2-O-01

Endothelin-1 stimulates transcription of cyclin D1 and Skp2 through activation of Stat3 in cultured rat astrocytes

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Activation of Stat3, a member of the Stat family of transcription factors, plays a pivotal role in induction of reactive astrocytes and glial scar formation. Endothelin-1 (ET-1) increases in brain disorders and promotes astrocytic proliferation through ET_B receptors. In this study, to clarify mechanisms underlying astrocytic proliferation, the effects of ET-1 on Stat3 were examined in rat cultured astrocytes. Treatment with ET-1 stimulated Ser727 phosphorylation of Stat3 in cultured astrocytes, although Tyr705 phosphorylation was not affected. ET-1 stimulated the binding of Stat3 protein to its consensus DNA fragments. ET-induced BrdU incorporation was reduced by Stat3 inhibitors and Stat3 siRNA. ET-1 increased the expression of cyclin D1 and Skp2 in cultured astrocytes. The effects of ET-1 on cyclin D1 and Skp2 expression were reduced by stattic, 5,15-DPP and Stat3 siRNA. ChIP-PCR analysis showed that ET-1 promoted the binding of Stat3 to 5'-flanking regions of rat cyclin D1 and Skp2 genes. These results suggest that cyclin D1 and Skp2 expression through Stat3-mediated mechanisms underlies ET-induced astrocytic proliferation.

2-O-02

Inositol 1,4,5-trisphosphate receptor type 2-independent Ca^{2+} release from the endoplasmic reticulum in astrocytes

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Astrocytes are actively involved in the physiological and pathophysiological functions of the brain. Ca^{2+} release from the endoplasmic reticulum (ER) is considered to be crucial for the regulation of astrocytic functions. Inositol 1,4,5-trisphosphate receptor type 2 (IP_3R2)-knockout (KO) mice are reportedly devoid of astrocytic Ca^{2+} signaling, and thus widely used to explore the significance of astrocytic Ca^{2+} signaling. However, functional deficits in IP_3R2-KO mice have been found in some reports, but not in others. To address this controversy, we re-evaluated the assumption that Ca^{2+} release from the ER is abolished in IP_3R2-KO astrocytes. We expressed the ER luminal Ca^{2+} indicator G-CEPIA1*er* in astrocytes to directly visualize Ca^{2+} release from the ER. We found attenuated but significant Ca^{2+} release in response to application of norepinephrine to IP_3R2-KO astrocytes. This IP_3R2-independent Ca^{2+} release induced only minimal cytosolic Ca^{2+} transients but induced robust Ca^{2+} increases in mitochondria that are frequently in close contact with the ER. These results indicate that ER Ca^{2+} release is retained and is sufficient to increase the Ca^{2+} concentration in close proximity to the ER in IP_3R2-KO astrocytes.

2-O-03

Peridinin suppresses zinc-enhanced M1 phenotype of microglia via inhibition of ROS generation

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[Aim] Extracellular zinc enhances pro-inflammatory cytokines secretion from M1 microglia via reactive oxygen species (ROS) generation. Here, we examined the effect of peridinin, a carotenoid in dinoflagellates, on the zinc-enhanced inflammatory M1 phenotype.
[Methods] M1 polarization of mouse microglia prepared was induced by lipopolysaccharide after $ZnCl_2$ treatment in the presence of peridinin, and cytokines secretion were assessed by ELISA. In addition, ROS detection assay and HPLC analysis were performed. Transient ischemia in mice was induced 5 min after peridinin injection. The levels of cytokines and M1 marker were examined by qPCR and immunostaining, respectively. Spatial memory was assessed by Y-maze test.
[Results] Peridinin prevented the zinc-induced aggravation of cytokines secretion from M1 microglia and increase in the microglial ROS levels. No shift in the absorption maximum of peridinin reacted with zinc was observed. Injection of peridinin suppressed ischemia-induced expression of cytokines and M1 marker, and memory impairment.
[Conclusion] These results suggest that peridinin exerts neuroprotective effects against the drastic post-ischemic inflammation by inhibiting the zinc-induced increase in the microglial ROS levels.

2-O-04
GPNMB$^+$ type 1 microglia in Alzheimer's disease

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The onset and progression of Alzheimer's disease (AD) correlate with neuroinflammatory processes, and inflammatory microglia (MG) are associated with AD-like pathology in a transgenic mouse model. However, the distinct role of MG subtypes in AD brain remains unclear. We recently developed a novel monoclonal antibody, 9F5, against one subtype (type 1) of rat primary MG, and identified the antigen molecule for 9F5: truncated form of rat GPNMB/osteoactivin (Kawahara et al., GLIA, 2016). In neonatal rat brain, GPNMB$^+$Iba1$^+$ MG were a portion of Iba1$^+$ MG, and were observed in specific brain areas including corpus callosum. However, the distribution and function of GPNMB$^+$ type 1 MG in AD brain are largely unknown. In the present study, we observed GPNMB $^+$Iba1$^+$ MG surrounding Aβ plaque in neocortex of amyloid precursor protein (APP23) transgenic mice. In addition, GPNMB$^+$Iba1$^+$ MG were observed in non-plaque areas of hippocampus of APP23 mice. We generated *Gpnmb* knockout mice to investigate the functional relevance of GPNMB for microglia *in vivo*. Homozygous *Gpnmb*-KO mice did not show any growth retardation including body weight loss, and the fertility was normal. We observed that AD-related brain dysfunction in APP23 mice were regulated by *Gpnmb* gene dosage. These finding suggest that GPNMB$^+$ type 1 MG may play a role in regulation of neuropathological process of AD.

2-O-05
3D imaging of Iba1-positive CNS macrophage

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Tissue macrophages in the central nervous system (CNS), including parenchymal microglia and non-parenchymal meningeal, perivascular and choroid-plexus macrophages, are important for the development and homeostasis of the healthy CNS. Also, there is a growing body of evidence that microglia plays a key role in neurological and psychiatric disorders. The aim of our study was to understand the spatial arrangement of these cells in the CNS and its change after peripheral inflammation, by using a genetic tool that can label Iba1-positive CNS macrophages (Nakayama et al., Nat Commun, 2018). Tissue clearing and 3D-imaging technique (CUBIC) clarified an activation of microglia in the spinal cord and brain region after peripheral inflammation.

2-O-06

Renin-angiotensin system-induced SPARC upregulation causes renal injury in hypertensive rats

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Background: Secreted protein acidic and rich in cysteine (SPARC), one of the ECMs, promotes inflammation in aging hearts. Pro-inflammatory and -fibrotic properties of ADAMTS1 is reported, and SPARC promotes collagen production via ADAMTS1 upregulation. This study investigated the roles of SPARC in hypertensive renal injury.
Methods: Uninephrectomized rats were treated with DOCA and salt for 0, 1, 2, or 3 weeks (DOCA-salt) with/without losartan (30 mg/kg). Blood pressure, proteinuria, CCr, and renal NADPH oxidase activity was measured. Fibrillar collagens and macrophages were detected by Masson's trichrome staining and immunohistochemistry, respectively. The protein levels of MCP-1, TGF-beta, collagen I, SPARC, and ADAMTS1 were examined by immunoblotting.
Results: Blood pressure increased time-dependently from 2w. Proteinuria increased from 2w and CCr decreased at 3w. NADPH oxidase activity, macrophage numbers, and MCP-1 increased in DOCA-salt. TGF-beta, tubulointerstitial fibrosis, and glomerulosclerosis increased time-dependently from 2w. The collagen I protein, in particular collagen I with larger molecular weight, increased in 3w. SPARC increased and peaked at 2w and reversed to the control levels at 3 weeks, and ADAMTS1 gradually increased until 3w, both of which were suppressed by Losartan.
Conclusions: Renal renin-angiotensin system-induced SPARC upregulation and subsequent ADAMTS1 production may mediate renal injury.

2-O-07

Effects of a non-steroidal selective of mineralocorticoid receptor antagonist, on blood pressure on hypertension and renal injury in low-renin salt-sensitive hypertensive rats

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Herein, we studied the effects of the novel nonsteroidal selective mineralocorticoid receptor (MR) blocker, esaxerenone, on blood pressure and renal injury in Dahl salt-sensitive (DSS) rats. We also monitored the urinary intact and total angiotensinogen (AGT). DSS rats were given a normal salt diet (NS: 0.4% NaCl, n = 10), high salt diet (HS: 8% NaCl), HS + esaxerenone (1 mg/kg/day, p.o.), or HS + losartan (angiotensin II receptor blocker, 10 mg/kg/day, p.o.) for 6 weeks. HS-treated DSS rats developed hypertension, albuminuria and glomerular injury, which were associated with increased glomerular desmin staining and reduced mRNA levels of glomerular *podocin* and *nephrin*. HS-treated DSS rats also showed tubulointerstitial fibrosis with an increase in renal oxidative stress (4-hydroxynonenal staining). The urinary (total AGT – intact AGT)/intact AGT ratio, an indicator of intrarenal renin activity, was significantly suppressed in HS-treated DSS rats. Treatment with esaxerenone significantly decreased blood pressure, while losartan did not. Furthermore, esaxerenone attenuated the development of albuminuria, glomerular injury and tubulointerstitial fibrosis more than losartan did, and this was associated with reduced renal oxidative stress. These data indicate that esaxerenone induces antihypertensive and renal protective effects in salt-dependent hypertensive rats with suppressed intrarenal renin activity, as indicated by low levels of the urinary (total AGT – intact-AGT)/intact AGT ratio.

2-O-08

Lipopolysaccharide induces filtrate leakage from tubular lumen to interstitial space via proximal tubular TLR4-dependent pathway

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We previously reported by using intravital imaging technique that lipopolysaccharide (LPS) slowed proximal tubular flow rate in an early phase of endotoxemia. Hereby, we hypothesized that LPS disrupts tight junction in proximal tubular cells and induce leakage of filtrate through a Toll-like receptor 4 (TLR4)-dependent mechanism. LPS at 5 mg/kg did not change glomerular filtration rate (GFR), and significantly reduced the washout rate of tubular fluid from the proximal tubules and urine output in the early phase, reflecting slowing down of tubular flow rate in the proximal tubules during oliguria. LPS at 15 mg/kg reduced both GFR and urine output. LPS (5 mg/kg) induced paracellular leakage of FITC-inulin and reduced tight junction mRNA expression (occludin and cldn2). LPS also increased water content, interstitial hydrostatic pressure and Na^+/K^+ ratio in the kidney, indicating the accumulation of extracellular fluid in the interstitium. The mice lacking TLR4 in proximal tubules showed markedly blunted aforementioned responses and an increased sensitivity to the fluid resuscitation. Our results suggest that LPS disrupted tight junction of proximal tubular cells via a TLR4-dependent mechanism, resulting in paracellular leakage of filtrate to interstitium, which blunted fluid sensitivity, during endotoxemia in mice.

2-O-09

GPR143, an L-DOPA receptor, may help control inflammation in adenine-induced chronic kidney disease

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L-3,4-dihydroxyphenylalanine (L-DOPA), a precursor of catecholamines, has been believed to be a pharmacologically inert amino acid. We previously showed that depressor and bradycardic responses to L-DOPA-microinjected into the nucleus tractus solitarii (NTS) were suppressed by shRNA knockdown of GPR143. The specific binding of [3H]-L-DOPA was detected in CHO cells expressing GPR143, which was displaced by L-DOPA CHE, an antagonist of L-DOPA. These findings suggest that L-DOPA itself can exert some of its actions through GPR143. GPR143 is expressed in both neuronal and non-neuronal organs including kidney. Although we reported that L-DOPA sensitize the vascular alpha 1 adrenergic receptor through activation of GPR143, peripheral functions of GPR143 are largely unknown. Here we focused on roles of GPR143 in adenine-induced chronic kidney disease model. *Gpr143* gene-deficient mice (*GPR143*-KO) and control mice were fed diets supplemented with 0.2% adenine. Although blood urea nitrogen and serum creatinine levels were similar, *Gpr143*-KO showed significant body weight loss of adenine diet than did wild type animals. Serum amyloid A gene expression was higher in *Gpr143*-KO of adenine diet, thereby suggesting that GPR143 may be involved in inflammation.

2-O-10

Inhibitory effects of SGLT2 inhibitor tofogliflozin on urate and glucose transport mediated by renal urate transporters

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SGLT2 is a sodium-coupled glucose cotransporter localized at the apical membrane of renal proximal tubule that uptakes glucose from urine into the cells in a Na^+-dependent manner. Although SGLT2 inhibitors are clinically used to reduce blood sugar, they exhibit uric acid lowering action at the same time. However, its molecular mechanism is still unknown. This study aims to elucidate its molecular mechanism behind the interaction between SGLT2 inhibitor and renal tubular transporters. cRNAs of URAT1 (*SLC22A12*), OAT10 (*SLC22A13*) and URATv1 (*SLC2A9*) were injected into *Xenopus* oocytes. 2-3 days later, we measured urate and glucose transport activities, the inhibition by glucose and SGLT2 inhibitor tofogliflozin (tofo) on uric acid transport, and the inhibition by urate on glucose transport. Urate transport by three was not inhibited by glucose. It was newly found that glucose is significantly transported by all, but since its transport inhibition by urate was not observed, any transporter has different substrate recognition sites for glucose and uric acid. In addition, urate transport by three was not inhibited by tofo.

Thus, urate-lowering action by SGLT2 inhibitor was considered unlikely to be due to the interaction with the existing major renal tubular urate transporter.

2-O-11

Zinc transporter ZIP7 regulates tumorigenic potential in human colorectal cancer cells

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Intestinal epithelium undergoes a continuous self-renewal to maintain the intestinal homeostasis. Recently, we identified ZIP7 as a novel zinc transporter, securing of vigorous proliferation of immature intestinal epithelial cells that are called transit-amplifying (TA) cells by resolving endoplasmic reticulum (ER) stress. Dysregulation of cell proliferation machinery has been implicated in tumorigenesis. In this study, we investigated the role of ZIP7 in colorectal cancer development. siRNA or shRNA knockdown of ZIP7 suppressed cell proliferation, which was assessed by cell counting assay or crystal violet analysis, in human colorectal cancer cell lines. Apoptotic cell death was caused by siRNA knockdown of ZIP7, suggesting that ZIP7 is required for survival of colon cancer cells. Xenograft assays in immunodeficient mice showed that tumors generated by ZIP7-depleted cells were smaller than those generated by control cells, showing the requirement of ZIP7 expression in cancer cells for tumorigenicity *in vivo*. Furthermore, we found that overexpression of ZIP7 substantially increased volumes and weights of the xenograft tumors. These findings suggest that ZIP7 not only is required for tumor formation but also promotes tumorigenesis and thus, represents an attractive therapeutic target for colorectal cancer.

2-O-12

Involvement of ALOX in resistance or sensitivity of cancer treatment via plasma membrane oxidation state.

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Although radiation therapy is one of the choices to treat cancers and is an excellent local treatment method, existence of radiation resistant cell is a major problem. We established clinically relevant radioresistant (CRR) cells that can survive exposing to 2 Gy/day X-rays for more than 30 days. However, the mechanism to obtain resistance has not been elucidated yet. We investigated the relationships between resistant mechanism to hydrogen peroxide (H_2O_2) and plasma membrane state in CRR cells.

CRR cells showed resistance to H_2O_2, but catalase enzyme activity was down-regulated. Plasma membrane potential were low, no internal H_2O_2 increase and no lipid peroxidation were seen even after 2 hours of H_2O_2 treatment in CRR cells. Administration of oxidized lipid led to further cell death after H_2O_2 treatment in CRR cells. The lipoxygenase (ALOX) gene and protein expressions were down-regulated in CRR cells. We also established stress-sensitive ρ^0 cells that lack mitochondrial DNA. Gene and protein expressions of ALOX were up-regulated in ρ^0 cells. Expression of cyclooxygenase-2 does not seem to be involved in this mechanism.

These results suggest that the involvement of ALOX in resistance or sensitivity of cancer treatment.

2-O-13

Discovery of anti-melanoma effect of flubendazole by zebrafish platform.

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Melanoma is one of the most deadly malignant diseases with the highest increase in incidence rate over the past 50 years. Even with new drug applications, its death rates have been stable over this decade. To discover new anti-melanoma drugs, we performed chemical screening using zebrafish melanoma allograft model. For preparing melanoma model of zebrafish, human BRAF mutation (BRAF[V600E]) -driven zebrafish melanoma cells were injected into the circulation of young zebrafish (48 hours-post-fertilization). The melanoma cells increased about 10-times on 6 days after implantation. We tested 2320 chemicals using this allograft zebrafish in combination with the fluorescence plate reader, and found that several chemicals can suppressed melanoma proliferation in vivo. Of these, flubendazole (FLBZ) also suppressed A375 melanoma cell proliferation in mouse xenografts. We further identified that the anti-melanoma function of FLBZ was responsible to inhibition of epithelial-to-mesenchymal transition, not to BRAF mutation or autophagy induction as previously reported. In summary, integrated cross-species analysis using zebrafish, mice and human cells revealed that FLBZ should be one of strong candidates for anti-melanoma drug.

2-O-14

An anti-podoplanin cancer-specific antibody LpMab-23 is a prognostic marker for oral cancer

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Purpose: Podoplanin (PDPN) is involved in cancer malignancy. PDPN is highly expressed in both cancer and normal cells. We aimed to develop cancer-specific anti-PDPN mAbs.
Methods: We immunized mice with PDPN-expressing cancer cells, and produced cancer-specific anti-PDPN monoclonal antibodies (mAbs). We characterized mAbs using flow cytometry (FCM) and immmunohistochemistry (IHC). Anti-tumor activities by a mouse-human chimeric mAb was examined using mouse xenograft models of oral cancer cells. We further investigated the possibility of PDPN as a diagnostic marker of oral cancers.
Results: One of established mAbs, LpMab-23 reacted with PDPN-expressing cancer cells, not with normal cells by FCM and IHC using oral cancers. LpMab-23 recognized a cancer-specific glycopeptide including Thr55/Ser56. chLpMab-23 revealed high ADCC and anti-tumor activities against oral cancers. We further showed that LpMab-23 is a prognostic marker of oral cancer. The Kaplan-Meier curves of the five-year new metastasis-free survival rate (nMFS) were significantly lower in LpMab-23-positive patients than in LpMab-23-negative ones.
Conclusion: LpMab-23 has antitumor activities, and LpMab-23-positive cases could be a useful predictor of poor prognosis for oral cancer.

2-O-15

Establishment of a culture method of dog bladder cancer organoids

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【Introduction】Bladder cancer (BC) is the most common neoplasm affecting the urinary tract of dogs. Stem cell-derived 3D organoid culture could recapitulate organ structure and physiology. In our previous study, urine sample-derived dog prostate cancer organoids have been established. However, urine-derived dog BC organoids have never been developed. We therefore generated dog BC organoids using the urine samples.

【Methods and Results】After dogs were clinically diagnosed with bladder tumor, urine samples were collected by catheterization and used for the organoid culture. Organoids from each BC dog were successfully generated. Expression of an epithelial cell marker (E-cadherin) and a myofibroblast marker (α-smooth muscle actin, (SMA)) was confirmed in the organoids. The organoids also expressed a stem cell marker (CD44). Injection of the BC organoids into immunodeficiency mice successfully generated tumor. While treatment with cisplatin and vinblastine decreased cell viability of organoids in a dose-dependent manner, treatment with gemcitabine and piroxicam had little effect on the cell viability of the organoids.

【Conclusions】These findings revealed that BC organoids derived from urine stem cells might become a useful tool to investigate the mechanisms of pathogenesis and treatment of dog BC

2-O-16

Noradrenaline reuptake inhibition enhances control of impulsivity by activating D_1-like receptors in the ventromedial prefrontal cortex

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Higher impulsivity is a risk factor for substance abuse and suicide, but only a few anti-impulsive drugs are clinically available. We recently proposed a strategy for identifying anti-impulsive drugs by investigating drugs that increase extracellular dopamine levels in the medial prefrontal cortex (mPFC) and stimulate dopamine D_1-like receptors, but not in the nucleus accumbens (NAc). To determine whether this strategy is promising, we examined the effects of duloxetine, a serotonin-noradrenaline reuptake inhibitor that might meet these criteria, on impulsive action in adult male Wistar/ST rats using a 3-choice serial reaction time task. The effects of duloxetine on the dopamine levels in the areas were evaluated using *in vivo* microdialysis, as the noradrenaline transporter mediates dopamine reuptake in some brain regions. We found that the administration of duloxetine reduced impulsive actions and increased dopamine levels in the mPFC but not in the NAc. Microinjection of a D_1-like receptor antagonist into the ventromedial prefrontal cortex blocked the suppression of impulsive action by duloxetine. These results support our proposed strategy for identifying and developing anti-impulsive drugs.

2-O-17

Activation of claustral glutamatergic neurons induces anxiety-like behaviors through catecholamine release

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Acute psychological stress dynamically changes functional brain networks and induces negative emotional states including anxiety through altered release of neuromodulators such as catecholamines. The underlying neuronal mechanisms of stress responses have been extensively investigated, however they remain unclear. Recently, we found that the neurons in the claustrum are critically involved in stress-induced anxiety-like behaviors as determined by whole-brain activity mapping and DREADD-based pharmacogenetic manipulation of these neurons. Here, we show that pharmacogenetic activation of glutamatergic neurons in a subregion of the claustrum induces anxiety-like behaviors and leads to activation of several nuclei involved in stress response. Activation of the claustral glutamatergic neurons induced a tonic release of dopamine and noradrenaline in the medial prefrontal cortex. Pharmacological inhibition of the catecholamine signal attenuated the claustral activation-induced anxiety-like behaviors. The present results suggest that glutamatergic neurons in the claustrum mediate stress-induced anxiety-like behaviors through modulating catecholamine release.

2-O-18

5-HT_{1A} and 5-HT_{2A} receptors in the amygdala, as target molecules of the anxiolytic action of SSRIs

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SSRIs are widely used as anxiolytics. Previously, we demonstrated that local injection of an SSRI into the basolateral nucleus of the amygdala (BLA) had anxiolytic effect in rats. In the present study, we investigated the effect of local co-administration of an SSRI and 5-HT_{1A} or 5-HT_{2A} antagonists into the BLA on conditioned fear in Wistar/ST rats, and indicated the expression of 5-HT_{1A} and 5-HT_{2A} receptor mRNAs in the BLA by *in situ* hybridization in C57BL/6 mice.

Local injection of citalopram (SSRI) into the BLA attenuated conditioned freezing, and this effect was blocked by local co-administration of WAY100635 (5-HT_{1A} antagonist) or MDL11939 (5-HT_{2A} antagonist). In *in situ* hybridization, 5-HT_{1A} mRNA was mainly expressed in GABAergic interneurons expressing somatostatin (SOM) mRNA, and 5-HT_{2A} mRNA was in those expressing parvalbumin (PV) or SOM mRNA.

From these results, it is speculated that SSRIs exert anxiolytic effect via 5-HT_{1A} and 5-HT_{2A} receptors in the BLA. Because PV- and SOM-positive GABAergic interneurons are known to form local neural circuits with glutamatergic pyramidal neurons, the anxiolytic action of SSRIs is likely to be mediated by serotonergic modulation of pyramidal neurons via these interneuron subclasses.

2-O-19

Functional activation of G(alpha)$_q$ proteins coupled with 5-HT$_{2A}$ and M$_1$ muscarinic acetylcholine receptor in prefrontal cortical membranes from suicide victims

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Suicide is a major public health concern and often associated with mental disorders, such as depression, bipolar illness, and schizophrenia. Although psychosocial stressors are major risk factors of suicide behavior, abnormalities in neurobiological mechanisms have been supposed to be another risk factor. In the present study, functional coupling of G(alpha)$_q$ proteins with 5-HT$_{2A}$ receptor and M$_1$ muscarinic acetylcholine receptor (mAChR) was determined by means of [^{35}S]GTPγS binding/immunoprecipitation assay in postmortem human brain membranes, and the effects of suicide on these measures were evaluated. Postmortem human brains were obtained from 20 patients with bipolar disorder, 20 depression, 20 schizophrenia, and 20 control subjects, and the patients groups included suicide victims in 14, 17, and 10 subjects, respectively. When these 80 subjects were divided into suicide victims (n = 41) and non-suicide (n = 39), there were no significant differences in %E$_{max}$, pEC$_{50}$, and slope factor values for each of the two measures between them. Our results indicate that 5-HT$_{2A}$ receptor- and M$_1$ mAChR-mediated signaling through G(alpha)$_q$ proteins is unaltered in suicide victims.

2-O-20

Implication of leukemia inhibitory factor in the formation of stress adaptation in mice

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Recent studies suggest that oligodendrocyte disruptions in the central nervous system can impact potentially to mood regulation in human psychiatric disorders. Leukemia inhibitory factor (LIF) has been shown to be involved in myelination. In the present study, we investigated whether LIF are involved in the formation of stress adaptation. A single exposure to restraint stress for 60 min induced a decrease in head-dipping behavior in the hole-board test. This emotional stress response was not observed in mice that had been exposed to repeated restraint stress for 60 min/day for 14 days, which confirmed the development of stress adaptation. In contrast, mice that were exposed to restraint stress for 240 min/days for 14 days did not develop the stress adaptation, and continued to show a decrease in head-dipping behavior. Major myelin proteins including myelin basic protein and myelin-associated glycoprotein expression were decreased in the hippocampus of stress-maladaptive, but not stress-adaptive, mice. Under these conditions, protein levels of LIF was significantly increased only in the hippocampus of stress-adaptive mice. These results indicate that increased LIF in the hippocampus may contribute to the development of stress adaptation.

2-O-21

Role of inward rectifier K^+ channel $K_{ir}2.1$ in mouse osteoblast differentiation

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Store-operated Ca^{2+} entry (SOCE) plays critical roles in intracellular Ca^{2+} ($[Ca^{2+}]_i$) homeostasis. Recent studies have shown that SOCE is essential for osteoblastic differentiation. In non-excitable cells, K^+ channels are key regulators of SOCE-mediated Ca^{2+} signaling and control cell proliferation, differentiation, and migration, however, the functional role of K^+ channels in osteoblast Ca^{2+} signaling remains unknown. In the present study, the contribution of K^+ channels to the SOCE activity in the osteoblastic cell line MC3T3-E1, established from mouse calvaria was investigated. We found that the expression levels of inward rectifier K^+ channel Kir2.1 transcripts were up-regulated in the differentiated MC3T3-E1 cells. The application of ML133, a Kir2 inhibitor, significantly reduced the SOCE-mediated $[Ca^{2+}]_i$ elevation in differentiated MC3T3-E1 cells, but not in immature MC3T3-E1 cells. In addition, the treatment with ML133 suppressed the expression of the differentiation markers in osteoblasts, and attenuated the endochondral ossification in murine embryonic metatarsals. These results suggest that Kir2.1 channels play essential roles in maintaining the bone homeostasis via modulating osteoblast differentiation.

2-O-22

Overexpression of KCNK9 exerts anti- and pro-apoptotic effect on PANC1 cells

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KCNK9, a member of the two-pore K^+channel family, is overexpressed in several types of human carcinomas, such as breast cancers and lung cancers, and is generally thought to be an oncogenic channel. However, it has been also reported that overexpression of KCNK9 induces apoptosis in some kinds of cells including cerebellar granular neurons. Thus, a paradox is observed in the effects of KCNK9 overexpression on apoptosis, which is considered to be due to differences in KCNK9 expression levels: low expression is anti-apoptotic and high expression is pro-apoptotic. In this study, we investigated whether it is true in the human pancreatic cancer cell lines, PANC1. For that purpose, KCNK9 was expressed transiently and stably in the cells. Transient overexpression of KCNK9 increased caspase activities in the transfected cells, whereas stable expression of KCNK9 made the cells resistant to hyperosmolarity-induced apoptosis. Although the mechanism is not fully understood, our data suggest that the opposite effect of KCNK9 overexpression on PANC1 apoptosis is not solely due to differential expression levels of the channels.

2-O-23

Prostanoid EP4 receptor-mediated augmentation of I_h currents in Abeta dorsal root ganglion neurons underlies neuropathic pain

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Neuropathic pain is refractory to conventional analgesics. Thus, the mechanism of neuropathic pain in rats with left L5 spinal nerve transection was reexamined. The patch-clamp technique was used on the isolated bilateral L5 dorsal root ganglion neurons. When rats exhibited established neuropathic pain, only the neurons with the diameter of 40-50 um (Abeta neurons) on the ipsilateral side showed a significantly larger density of an inward current at <–80 mV (I_h current) than that on the contralateral side. Ivabradine—an I_h current inhibitor—inhibited I_h currents in these neurons on both the sides in a similar concentration-dependent manner, with an IC_{50} of ～3 uM. Moreover, the oral administration of ivabradine significantly alleviated the neuropathic pain on the ipsilateral side. An inhibitor of adenylyl cyclase or an antagonist of prostanoid EP4 receptors (CJ-023423) inhibited ipsilateral, but not contralateral I_h currents in these neurons. Furthermore, the intrathecal administration of CJ -023423 significantly attenuated neuropathic pain on the ipsilateral side. Thus, ivabradine and/or CJ -023423 may be a lead compound for the development of novel therapeutics against neuropathic pain.

2-O-24

A novel binding site of allosteric modulators of G protein-gated inwardly rectifying K^+ (GIRK) channels

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GIRK channels regulate membrane excitability dependent on GPCR activity. Gain-of-function mutations in the channels have been identified in patients suffering from several diseases; e.g. primary aldsteronism. Since gene elimination of these channels yielded mild phenotypes of the mice, the channel blockage could be expected as therapeutic strategy. While channel blockers used to bind at a central cavity of ion permeation pathway located at the membrane domain, a loose spatial constraint of the mode of binding leads to a low selectivity of drug-channel interaction. Wide distribution of binding sites of their activators and blockers suggests that the prerequisite of conformational changes of entire molecules for functioning and the possibility to design allosteric modulators that bind to sites out of the central cavity. In an electron density map of a high-resolution crystal structure of Kir3.2 cytoplasmic region, we identified an electron density which was not belonging to the polypeptide chain. A model compound related to the shape of the density inhibited Kir3.2 activity. These results strongly suggested that the electron density and its surrounding area correspond to an allosteric modulator and its binding site.

2-O-25

Structure development of oxolinic acid, a novel inhibitor of type 1 ryanodine receptor

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Type 1 ryanodine receptor (RyR1) is a Ca^{2+} release channel on the sarcoplasmic reticulum in the skeletal muscle. Mutations in RyR1 cause various muscle diseases including malignant hyperthermia (MH) and central core disease (CCD). Although dantrolene is the only therapeutic drugs for MH, it cannot be used for CCD due to its lower solubility and side effects. It is therefore urgent to discover novel RyR1 inhibitors. We have recently identified oxolinic acid as a novel RyR1 inhibitor. However, affinity of oxolinic acid was much lower than that of dantrolene. In this study, we designed and synthesized a series of quinolone derivatives using oxolinic acid as a lead compound. Dose-dependent inhibitory effects were evaluated by ER Ca^{2+} measurement using HEK293 cells expressing R-CEPIA1er, a genetically-encoded ER Ca^{2+} indicator, and RyR1 carrying an MH mutation (R2163C). Compounds bearing a longer alkyl chain at the nitrogen atom of quinolone ring exhibited stronger RyR1 inhibiting activity. Modification at 6 and 7 positions of quinolone ring greatly affected the inhibitory activity. Derivatives of oxolinic acid may be good candidates for treatment of RyR1-related diseases.

2-O-26

PDGF-induced migration of synthetic vascular smooth muscle cells through c-Src-activated L-type Ca^{2+} channels with full-length $Ca_V1.2$ C-terminus

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Platelet-derived growth factor (PDGF) potently induces migration of vascular smooth muscle cells (VSMC); however, molecular mechanism underlying this phenomenon remains unclear. The migration of rat aorta-derived synthetic VSMCs, A7r5, in response to PDGF was potently inhibited by a $Ca_V1.2$ channel inhibitor, nifedipine, and a Src family tyrosine kinase inhibitor, bosutinib, in a less-than-additive manner. In contractile VSMCs, the C-terminus of $Ca_V1.2$ is proteolytically cleaved into proximal and distal C-termini (PCT and DCT, respectively). Clipped DCT is noncovalently reassociated with PCT to autoinhibit the channel activity. Conversely, in synthetic A7r5 cells, full-length $Ca_V1.2$ ($Ca_V1.2$FL) is expressed much more abundantly than truncated $Ca_V1.2$. In a heterologous expression system, c-Src was bound to, phosphorylated Tyr1709 and Tyr1758 in PCT and activated $Ca_V1.2$ channels composed of $Ca_V1.2$FL significantly more efficiently than $Ca_V1.2$ ($Ca_V1.2$delta1763) or $Ca_V1.2$delta1763 plus clipped DCT. Therefore, in atherosclerotic lesions, phenotypic switching of VSMCs may facilitate pro-migratory effects of PDGF on VSMCs by suppressing posttranslational $Ca_V1.2$ modifications.

2-O-27

Caveolae control excitation-transcription coupling in vascular smooth muscle cells.

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In smooth muscle cells (SMCs), caveolin (cav)-1, an essential component of caveolae, forms Ca^{2+} microdomain accumulating voltage-dependent Ca^{2+} channels (VDCC) and ryanodine receptors (RyR). The functional coupling between VDCC and RyR (Ca^{2+}-induced Ca^{2+} release: CICR) causes SMC contraction, i.e. excitation-contraction (E-C) coupling. On the other hand, Ca^{2+} influx through VDCC activates Ca^{2+}/calmodulin-dependent protein kinase, and promotes gene transcription in neurons, i.e. excitation-transcription (E-T) coupling. E-T coupling is known in SMCs, but its structural basis and physiologic function are unknown. Therefore, we examined the relationships between Ca^{2+} microdomain formed by caveolae and E-T coupling in SMCs. When the mesenteric artery was depolarized, the phosphorylation of CREB was detected in the nuclei of SMCs. This response was not observed in tissues from cav-1 KO mice that lack caveolae in SMCs and those in which caveolae were destroyed by methyl beta cyclodextrin. Inhibition of RyR by tetracaine also reduced the CREB phosphorylation. These results suggest that CICR in caveolae is necessary for the E-T coupling in SMCs. Caveolae can control not only SMC contractility but also gene expression by regulating Ca^{2+} signaling.

2-O-28

PDGF upregulates Ca^{2+}-sensing receptors in idiopathic pulmonary arterial hypertension

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Idiopathic pulmonary arterial hypertension (IPAH) is a rare and progressive disease of unknown pathogenesis. We previously reported that the Ca^{2+}-sensing receptor (CaSR) is upregulated in pulmonary arterial smooth muscle cells (PASMCs) from patients with IPAH, contributing to an enhanced Ca^{2+} response and excessive cell proliferation in IPAH-PASMCs. However, the mechanisms underlying the upregulation of CaSR in IPAH-PASMCs have not yet been elucidated. The present results demonstrated that platelet-derived growth factor (PDGF) and its signaling pathway promote the expression of CaSR in PASMCs, thereby facilitating Ca^{2+} responses, cell proliferation, and migration followed by pulmonary vascular remodeling. Therefore, siRNA knockdown of PDGFα/β receptors and STAT1/3 or imatinib (a tyrosine kinase inhibitor including PDGF receptors) blocked the CaSR upregulation and functions in IPAH-PASMCs. The combination of NPS2143 (a CaSR antagonist) and imatinib acted additively to inhibit the development of pulmonary hypertension in monocrotaline-treated rats. In conclusion, the crosslink between CaSR and PDGF signals is a novel pathophysiological mechanism contributing to the development of IPAH.

2-O-29

Angiotensin II-induced constriction via Rho kinase activation in pressure-overloaded rat thoracic aortas

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Inactivation of myosin light chain phosphatase thorough myosin phosphatase targeting subunit 1 (MYPT1) phosphorylation by Rho kinase is important for angiotensin II (Ang II)-induced constriction. However, the mechanism of Rho kinase activation by Ang II is unknown. We investigated whether Ang II-induced constriction in pressure-overloaded rat thoracic aortas is mediated by Rho kinase activation through Src, epidermal growth factor receptor (EGFR), extracellular signal-regulated kinase (Erk), and janus kinase (JAK).

The pressure-overload in rat thoracic aortas was produced by suprarenal abdominal aortic coarctation. After 4 weeks, thoracic aortas were excised and performed organ chamber experiments. Protein levels were measured by Western blotting.

Contractile response to Ang II significantly attenuated by inhibitors of Rho kinase, Erk1/2, Src, and EGFR in sham-treated and pressure-overloaded rats. Total and phosphorylated levels of MYPT1 and Src were increased in pressure-overloaded rat thoracic aortas. These data suggest that Ang II-induced constriction is mediated by Rho kinase activation via Src, EGFR, and Erk in pressure-overloaded rat thoracic aortas.

2-O-30

Proteinase-activated receptor 1 (PAR_1)-mediated cellular effects of coagulation factor XI in vascular smooth muscle cells

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Objectives: Coagulation factor XI (FXI) was reported to contribute to atherogenesis. The underlying mechanism remains unknown. We investigated the cellular effect of FXI and its mechanism in vascular smooth muscle cells.

Methods and main results: Activated FXI (FXIa) induced intracellular Ca^{2+} signaling mainly due to Ca^{2+} influx in the fura-2-loaded A7r5 cells, rat embryonic aorta smooth muscle cells. Pharmacological inhibitor (diltiazem) and genetic knockdown of L-type Ca^{2+} channel abolished the FXIa-induced Ca^{2+} influx. FXIa-induced Ca^{2+} signaling was abolished by atopaxar, a PAR_1 antagonist. FXIa, when pre-incubated with a proteinase inhibitor p-APMSF, failed to elicit Ca^{2+} signaling in A7r5 cells. FXIa failed to induce Ca^{2+} signal in embryonic fibroblasts derived from PAR_1-knockout mice. *In vitro* digestion assay revealed that FXIa cleaved the extracellular domain of PAR_1 at the same site that thrombin cleaved. FXIa accelerated cell migration of A7r5 cells in a wound healing assay. The acceleration of cell migration was partly inhibited by atopaxar and diltiazem.

Conclusions: We provides the first evidence that FXIa exerts cellular effect via PAR_1 in vascular smooth muscle cells.

2-O-31

Iron accumulation negatively regulates skeletal muscle myogenesis

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Background: Skeletal muscle mass is defined by the homeostatic coordination of muscle degeneration and regeneration under various pathophysiological conditions. We have previously reported that iron accumulation induces skeletal muscle atrophy via the ubiquitin-ligase dependent pathway. However, the actionof iron on muscle myogenesis has remained unclear. In the present study, we investigated the effect of iron on skeletal muscle myogenesis.

Methods: We examined muscle regeneration by using cardiotoxin (CTX)-induced muscle injury mice model with or without iron overload in *in vivo* experiments, and C2C12 mice myoblast cells for *in vitro* study.

Results: In mice with iron overload, the skeletal muscles exhibited an increase in oxidative stress and a decrease in the expression of satellite cells markers such as Pax-7 and MyoD expression. Following CTX-induced muscle injury, mice with iron overload also exhibited delay in muscle regeneration with the reduced size of regenerating myofibres, decreased expression of myogenin and myosin heavy chain, and less phosphorylation of the p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) 1/2. Similarly to the findings in the *in vivo* experiments, iron treatment also inhibited C2C12 myoblast cells differentiation, and it was ameliorated by a superoxide scavenger drug.

Conclusion: Iron accumulation suppresses skeletal muscle myogenesis through inhibiting MAPKs signalling via oxidative stress, causing an imbalance in the skeletal muscle homeostasis.

2-O-32
Roles of heparan sulfate in skeletal muscle differentiation

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Heparan sulfate (HS) is a sulfated linear polysaccharide around cell surface. HS is involved in various physiological processes. Recent studies revealed that lack of HS induced autism-like behaviors and hyperglycemia. However, the roles of HS in skeletal muscles remained to be elucidated.
First, we examined the role of HS in the differentiation of muscle cells using C2C12 cells, a mouse myoblast cell line. CRISPR/CAS9 technology was used to delete Ext1, which encodes a heparan sulfate synthase, to generate a HS-deleted C2C12 cell line. HS deletion dramatically impaired myoblast differentiation, demonstrating the essential role of HS in myoblast differentiation. Next, we generated skeletal muscle-specific Ext1 deleted mice (cKO). Muscle weakness of cKO was revealed in treadmill tests and wire hang tests. Histochemical analysis of skeletal muscles revealed that the cross sectional area of each muscle was smaller in cKO. Electromicroscopic observation showed that myofibrils were thinner in cKO. Finally, we examined muscle differentiation after muscle injury Myosin heavy chain expression, one of the marker proteins for muscle differentiation, was significantly decreased in cKO muscles. These results demonstrate that HS plays an important role in skeletal muscle, especially in differentiation.

2-O-33

Effects of the gabapentin, a newer antiepileptic drug, on bone metabolism in rat

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The relationship between the uses of antiepileptic drugs (AEDs) and the risk of fractures have been reported. While, there is only limited data concerning the effects of the newer AEDs on bone metabolism. In this study, we investigated the effects of gabapentin, a newer antiepileptic drug on bone metabolism. Male SD rats were administrated with gabapentin (30, 150 mg/kg) orally every morning for 12 weeks. Bone histomorphometry was analyzed using a semiautomatic image analyzing system, and bone strength was evaluated using a three-point bending method. Bone mineral density was measured using quantitative computed tomography, and serum biochemical markers (osteocalcin, tartrate-resistant acid phosphatase-5b) were examined. Administrated of gabapentin significantly decreased bone volume and increased trabecular separation, as shown by bone histomorphometric analysis. Moreover, the bone formation parameters, osteoid volume and mineralizing surface, were decreased after gabapentin treatment, whereas the bone resorption parameters, osteoclast surface and number, were increased. These results suggested that gabapentin may act on bone metabolism through the suppression of bone formation and enhancement of bone resorption.

2-O-34
The effects of Eucommia leaf extract (ELE) for rat bone marrow.

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We have reported that Eucommia leaf extract (ELE) influences the early stage of differentiation of both osteoblast and chondrocyte cells. Therefore, in this study, we focused on the bone marrow containing mesenchymal stem cells, which are the origin of both cells, and analyzed protein composition in bone marrow of rat administered with ELE. Femoral bones were extirpated from 2-month-old wistar rats orally administered with 10% ELE for 3 days. Bone marrow was washed out from the diaphysis of femur by lysis buffer and protein was extracted from the obtained bone marrow and fluorescent labeling was performed. Protein separation was then carried out by the immobilized pH gradient method. Subsequently, protein separation was carried out by molecular weight by performing SDS-PAGE. After electrophoresis, a fluorescence image of the gel was captured and image analysis was performed using ImageMsater 2D Platinum 7.0. Proteins were then extracted from significantly changed spots and mass spectrometry was performed by MALDI-TOF-MS. As a result of image analysis, 1,567 spots changed in expression level, and 74 spots were detected in which protein amount was changed more than 2 times. In addition, 20 spots were detected in which the expression was changed at 0.05> P.

2-O-35

Platelet aggregation of *quinonoid* dihydropteridine reductase knockout mice.

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Quinonoid dihydropteridine reductase (QDPR) regenerates tetrahydrobiopterin (BH4), an indispensable cofactor for synthesizing catecholamines and serotonin. We evaluated the platelet aggregation of QDPR gene knockout (*Qdpr* $^{-/-}$) mice. Citrated blood was collected from wild type (*Qdpr* $^{+/+}$) mice and *Qdpr* $^{-/-}$ mice (OYC35, Lexicon Pharmaceuticals Inc.). Platelet rich plasma (PRP) was obtained by centrifuging the blood, and adjusted platelet count to 250,000/μL. Platelets were stimulated with collagen (3～4 μg/mL) or ADP (5～20 μM) and aggregation was measured by light transmission method for 10 minutes. Intraplatelet serotonin was quantified by high-performance liquid chromatography (HPLC) with electrochemical detection. The *Qdpr* $^{-/-}$ mice significantly decreased the area under the aggregation curve (AUC) stimulated with collagen (*Qdpr* $^{+/+}$: 6,061±600, *Qdpr* $^{-/-}$: 3,424±514, $p<0.05$ by Tukey-Kramer test) and ADP (*Qdpr* $^{+/+}$: 4,197±295, *Qdpr* $^{-/-}$: 2,452 ±266, $p<0.05$). The intraplatelet serotonin content was significantly decreased in the *Qdpr* $^{-/-}$ mice (*Qdpr* $^{+/+}$: 48.1±4.0 pmol/10^6 platelets, *Qdpr* $^{-/-}$: 22.1±2.8 pmol/10^6 platelets, $p<0.01$). These results indicate that the *Qdpr* $^{-/-}$ mice suppresses secondary platelet aggregation via serotonin.

2-O-36

Intrinsic expression of G protein-coupled receptor 3 accelerates the axon specification in cultured hippocampal neurons

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During the course of neuronal development, an axon is specified by the PI3 kinase- and PKA-dependent signaling pathways. However, the up-stream factors that modulate the activity of these kinases remain to be fully elucidated. Recently, we clarified that the subcellular dynamics of Gαs activating G protein-coupled receptor 3 (GPR3) are associated with the local activation of PKA in cerebellar granular neurons. In the current study, we focused on the possible involvement of GPR3 in axon specification. The numbers of neurons with Tau-1-positive neurites significantly decreased at 48–60 h after GPR3 siRNA transfection in rat hippocampal neurons. In contrast, the upregulated expression of GPR3 resulted in an accelerated formation of Tau-1-positive neurites at 24 h after transfection. Similarly, a delayed formation of Tau-1-positive neurites was observed in the hippocampal neurons from GPR3-knockout mice. GPR3-mediated acceleration of axon formation was significantly reduced by the administration of PI3-kinase inhibitor and not by PKA inhibitor. Subsequently, we evaluated whether the GPR3 expression affects the de-phosphorylation of CRMP2, which is downstream in the PI3-kinase signaling pathway. The number of neurons with pCRMP2-negative neurites significantly decreased 60 h after GPR3 siRNA transfection. Furthermore, the staining intensity of pCRMP2 at the neurite tip was significantly stronger in GPR3-knockout mice than in the wild-type mice. Our cumulative results indicate the potential role of GPR3 in the axon specification in cultured hippocampal neurons.

2-O-37

The mechanism of long-term functional maintenance of neural stem cells by 3D culture system.

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The hippocampus that includes neural stem cells (NSCs) is an important organ associated with memory and learning. As a simple retrospective assay to identify NSC activity, neurosphere culture is generally employed. However, it is difficult to expand stemness-possessing neurosphere from the adult hippocampus. To better understand the nature of adult hippocampal NSCs, establishment of the specified *in vitro* expansion system is needed.

Here, we tried the culture of NSCs on a polyimide sheet possessing pores ranging from 5 to 50 μm. The NSCs derived from the adult hippocampus efficiently proliferate and sparsely formed sphere-like aggregates on the sheet. Those aggregates detached from the sheet could differentiate into the three cell lineages in response to differentiating agents. According to this procedure, we enabled the adult hippocampus-derived NSCs to maintain their stemness at least over 400 days. To evaluate the molecular mechanism, we performed a DNA microarray analysis. Notably, there was a big difference in expression profiles of mRNAs from NSCs between the long-term 3D culture and the short-term spheroid culture. We will discuss how the stemness of NSC can be maintained by the culture with polyimide sheet.

2-O-38

Prenylated quinolinecarboxylic acid derivative prevents neuronal cell death through inhibition of MKK4.

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The development of neuroprotective agents is necessary for the treatment of neurodegenerative diseases. Here, we report PQA-11, a prenylated quinolinecarboxylic acid (PQA) derivative, as a potent neuroprotectant. PQA-11 inhibits glutamate-induced cell death and caspase-3 activation in hippocampal cultures, as well as inhibits N-Methyl-4-phenylpyridinium iodide- and amyloid β_{1-42}-induced cell death in SH-SY5Y cells. PQA-11 also suppresses mitogen-activated protein kinase kinase 4 (MKK4) and c-jun N-terminal kinase (JNK) signaling activated by these neurotoxins. Quartz crystal microbalance analysis and *in vitro* kinase assay reveal that PQA-11 interacts with MKK4, and inhibits its sphingosine-induced activation. The administration of PQA-11 by intraperitoneal injection alleviates 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced degeneration of nigrostriatal dopaminergic neurons in mice. These results suggest that PQA-11 is a unique MKK4 inhibitor with potent neuroprotective effects *in vitro* and *in vivo*. PQA-11 may be a valuable lead for the development of novel neuroprotectants.

2-O-39

An apelin receptor agonist prevents retinal ganglion cell death in the diabetes model mouse fed the high-fat diet

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Diabetic retinopathy results in visual dysfunction through retinal ganglion cell (RGC) death. We have previously shown that APJ, an apelin receptor, is expressed in the RGCs and intravitreal injection of apelin protects against NMDA-induced RGC death. In the present study, we investigated whether systemic administration of an APJ agonist prevents RGC death in the retina of the diabetes model mouse fed the high fat diet (HFD). We used Insulin2 mutant (Ins2+/-) Akita mouse, which is a mouse model of type 1 diabetes. The Akita mouse was fed the HFD from 5 weeks after birth. The APJ agonist ML233 (5 mg/kg) was administered intraperitoneally on every other day for 4 weeks from 5 weeks after birth. The electro-responses of the RGCs were measured by electroretinography. ML233 suppressed the reduction of both the electro-response and the number of Brn-3a positive RGCs in the retina of the Akita mice fed HFD for 4 weeks. These effects were blocked by an APJ antagonist ML221 (5 mg/kg, i.p.). The present study showed that the APJ agonist protected against RGC death via APJ in the diabetes model mouse fed the HFD, suggesting that systemic administration of APJ agonists may prevent RGC degeneration in diabetic retinopathy.

2-O-40

Oxidative stress-mediated cell death induced by silica nanoparticles in neural cells.

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Nanomaterials have a variety of unique physical and chemical properties, and are being studied for biotechnological, pharmacological, and medical applications. Silica nanoparticles (SiNPs) are produced on an industrial scale and used in various fields. Despite these benefits, there is concern that exposure to SiNPs may lead to adverse effects in certain types of cells or tissues, such as developmental abnormalities in the brain and developing embryos. Although investigations on the toxicity of SiNPs against neurons are essential for medicinal use, a few studies have assessed the direct effects of SiNPs on cells derived from the central nervous system. In this study, we investigated the toxic effects of SiNPs on primary cultures of hippocampal cells. We showed that treatment with SiNPs caused oxidative stress and cell death. Furthermore, we found that these cytotoxicities were dependent on the particle size, concentration, and surface charge of SiNPs. The toxicity was reduced by SiNP surface functionalization or protein coating and by pretreating cells with an antioxidant, suggesting that contact-induced oxidative stress may be responsible for SiNP-induced cell death. These data will be valuable for utilizing SiNPs in biomedical applications.

2-O-41

Contribution of GRP-GRPR system in the spinal dorsal horn to pathological itch

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Gastrin-releasing peptide (GRP) receptor (GRPR)$^+$ neurons in the spinal dorsal horn (SDH) are itch-responsive, but their regulatory mechanisms are not yet clarified. Recently, we found that not only GRP but also glutamate directly activate GRPR$^+$ neurons through AMPA receptors (AMPAR), and those transmitters play fundamental roles in the spinal transmission of physiological itch. Here, we determined whether GRP-GRPR system underlies pathological itch in mice. To induce contact dermatitis, diphenylcyclopropenone (DCP) was applied to C57BL mice after having shaved on the back. The mRNA expression levels of GRP and GRPR in the cervical SDH were upregulated after DCP application. DCP-induced scratching behaviors were prevented by ablation of GRPR$^+$ AMPAR$^+$ neurons following bombesin-saporin treatment. Moreover, chemogenetic silencing of GRP$^+$/glutamate$^+$ neurons using Cre-dependent Gi- designer receptors exclusively activated by designer drugs (DREADD) also attenuated DCP-induced scratching behaviors. These results suggest that GRP-GRPR system and glutamate-AMPAR system in the SDH might cooperatively regulate not only physiological but also pathological itch.

2-O-42
Gangliosides modulate the nociceptive behavior and pruritus.

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Glycosphingolipids that have diverse variations in their carbohydrate-chains are abundant in neural tissues and previous studies revealed the important roles in neuronal functions. However, the roles of glycosphingolipids in the pain sensing pathway remain unclear. We reported that sialic acid-containing glycosphingolipids, gangliosides, are involved in nociceptive behavior. Depending on carbohydrate structures of gangliosides, they are divided into four groups such as o-, a-, b-, and c-series. Intraplantar injection of GT1b (b-series gangliosides) but not GM1a (a-series gangliosides) caused mechanical allodynia that was attenuated by the TRPV1 antagonist capsazepine. In addition, pretreatment of GT1b enhanced capsaicin induced nociception. On the other hand, intradermal injection of GM1a enhanced chloroquine induced pruritus.
These results suggested that the differences in carbohydrate structures of gangliosides lead to different effects on cutaneous sensation.

2-O-43

The role of BK channels in spinal microglia for the induction of neuropathic pain in mice

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The large-conductance Ca^{2+}- and voltage-activated K^{+} (BK) channel is formed by four pore-forming subunits encoded by a single *Slo1* gene and dually regulated by membrane voltage and intracellular Ca^{2+} levels. Recently, accumulating evidence using pharmacological methods suggests that the BK channel are associated with nociceptive sensitization. However, mechanisms underlying the effect of the BK channel on the regulation of pain signaling is still largely unknown. Here, we studied the role of the BK channel in the pain signaling *in vivo* using mice deficient for *Slo1*. In a partial sciatic nerve ligation (PSNL) model, *Slo1* KO mice showed partially restored thermal hyperalgesia compared to their WT littermates. In microglia from WT mice, p38 MAPK phosphorylation was significantly increased after lysophosphatidic acid (LPA) treatment; conversely, it was not significantly upregulated in microglia from *Slo1* KO mice, suggesting that the BK channel contributes to the signaling of microglial hyperactivation. Notably, intrathecal (i.t.) injection of microglia derived from WT mice into *Slo1* KO mice before PSNL induced the normal development of hyperalgesia in *Slo1* KO mice. These results indicate that the BK channel activation in spinal microglia, but not in neuron, contribute to the induction of neuropathic pain in this PSNL model.

2-O-44

Improvement by a phosphodiesterase type 5 inhibitor in the vascular impairment prevents oxaliplatin-induced peripheral neuropathy

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Oxaliplatin, a platinum-based chemotherapy drug, often induces cumulative sensory peripheral neuropathy. Accumulating evidences suggest a possible relationship between vascular impairment and peripheral neuropathy. In this study, we examined the effects of vasodilators on the oxaliplatin-induced peripheral neuropathy in mice. Mice injected with oxaliplatin (10 mg/kg, i.p.) once a week for 8 weeks showed mechanical (von Frey filament test) and cold (acetone test) hypersensitivities. A single injection of vasodilators (PDE5 inhibitor, endothelin receptor antagonist and prostaglandin analog) 1 h before tests improved the decreased skin blood flow and cold hypersensitivity induced by oxaliplatin, while it had no effect on mechanical hypersensitivity. When mice were fed with diets containing PDE5 inhibitor for 8 weeks during oxaliplatin treatment, the vascular impairment and hypersensitivities to mechanical, cold and transcutaneous current stimuli were suppressed. Furthermore, it prevented the decrease in nerve conduction velocity and axonal degeneration induced by oxaliplatin. These results suggest improvement by PDE5 inhibitor in vascular impairment can prevent oxaliplatin-induced peripheral neuropathy.

2-O-45

Hypoxia induced factor-1α participates in Schwann cell differentiation in peripheral nerves.

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Schwann cells (SC) generate myelin sheath in the peripheral nervous system. SC differentiation stages observed during development as well as degeneration/regeneration of the peripheral nerve have been characterized by the expression of genes specific for each stage. However, it remains unclear how these genes regulate SC differentiation and peripheral myelination. Here, we show the involvement of hypoxia induced factor-1α (HIF-1α) in the regulation of SC differentiation and myelination. Expression of HIF-1α in SC increased during development of peripheral nerves. Hypoxic treatment, application of a HIF-1α stabilizing drug, and overexpression of HIF-1α, bearing a mutation which gives resistance to proteasomal degradation, activated the expression of myelin related genes in SC and facilitated myelination *in vitro*. Expression of HIF-1α was also observed in SC in peripheral nerves after injury. In addition, the number of myelinating axons was increased by an local application of a HIF-1α stabilizing drug. These findings suggest that HIF-1αmight be involved in SC differentiation and peripheral myelination during development as well as regeneration after injury. We are now investigating whether HIF might be a potential therapeutic target for inherited neuropathy such as Charcot-Marie-Tooth disease.

2-O-46

Assessing cell viability for physiological concentration of uric acid in normal cells

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Uric acid (UA) is well known as the end product of purine metabolism in human. Hyperuricemia is defined as a serum UA level of ≥ 7.0 mg/dl (416 μM) in Japanese guideline for the management of hyperuricemia and gout (second edition), which is known as a risk factor for cardiovascular disease. Recently, it has been reported that low UA levels as well as high UA levels are predictive markers of increased mortality in epidemiologic studies. Hence, the aim of this study is to assess the role of UA at physiological concentration (conc) in normal cells. Normal human dermal fibroblasts were treated with UA conc at 62.5 - 500 μM (1.1 - 8.4 mg/dl). Cell viability of UA treated groups within the normal range was higher than that of control group. There was no change in cell number among groups, but total protein levels in UA treated groups increased at 72 hours. On the other hand, UA is known to have the potential antioxidant effects. Physiological conc of UA treatment decreased reactive oxygen species in the present study. Additionally, the expression of an oxidative stress-related protein was increased by UA treatment. Taken together, these findings suggest that physiological conc of UA in normal cells is probably implicated in cell viability.

2-O-47

Effects of purine/nonpurine type xanthine oxidoreductase inhibitors (Allopurinol/Topiroxostat) on high hypoxanthine phosphoribosyltransferase activity-uricase knockout mice

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The differences of purine metabolism of mice from that of human are known to be not only conserved uricase (Uox), but also low hypoxanthine phosphoribosyltransferase (HPRT) activity. The aim of this study is the establishment of high HPRT activity-Uox knockout mice as mouse model for the study of purine metabolism in human and the investigation of the effect of Allopurinol and Topiroxostat on HPRT. Allopurinol 30 mg/kg (Allo) and Topiroxostat 1 mg/kg (Top) were administered to the model mice for 7 days by feeding diet. Oxypurines (urate (UA), hypoxanthine (HX) and xanthine (XA)) and creatinine (Cr) in plasma and urine were measured by HPLC. Plasma UA value and urinary UA/Cr ratio significantly decreased in Allo and Top. Although the plasma UA-lowering effect was equivalent in Allo and Top, the urinary HX+XA/Cr ratio in Top 1 was significantly lower than those in Allo. Moreover, urinary oxypurine/Cr ratio demonstrated a significant lowering effect in Top, but not in Allo. In conclusion, Topiroxostat has a potent plasma UA-lowering effect and didn't affect the salvage pathway unlike Allopurinol, efficiently resulted in decreased total oxypurine excretion.

2-O-48

Life span extension of XDH knockout mouse with high HPRT activity

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Although xanthinuria is asymptomatic in humans, xanthine dehydrogenase knockout (XdhKO) mice die from renal failure. The activities of hypoxanthine phosphoribosyl transferase (HPRT) of human and wild mice are higher than those of labolatory mice. The aim of this study is to compare the life span between the XdhKO mice with high and low HPRT activity. High HPRT activity XdhKO mice were produced by crossbreeding with a consomic C57BL/6 mice whose Hprt allele is derived from wild mice. Urinary substances of high or low HPRT activity XdhKO mice were separated by HPLC. In a low HPRT mice, which died at 7 weeks of age, the excretion of xanthine (XA) decreased with increasing excretion of hypoxanthine (HX) without changing the total amount of urinary oxypurine excretion. At one week before death, HX excretion of low HPRT mice were 2.5 times higher than those of high HPRT mice. Prior to death of high HPRT activity mice, the excretion of XA and its precursor xanthosine decreased as HX excretion increased. In conclusion, the change of urinary oxypurine excretion from xanthine to hypoxanthine might be a cause of death of XdhKO mice, suggesting involvement of the reduction of IMP dehydrogenase activity due to NAD^+ deficiency or competitive inhibition.

2-O-49
Advanced glycation end-products uptake by macrophage

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Advanced glycation end-products (AGEs), which comprise non-enzymatically glycosylated proteins, play an important role in several diseases and aging processes including angiopathy, renal failure, diabetic complications, and neurodegenerative diseases. Among AGE-associated phenotypes, toxic AGEs, glyceraldehyde-derived AGE-2, and glycolaldehyde-derived AGE-3 are involved in the pathogenesis of diabetic complications. In addition, macrophages are reported to remove extracellular AGEs from tissues via scavenger receptors, leading to the progression of atherosclerosis. In the present study, we found that AGE-2 and AGE-3 enhanced their own endocytic uptake by RAW264.7 mouse macrophage-like cells in a concentration-dependent manner. Furthermore, we demonstrated the morphology of phagocytic macrophages and the endocytosis of AGE particles. The toxic AGEs induced the expression of a scavenger receptor, CD204/scavenger receptors-1 class A (SR-A). Notably, an antibody against CD204 significantly prevented toxic AGE uptake. Moreover, an SR-A antagonistic ligand, fucoidan, also attenuated the AGE-2- and AGE-3-evoked uptake in a concentration-dependent manner. These results indicated that SR-A stimulation, at least in part, plays a role in AGE uptake.

2-O-50
Possible Anti-Oxidative Effect of Juzen-taiho-to in Dogs

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【Background】
Recently, even dogs have developed an aging society. It is recognized that oxidative stress is relative to aging and disease. Many traditional Chinese medicines have been said that they had anti-oxidantive effect. But, there are no repot that these medicines have anti-oxidative effect in dogs. In this study, therefore, I examined anti-oxidative effect of Juzen-taiho-to, one of the traditional Chinese medicines, in dogs.
【Material and Method】
Five healthy female beagle dogs (38-41months of age and weighing 8.6-10.7 kg) were orally administered Juzen-taiho-to at 450 mg/kg with food for 28 days. Blood samples were taken from five dogs at day0, 7, 14, 21 and 28. Using the blood samples, anti-oxidant level, oxidative stress level and blood fluidity were determined.
【Results / Discussion】
Although improvement tendency was seen in anti-oxidant level and blood fluidity, the significant difference was not observed. But oxidative stress level at day 14, 21, and 28 was significantly lower than day 0. So, Juzen-taiho-to may have anti-oxidative effect in dogs by reducing oxidative stress. In addition, because traditional Chinese medicine is habitually administered for long term, further effects may be expected by administering Juzen-taiho-to in dogs for longer term.