of GJIC in the cochlear spiral ligament is an important cause of cochlear OHC loss in models of noise-induced hearing loss.

1-O-28

KNT-127, a delta-opioid receptor agonist, modulates heart rate via the insular cortex in mice.

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Delta-opioid receptors (DOR) are highly expressed in the insular cortex, which regulates various autonomic functions including cardiovascular responses. However, the functional roles of DOR in the insular cortex are yet unknown. In the present study we investigated the effects of KNT-127, an agonist of DOR, on the heart rate and neuronal activities in the insular cortex of mice. ddY male mice (5-9 weeks) were used. The electrocardiogram was recorded using wire electrodes inserted subcutaneously in freely moving mice. The extracellular neuronal activities were recorded in the insular cortex in anesthetized mice. The field excitatory postsynaptic potentials (fEPSP) were recorded in the acute insular cortex slices. Microinjection of KNT-127 (46 ng /mouse) in the right, but not left, side of the insular cortex significantly decreased the heart rate. The firing frequency of insular neurons was reduced by intracerebroventricular administration of KNT-127 (230 ng /mouse). Moreover, the fEPSP amplitude was reduced by bath-application of KNT-127 (1-100 μM). Together, activation of DOR in the insular cortex modulates the heart rate via the suppression of neuronal activities.

1-O-29

Sepiapterin reductase gene disrupted mice suffered from severe priapism.

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(6R)-L-erythro-5,6,7,8-tetrahydrobiopterin (BH4) is an essential cofactor for phenylalanine-catabolism, and production of monoamines and nitric oxide (NO). Sepiapterin reductase (SPR) catalysis the final step of BH4 biosynthesis. Previously, we established SPR gene disrupted (*Spr*$^{-/-}$) mice (OYC32, Lexicon Pharmaceuticals Inc.) and reported that they exhibited dystonic posture, low body weight, hyperphenylalaninemia and unstable hypertension with bradycardia. Recently, we found that *Spr*$^{-/-}$ mice suffered from severe priapism (persistent erection) at high incidence (69.2%) and analyzed their penile tissues to reveal the mechanism. The content of BH4 and noradrenaline in the penile homogenate of *Spr*$^{-/-}$ mice significantly decreased compared to those of wild type (*Spr*$^{+/+}$) mice, which were 0.69% and 17.4%, respectively. There was no significant difference in the protein amount of eNOS, PDE5A, p-PDE5A between two genotypes. Tyrosine hydroxylase significantly decreased and, on the contrary, that of nNOS significantly increased in the penis of *Spr*$^{-/-}$ mice. NO metabolites significantly increased in the penis of *Spr*$^{-/-}$ also. Thus, we concluded that sympathetic nerve failure and up-regulation of NO production contribute to the severe priapism of *Spr*$^{-/-}$ mice.

1-O-30

The efficacy and safety of a laser thrombolytic system in animal thrombosis models

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For treating acute cerebral infarction, we developed a laser thrombolytic system with the second harmonic generation of microsecond Nd:YAG laser, and investigated its effectiveness, safety and mechanisms in animal thrombosis models. The dynamics of laser-induced thrombolysis in a gelatin phantom model was investigated with a high speed camera. The observation revealed that laser irradiation generated a bubble in the gelatin phantom. In vivo thrombolytic efficacy was investigated using animal thrombosis models. Thrombi in the vena cava inferior of rats or in the carotid artery of rabbits were induced by an application of ferric chloride ($FeCl_3$). Laser irradiation was then carried out through an optical fiber inserted from the femoral vein or artery. Laser irradiation induced significant thrombolysis in the rat thrombosis model. Laser irradiation also resulted in recanalization in the rabbit thrombosis model. One day after the recanalization, neurological disorders, cerebral ischemia and cerebral hemorrhage were not observed. No vascular endothelial damage evaluated by Evans blue staining after laser irradiation was observed. The irradiation of the pulsed green laser can induce bubbles that fragment thrombi without vessel or brain damage.

1-O-31

Expression and Roles of N-type Ca^{2+} Channel in Cardiac Myocytes

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[**Background**] Voltage dependent Ca^{2+} channels are divided to L-, T-, N-, P/Q-, and R- types, and N-type Ca^{2+} channel (NCC) are mainly expressed in nerve terminal. Recently, NCC has been reported to express in adrenal gland and renal tubular cells. We examined whether NCC is expressed in cardiac myocytes and if so, the roles of this channel. [**Methods and Results**] NCC mRNA and protein are expressed in neonatal rat cardiac myocytes, using real time PCR and Western blot analysis. Immunocytochemistry showed this channel was expressed on myocyte plasma membrane. After birth the expression level of this channel in cardiac tissue was gradually decreased within 2 weeks. In pathological condition, such as 5 hours of hypoxia followed by 30 minutes of reoxygenation (H/R) and norepinephrine (10^{-5} mol/L, 24 hours) increased NCC expression in neonatal cultured myocytes. In addition, in adult rats (12 weeks) mRNA level of this channel was also increased in non-infarcted myocardium after 4 weeks of myocardial infarction. Furthermore, to clarify the role of NCC in myocyte, we examine the effect of ω-conotoxin, a selective NCC blocker. ω-conotoxin significantly suppressed H/R- and norepinephrine-induced lethal myocytes injury, assessed by LDH activity in cultured medium and caspase 3 activity in myocytes. [**Conclusion**] NCC is expressed in neonatal and pathological cardiac tissue, and augmented myocyte lethal injury.

1-O-32

Analysis of electropharmacological effects of intracellular Ca^{2+} handling modulator caldaret on the canine heart

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Since information is lacking regarding how the enhancement of net sarcoplasmic reticulum (SR) Ca^{2+} uptake may affect cardiac electrophysiological properties in vivo, we analyzed it with caldaret which can decrease SR Ca^{2+} leak, enhance SR Ca^{2+} reuptake and inhibit reverse-mode Na^{+}/Ca^{2+} exchanger. Caldaret in doses of 0.5, 5 and 50 μg/kg was intravenously administered over 10 min to the halothane-anesthetized beagle dogs (n=4), possibly providing pharmacologically effective plasma concentration. The low and middle doses increased the ventricular contraction, which can be explained by its on-target pharmacological activities. The high dose enhanced the sinus automaticity followed by its suppression in addition to the increase of the total peripheral resistance. The low and middle doses enhanced the atrioventricular conduction. The middle and high doses prolonged the ventricular effective refractory period without altering the intraventricular conduction or repolarization period. Thus, modulation of intracellular Ca^{2+} handling by caldaret can induce not only inotropic effect, but also various electrophysiological actions on the in situ heart.

1-O-33

Atrial natriuretic peptide exerts hypotensive effect via regulator of G-protein signaling 2-mediated endothelial hyperpolarization

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Objective: Atrial natriuretic peptide (ANP) functions via guanylyl cyclase (GC)-A, a single transmembrane receptor, resulting in diuresis, natriuresis, and lowering of blood pressure. However, molecular mechanism of hypotensive effect of ANP is not well understood. ***Results***: Immunohistochemistry indicated that GC-A is abundantly expressed in endothelial cells. Intravenous infusion of ANP in wild-type mice significantly lowed systolic blood pressure. ANP failed to lowering systolic blood pressure in endothelial cell-specific GC-A knockout mice. In endothelial nitric oxide synthase knockout mice, ANP also significantly lowed systolic blood pressure. In in vitro study, ANP treatment significantly hyperpolarized cultured human umbilical vein endothelial cells (HUVECs), but not changed intracellular Ca^{2+} concentration in HUVECs. ANP-induced hyperpolarize of HUVECs was dependent of cyclic GMP-Protein Kinase G and regulator of G-protein signaling2 (RGS2) pathway but not dependent of Ca^{2}-activated K^{+} channels. ***Conclusions***: These results suggest that hypotensive effect of acute ANP administration can be caused by endothelial GC-A-mediated RGS2 pathway-dependent endothelial hyperpolarization.

1-O-34

Analysis of pregnancy hypertensive phenotype in histidine-rich glycoprotein gene-deficient mice.

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Hypertensive disorders of pregnancy (HDP) is a pathological condition with hypertension and vascular endothelial cell dysfunction (inflammation) during the gestational period. Recently, it was reported that reduction level of plasma histidine-rich glycoprotein (HRG) in human pregnant patients was correlated with the HDP seriousness. HRG is an anti-inflammatory factor that controls the progression of systemic inflammatory pathology such as sepsis syndrome. However, HRG functions in HDP pathology and perinatal physiology have not been clarified yet. In this study, we examined the pregnancy phenotype of HRG gene-deficient (HRG KO) mice to clarify the involvement of plasma HRG on placental formation and hypertensive event during the gestational period. Pregnant HRG KO mice had fetal growth disorder and placental hyperplasia. There was no change in the expression levels of inflammatory factors (IL-1beta, TNF-alpha) in the placenta. On the other hand, the expression level of angiogenic factors (VEGF, PlGF) in placenta increased. In addition, pregnant HRG KO mice had hypertension. These results suggest that HRG may have a physiological function in the gestational period. Pregnant HRG KO mice may be a HDP-like pathological model that causes abnormalities in the placental formation mechanism controlled by HRG.

1-O-35

Identification of novel mechanisms in pulmonary hypertension using omics data

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Pulmonary hypertension (PH) is a heterogeneous disorder associated with a progressive increase in pulmonary artery resistance and pressure. Although various therapies have been developed, the 5-year survival rate of PH patients still remains low. There is thus an important need to identify novel molecular networks involved in the pathogenesis of PH. In this study, we performed comparative transcriptome analysis of two mammalian PH models. In the one model, PH was caused by chronic hypoxia (Hx model). In the other model, PH was caused by the combination of a vascular endothelial growth factor receptor antagonist Sugen 5416 and chronic hypoxia (SuHx model). Intima and media proliferation and its consequent pulmonary vascular obstruction were prominent in SuHx model. Comparative transcriptome analysis revealed that five genes were significantly dysregulated in SuHx model and these genes might be regulated by Fos-related antigen-2（Fra-2）. The immunohistochemal analysis confirmed that the expression of Fra-2 were induced and localized within the cell nucleus in the pulmonary vascular lesions in SuHx model but not Hx model. These results suggest that Fra-2 may be involved in the pathophysiology of PH and a novel therapeutic target for the treatment of PH.