Membrane proteome analysis for clarification of sex difference formation mechanism of pharmacokinetics in the kidney

腎臓における薬物動態の性差形成メカニズム解明に向けた膜タンパク質プロテオーム解析

○清水 聡史[1,2]、橋口 丈晃[1]、Pornparn Kongpracha[2]、宮坂 政紀[2]、Pattama Wiriyasermkul[2]、坂本 多穂[1]、永森 收志[2]、黒川 洵子[1]

[1]静岡県立大・薬・生体情報分子解析学、[2]東京慈恵会医科大・医・臨床検査医学

The kidney are the major organs determining the drug elimination, and membrane transporters play a central role. There are reports that there are sex differences in the expression levels of transporters that are important in drug elimination function. However, the mechanism of sex difference formation is unclear. The one of reason is that the low abundance and hydrophobic features of membrane proteins, such as transporters, make it difficult to analyze the proteins. Therefore, we developed an original membrane proteomics method to analyze membrane proteins comprehensively with high sensitivity. In this study, we used this method to comprehensively analyze the membrane protein expression level of the Four Core Genotypes (FCG) model, in which the *Sry* gene was moved from the Y-chromosome to an autosome, allowing distinction between gonadal and sex chromosome effects. Membrane proteomics was performed on brush border membrane vesicles from FCG mice and obtained a membrane protein profile according to gonad and sex chromosomes. Next, bioinformatics analyses were conducted to identify proteins and biological pathways that are important for gonad-derived and sex chromosome-derived sex differentiation. It is expected that this result will be an important information on sex differences of drug elimination in the kidney.

A pseudo-irreversible inhibition elicits a persistent effect of the sphingosine 1-phosphate receptor-1 antagonist

スフィンゴシン1−リン酸受容体−1拮抗薬は偽非可逆性の阻害作用により持続的な作用を示す

○丸山 祐哉[1,2,4]、大澤 雄亮[1]、鈴木 孝幸[1]、山内 裕子[1]、大野 孝介[1]、井上 仁史[1]、山本 明俊[1]、林 守道[1]、宮内 真紀[2,4]、広川 貴次[3]、秋山 泰身[2,4]

[1]キッセイ薬品工業・中央研究所、[2]横浜市立大・院生命医科学・免疫生物学研究室、[3]筑波大・医学医療系、[4]理研・生命医科学研究センター・免疫恒常性研究チーム

Sphingosine 1-phosphate receptor-1 (S1P1), a G protein-coupled receptor, is required for the egress of lymphocytes from lymphoid organs into blood. Blocking S1P1 signaling is a promising therapeutic approach to inflammatory diseases, and the understanding of the structural basis for enhancing inhibitory activity is critical to find potent S1P1 antagonists. We discovered a novel competitive antagonist, KSI-6666, that persistently suppresses S1P1 signaling in vivo and effectively inhibit the pathogenesis in mouse colitis models. In silico studies of molecular docking and molecular dynamics suggested that the dissociation of KSI-6666 from S1P1 is obstructed by the interaction of its bulky substituent with a methionine residue in the ligand-binding pocket of S1P1. In vitro analysis revealed a pseudo-irreversible inhibition of S1P1 signaling by KSI-6666 and the structural components that hindered its dissociation from receptors, corresponding to the predicted outcomes by molecular dynamics simulation. Moreover, in vivo studies suggested that the pseudo-irreversible inhibition contributes to the persistent effect of KSI-6666. These findings would help the rationale design of potent S1P1 antagonists for the treatment of inflammatory disorders.

Extracellular vesicles expressing brain-derived TrkB is detectable in serum: Its potential as biomarker for cognitive enhancement

脳由来TrkBを発現した細胞外小胞は血清中に検出される：認知機能改善作用のバイオマーカーとしての可能性

○山下 怜矢[1]、石本 尚大[1]、松本 聡[2]、増尾 友佑[1]、鈴木 誠[2]、加藤 将夫[1]

[1]金沢大・薬学系、[2]エル・エスコーポレーション（株）

Brain-derived neurotrophic factor/TrkB signaling plays important roles in cognitive enhancement by promoting neuroplasticity such as neurogenesis. Evaluation of the TrkB activation would be a potential biomarker for cognitive enhancement although its noninvasive evaluation is quite difficult. We hypothesized that TrkB would be detected in serum extracellular vesicles (EVs) leaked from the brain, which would be a useful biomarker for cognitive enhancement. First, we checked secretion of TrkB-expressing EVs from the neuronal cells Neuro2a transfected with a plasmid encoding a flag tagged mouse TrkB (TrkB-flag). TrkB-flag was detected in EVs isolated from the culture medium. Interestingly, intrahippocampal injection of adeno-associated virus serotype PHP.eB vector encoding TrkB-flag gene (AAV-PHP.eB-mTrkB-flag) in mice showed that TrkB-flag was detected in the serum EVs, implying secretion of TrkB-expressing EVs from the brain to circulating blood. Finally, we analyzed the correlation between TrkB activation in serum EVs and cognitive enhancement in humans administered ergothioneine (ERGO)-containing tablets, which are reported to activate TrkB in mice. Oral administration of the ERGO tablets increased ratio of phosphorylated TrkB to TrkB, an indicator of TrkB activation, in serum EVs, which was correlated with plasma ERGO levels and cognitive enhancement. These findings suggest that TrkB-expressing EVs would be secreted into circulating blood, and its phosphorylated states may help quantitative evaluation of cognitive enhancement.

Exploring the novel role of mitochondria dynamics in neuron and oligodendrocyte differentiation

ミトコンドリアダイナミクスによる神経およびオリゴデンドロサイト分化制御機構の探索

○岩田 圭子[1]、松﨑 秀夫[2]、野口 雅史[1]、新谷 紀人[1]

[1]和歌山県立医大・薬・薬品作用、[2]福井大・子どもセ・脳機能発達研

Emerging evidence indicates that cell fate is pleiotropically regulated by mitochondria, which undergo specific dynamics (biogenesis, fission, fusion, and mitophagy) in the cells. In this study, we explored the possible roles of the mitochondrial dynamics (mtDYN) in neuronal and glial differentiation using *in vitro* models. As models, we used human-derived cell lines SH-SY and MO3.13 cells, having the potential to differentiate into neurons and oligodendrocytes (OL), respectively. First, the transcriptome analysis on the mtDYN-related genes was performed, and a common and marked increase was highlighted in the expression levels of peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1 alpha) in both differentiation models. The differentiation-related increase of PGC-1 alpha, a key regulator of mitochondrial biogenesis, was also confirmed by an immunoblot study, and interestingly, mRNA analysis suggested the protein was produced from a novel transcript variant, named OL-PCC-1 alpha. Further analyses in the OL differentiation model revealed that the mitochondrial mass was dramatically increased, and moreover, specific knockdown of OL-PGC-1 alpha resulted in a significant decrease of the mitochondrial mass and the expression OL differentiation marker proteins. Collectively, the present data at least indicate that OL-PGC-1 alpha-related mitochondria biogenesis plays promotive roles in OL differentiation, and open an avenue to study OL mtDYN in the pathophysiology underlying brain dysfunction.

Multistep regulation of mammalian sleep by phosphorylation states of CaMKII

CaMKIIのリン酸化状態は睡眠覚醒サイクルを多段階に制御する

○戸根 大輔[1,2]、大出 晃士[1,2]、張 千惠[1]、上田 泰己[1,2]

[1]東京大・院医・システムズ薬理学、[2]理研・BDR・合成生物学

The sleep-wake cycle is an organism-level physiological phenomenon conserved in a wide range of species, however, the molecular mechanisms remain largely unexplored. We have previously shown that Ca^{2+}/calmodulin-dependent protein kinase II (CaMKII), one of the protein kinases regulated by intracellular Ca^{2+}, plays a critical role in promoting sleep (Tatsuki et al, 2016). CaMKII function is precisely controlled by phosphorylation in response to neural activity, suggesting that transitions of the CaMKII phosphorylation states are deeply involved in driving the sleep-wake cycle. In this study, we comprehensively analyzed the effects of CaMKII phosphomimetic mutations on sleep phenotypes in mice. We found that the phosphorylation of a single residue in CaMKII could induce sleep from wakefulness. We further revealed that additional phosphorylation switches the CaMKII function from sleep induction to sleep maintenance. These results provide evidence that CaMKII plays multiple roles in sleep-wake regulation, depending on its phosphorylation state.

Exploration of proliferation-promoting factors for human pancreatic beta cells

ヒト膵β細胞増殖促進因子の探索

○前田 貴広[1,2]、木村 東[2]、伊藤 遼[2]、長船 健二[2]

[1]京都大・院医・医科学、[2]京都大・iPS研・増殖分化機構研究分野

Approaches that induce the proliferation of insulin-producing pancreatic beta cells hold promising therapeutic potential to treat both insulin-dependent and insulin-independent diabetes. However, the factors that regulate beta cell proliferation in human remain unknown, and drugs that control proper beta cell mass are in high demand. Here, we aimed to identify small molecules that induce the proliferation of pancreatic beta cells as a novel therapy to treat diabetes. With this aim, we focused on *c-Myc* gene, which is an essential driver of proliferation in beta cells, and generated multiple human iPSC lines constitutively expressing a luciferase reporter under the control of human *c-Myc* promoter. Then, we developed a luciferase-based high-throughput screening (HTS) system to detect small molecules that directly or indirectly enhance the activity of *c-Myc* promoter in beta cells derived from the human iPSCs. Screens of 5,120 compounds using this system identified several candidates with enhanced *c-Myc* promoter activity, and we confirmed that one compound promotes human iPSC-derived beta cell proliferation. We are currently examining the mechanisms of action of the compound. Our finding could contribute to the identification of novel targets for increasing beta cell mass.

Investigation of Anti-Inflammation and Metabolome Shifts induced by SGLT2 Inhibitors to Elucidate the Mechanism of Cardioprotective Effects

SGLT2阻害剤による心保護作用のメカニズム解明のための脂肪組織の抗炎症および代謝シフトに関する検討

○豊島 拓斗

千葉大・院薬・臨床薬理学研究室

To investigate the possibility that Sodium-Glucose Cotransporter 2 Inhibitor (SGLT2i) reduces the risk of cardiovascular events by suppressing inflammation in adipose tissue, this study examined the effects of SGLT2i on inflammatory factors (IL-6, iNOS, MCP-1) in THP-1 derived macrophages and 3T3-L1 derived white adipocyte cells to observe the effect of SGLT2i on inflammatory factors (IL-6, iNOS, and MCP-1). We also observed the effect of SGLT2i on the comprehensive metabolome of white adipocytes: in THP-1, dapagliflozin (Dapa) and empagliflozin (Empa) showed a tendency to decrease IL-6 mRNA. In white adipocytes, 30 μM Dapa significantly decreased IL-6 and iNOS mRNA and significantly increased methotionine (Met) and GABA. Metabolomic pathway analysis also showed significant variation in Glu-related metabolism. Furthermore, Met and GABA on white adipocytes tended to decrease IL-6 and MCP-1 in a concentration-dependent manner, similar to SGLT2i. These findings suggest that SGLT2i may act on both adipocytes and macrophages. Future studies are expected to elucidate the mechanism of action of SGLT2i via Met and GABA, which were found to correlate with the suppression of inflammation.

Branched-chain amino acid metabolism regulates gluconeogenesis via mitochondrial pyruvate transport

分岐鎖アミノ酸代謝はミトコンドリアのピルビン酸輸送を介して糖新生を制御する

○西 清人[1]、大野 美紀子[1]、西 英一郎[1]、Tian Rong[2]

[1]滋賀医科大・薬理、[2]ワシントン大・医・麻酔

While it is well established that branched-chain amino acid (BCAA) catabolism is impaired in obesity, detailed mechanisms how BCAA catabolism regulates glucose homeostasis is still elusive. While impaired BCAA catabolism induces glucose intolerance in skeletal muscle or brown adipose tissues, the role of BCAAs and its catabolism on liver metabolism is under-investigated.

The rate limiting step of BCAA catabolism is positively regulated by PPM1K through the dephosphrylation of branched-chain keto acid dehydrogenase (BCKDH) complex. We found that Ppm1k-deficient (KO) mice, a model of impaired BCAA catabolism, showed reduced gluconeogenesis and were protected from glucose intolerance induced by high-fat diet feeding. In primary hepatocytes, accumulation of branched chain keto acids (BCKAs), downstream metabolites of BCAAs, due to Ppm1k deficiency inhibited hepatic glucose production in a cell-autonomous manner. Interestingly, pyruvate-supported glucose production was specifically suppressed in KO mice or hepatocytes. Mechanistically, BCKAs directly inhibited liver mitochondrial pyruvate carrier (MPC) activity resulting in selective suppression of pyruvate-supported gluconeogenesis or mitochondrial respiration. Moreover, in non-hepatic mitochondria, BCKA accumulation was alleviated via the reversible reaction of branched-chain amino transferase, rendering them less susceptible to BCKA-mediated inhibition of MPC. Together, these results suggest that BCAA catabolism regulates pyruvate and energy homeostasis in liver via BCKAs.

Specific binding of uric acid to NDFIP1 associates with hyperuricemia-induced liver fat accumulation

高尿酸血症誘発性の肝脂肪蓄積における尿酸センサーの探索

○朱 秋楠、荒川 大、谷口 愛美、長尾 優里佳、白坂 善之、玉井 郁巳

金沢大・医薬保健研究域薬学系・薬物動態学

Uric acid (UA) is uniquely maintained at high concentration in higher primates. Contrastingly, hyperuricemia (HU) is a risk factor for gout and associates with diverse diseases, including NAFLD, while its mechanism remains unclear. In this study, we aim to explain UA effect based on the hypothesis that UA-binding proteins function as UA sensors and regulate various physiological processes.

To find UA sensors, pull-down assay using UA-binding beads and cell homogenate was carried out and 6 proteins were found as UA binding proteins. Specific binding of UA to these proteins was confirmed by binding assay to *in vitro* synthesized proteins, including NDFIP1. Meanwhile, we performed a proteomic analysis of human primary hepatocytes treated with and without UA, followed by an enrichment analysis which showed NAFLD is one of the remarkable diseases. When NDFIP1 was knocked down in HepG2 cells, an increase of fat accumulation by UA exposure was attenuated, suggesting NDFIP1 plays a role in the HU-induced NAFLD. Additionally, the proteomic analysis also showed UA exposure stabilized PTEN, whose ubiquitination is promoted by NDFIP1. Furthermore, a decrease in PTEN ubiquitination and an increase in the protein level were observed after UA exposure. Since the change of PTEN expression is reported to promote insulin resistance that induces NAFLD, binding of UA to NDFIP1 is a possible mechanism of HU-induced NAFLD. In conclusion, NDFIP1 was found as UA binding protein and was suggested as a UA sensor that regulates HU-induced NAFLD by modulating PTEN.

Wnt5a, produced by mechanically stimulated periodontal ligament cells, modulates differentiation of trigeminal ganglion cells

機械刺激を負荷された歯根膜細胞由来Wnt5aが調節する三叉神経節細胞の分化機構の解明

○高橋 かおり[1]、吉田 卓史[2]、中村 卓史[1]、若森 実[1]

[1]東北大・院歯、[2]帝京平成大・薬・薬理

The periodontal ligaments (PDLs), located between the tooth and alveolar bones, anchor the tooth and absorb the daily chewing force by mastication. In addition, the Ruffini's corpuscles in the PDLs play a pivotal role in pressure sensing to identify various food properties and to adjust occlusal force. Although recent studies have shown that branches of Ruffini's corpuscles are not formed without mechanical stimulation in the rat PDL (rPDL), there is no report to assess the regulatory mechanisms for the peripheral axonal structure by mechanically stimulated PDL cells.

We established primary PDL cell lines derived from rat molar tooth. The rPDL cells were loaded with periodic mechanical stimulation (0.5 Hz, 15% expansion). The supernatant media of the mechanically stimulated PDL cells enhanced neurite elongation, sprouting, and branching in trigeminal ganglion (TG) cells. Neurotrophic factors such as NGF and BDNF and axon guidance proteins including Wnt family are known to be involved in neurite outgrowth. The rPDL cells expressed NGF, BDNF, NT-4 and Wnt5a mRNA. The mechanical stimulation increased only Wnt5a in rPDL cells by the qPCR analysis and ELISA. Moreover, AP7677a (neutralizing anti-Ryk antibody), or strictinin (Ror1 inhibitor) suppressed the morphological changes. These findings indicate the indirect mechanisms where Wnt5a, released from the connective tissues in response to mechanical stimulation, enhances the outgrowth of the peripheral nerves.

Involvement of *Pgc-1α* in the skeletal muscle on glucose uptake via the peripheral sympathetic nervous system in rats

ラット末梢交感神経系を介した糖取り込みにおける骨格筋*Pgc-1α*の関与

○佐藤 大介[1]、今泉 希[1]、楠 正隆[2]、宮本 理人[3]

[1]山形大・院理工・化学・バイオ工学、[2]名古屋大・総合保体セ、[3]神奈川工大・健康医療・管理栄養学

It has been suggested that sympathetic nerve activity is involved with not only blood pressure control but also enhancement of peripheral glucose uptake. To evaluate the effects of peripheral sympathetic activation on glucose uptake and skeletal muscle mRNA expression involved with energy metabolism, in the present study, we detected sympathetic nervous signal with a microelectrode in the unilateral sciatic nerve under anesthetic condition in normal and high-fat diet-fed (HFD) rats, and applied electrical microstimulation (MS) via the microelectrode. Glucose uptake was assessed as glucose infusion rate (GIR) during the hyperinsulinemic-euglycemic clamp. The MS significantly increased the GIR in both groups ($P<0.01$) whereas we observed no significant change in plasma insulin level. Furthermore, the GIR in HFD group was lower than that in the normal group. As a result of the MS for 60 min, in the normal group, *Pgc-1α* expressions in the bilateral soleus and extensor digitorum longus (EDL) tended to be elevated while *Glut4* expression in the bilateral EDL was significantly suppressed ($P<0.05$). In contrast, these changes were not seen in the HFD group. These results suggest that the MS enhances non-insulin-mediated glucose uptake, and the effects may be mediated by several pathways including *Pgc-1α* in the soleus but not in the EDL.

15-hydroxyeicosatrienoic acid increases vascular permeability in nasal mucosa

15-ヒドロキシエイコサトリエン酸は鼻粘膜血管の透過性を上昇させる

○尾崎 乃理子[1,2]、堀上 大貴[1]、平山 和宏[2]、村田 幸久[1,3]

[1]東京大・農・農学生命科学研究科 放射線動物科学研究室、[2]東京大・農・農学生命科学研究科 獣医公衆衛生学研究室、[3]東京大・農・農学生命科学研究科 食と動物のシステム学

Upon allergic rhinitis (AR), various inflammatory mediators increase blood flow and vascular permeability in nasal mucosa, which result in nasal congestion.

We here investigated the effects of 15-hydroxyeicosatrienoic acid (15-HETrE) on functional changes of vasculature and nasal congestion in mice. In isolated mouse aorta, the treatment of 0.1-3 μM 15-HETrE itself did not induce any contraction but inhibited the TP agonist (U46619)-induced contraction in a dose-dependent manner. Several lipid mediators are known to cause vasodilation by activating K^+ channels. Consistently, a pre-treatment of BKCa/IKCa, K_V, or K_{ATP} channel inhibitor inhibited the 15-HETrE-induced relaxation. In vivo, the topical administration of 1 μg U46619 constricted vein in mouse ear. A pre-administration of 1 μg 15-HETrE also inhibited the U46619-induced vein constriction. In modified Mile's assay, an intranasal administration of 20 μg 15-HETrE increased dye extravasation in the nasal mucosa. This administration also induced abdominal breathing, immobility, and lying down, which can be caused by nasal congestion. Thus, 15-HETrE induced vasorelaxation and vascular hyperpermeability. These phenomena presumably contribute to nasal congestion.

2, 5-Dimethylcelecoxib induces accumulation of anti-inflammatory macrophages and attenuates cardiac fibrosis in a mouse model of cryoinjury-induced myocardial infarction

2,5−ジメチルセレコキシブは抗炎症性マクロファージを集簇させ、冷凍障害心筋梗塞モデルマウスにおいて心臓線維化を抑制する

○岸上 赳大、石兼 真、有岡 将基、高橋 富美

産業医科大・薬理学講座

We previously reported that 2, 5-dimethylcelecoxib (DMC), a derivative of celecoxib, suppresses cardiac remodeling by suppression of fibroblast-myofibroblast transformation. However, its effects on the immunoreactive responses remain unclear. As macrophages are known to play critical roles in the process of fibrosis after myocardial damage, we evaluated the effect of DMC on macrophages using a cryoinjury-induced myocardial infarction (CMI) model mouse. The anterior left ventricular was cryo-injured by a liquid nitrogen-cooled aluminum probe in male C57 BL/6 mice. The mice were provided feed containing DMC or vehicle starting 3 days before the operation. Echocardiography showed that DMC attenuated the impairment of cardiac function, and Masson's trichrome staining of cross-section heart showed DMC reduced fibrosis area at the 14 days post-operation. In the cryo-injured damage area, DMC increased CD163-positive anti-inflammatory (M2) macrophages 3 days after operation, but not CD86-positive pro-inflammatory (M1) macrophages. Real-time PCR showed that DMC suppressed mRNA expression of interleukin (IL) -1β, IL-6, and monocyte chemoattractant protein (MCP) -1, which are known as inflammatory cytokines, in the damaged myocardium. These results suggested that DMC could attenuate impairment of cardiac function and fibrosis after the cardiac damage through increasing accumulation of M2 macrophages and decreasing inflammatory cytokines. Thus, DMC has potential against cardiac fibrosis and could be useful for the treatment of cardiac remodeling.

β-arrestin-biased AT_1R agonist improves health- and lifespan in mice with congenital dilated cardiomyopathy

βアレスチンバイアスAT_1受容体アゴニストは先天性拡張型心筋症モデルマウスの生命予後を改善する

○川岸 裕幸[1,2]、Ramadhiani Risa[3]、冨田（沼賀） 拓郎[2]、中田 勉[4]、江本 憲昭[3]、山田 充彦[2]

[1]信州大・バイオメディカル研・バイオテクノロジー、[2]信州大・医・分子薬理、[3]神戸薬科大・薬・臨床薬学、[4]信州大・基盤共通支援セ・機器分析

Pediatric heart failure is an important cause of death in childhood; however, therapeutic drugs specific for pediatric heart failure have not been developed. Angiotensin II modulates cellular function by associating with its receptor, mainly angiotensin II type 1 receptor (AT_1R) and activates G protein or β-arrestin signaling. We reported that a β-arrestin-biased AT_1R agonist (BBA) peptide, TRV027 induced a strong inotropic effect on preweaning mice suffering from congenital dilated cardiomyopathy (DCM). Remarkably, this inotropic effect was not associated with either arrhythmia or an increase in cardiac oxygen consumption. Here, we examined the prosurvival effect of TRV027 in DCM model mice. Daily subcutaneous administration of TRV027, but not an AT_1R blocker from postnatal day 1 significantly improved the survival rate and contractility of the left ventricle. Hematologic and pathological analyses revealed no detectable abnormalities in TRV027-treated DCM model mice except for hypertrophic heart. These results suggested that TRV027 has a beneficial effect to prolong healthy lifespan of pediatric heart failure patients. We also performed a high-throughput screening of one million compounds in order to discover small molecule BBA and found some hit compounds.

TMEM182 maintains the activated state of Wnt/β-catenin signaling by increasing ILK and inhibits cardiac differentiation in human iPS cells

TMEM182 は integrin-linked kinase を増加させることで Wnt/β-catenin シグナルの活性化状態を維持しヒトiPS細胞の心筋分化を抑制する

○森原 啓文[1]、若林 繁夫[1,2]、朝日 通雄[1]

[1]大阪医科薬科大・医・薬理、[2]大阪青山大・健康科学・看護

Transmembrane protein 182 (TMEM182) is known to be specifically expressed in muscle and adipose tissue. Previous reports suggest that TMEM182 plays an important role in skeletal muscle development. However, the role of TMEM182 in cardiac muscle is still unknown, and this study aims to elucidate the role of TMEM182 in the process of cardiomyogenesis. Human induced pluripotent stem cells (hiPSCs) can be converted into functional cardiomyocytes along the mesoderm lineage. Therefore, we generated hiPSCs overexpressing TMEM182 in a doxycycline-inducible manner and induced differentiation into cardiomyocytes. At day12 of differentiation, the expression of cardiomyocyte markers TNNT2 and MYH6 was significantly decreased in TMEM182-overexpressing cells. Wnt/β-catenin signaling is activated during early differentiation and repressed after mesoderm formation during cardiac differentiation. Therefore, we investigated Wnt/β-catenin signaling during TMEM182 overexpression. We found that phosphorylation of GSK-3β (Ser9) and β-catenin (Ser552) was increased during TMEM182 overexpression, suggesting activation of Wnt/β-catenin signaling. We further focused on integrin-linked kinase (ILK) as mechanisms by which TMEM182 activates Wnt/β-catenin signaling. The results showed that ILK expression was increased in cells overexpressing TMEM182. These results suggest that TMEM182 maintains Wnt/β-catenin signaling activation after mesoderm formation by increasing ILK expression and suppresses hiPSCs differentiation into cardiomyocytes.

Nucleotide metabolism as a novel and potential target to regulate cardiomyocyte proliferation

心筋細胞増殖制御の新規標的としての核酸代謝

○齋藤 祐一、木村 航

理研・生命機能科学研究センター・心臓再生研究チーム

Heart failure is a life-threatening disease with a poor prognosis. The most common cause of heart failure is myocardial infarction, in which a large number of cardiomyocytes are lost by occlusion of coronary blood flow. In adult mammals, injured hearts cannot regenerate themselves because of the lack of proliferative capacity in cardiomyocytes. In mice, cardiomyocytes permanently exit the cell cycle within 2 weeks after birth. Therefore, understanding what molecular mechanisms underlying this permanent cell cycle arrest would provide an important clue to identify potential therapeutic targets to induce heart regeneration. By integrated analysis of metabolome and transcriptome, we found a drastic increase in nucleotide metabolism in the mouse heart during this postnatal cell cycle withdrawal. Pharmacological inhibition of nucleotide metabolism in neonatal mice extended postnatal cardiomyocyte proliferation window with reduced oxidative DNA damage. Our findings suggest that the nucleotide metabolism is a novel regulator of postnatal cardiomyocyte cell cycle.

Effects of dexamethasone on nasal allergic response and hyperresponsiveness induced by Japanese cedar pollen in mice

マウスにおけるスギ花粉誘発鼻炎様症状および鼻粘膜過敏性に対するdexamethasoneの効果

○星野 楓月[1]、山下 道生[2]、安藤 祐介[3]、笠井 菜穂子[1]、田中 淑媛[1]、牧野 春香[1]、松尾 香寿美[1]、山下 恵梨華[1]、山田 萌恵[1]、吉田 夏子[1]、竹ノ谷 文子[2]、渡辺 知恵[3]、酒井 寛泰[4]、塩田 清二[5]、千葉 義彦[1]

[1]星薬科大・薬・分子生物学、[2]星薬科大・薬・運動科学、[3]城西大・薬・臨床病理学、[4]星薬科大・薬・生体分子薬理学、[5]湘南医療大学・薬・解剖生理学

In the present study, effects of dexamethasone (Dex) on allergic rhinitis (AR) induced by Japanese cedar pollen (JCP) were investigated. Mice were sensitized by *i.p.* injections with JCP+alum on days 0, 7 and 14. From day 21, the sensitized mice were challenged by intranasal (*i.n.*) administrations of JCP for 4 consecutive days. Animals were also treated with Dex (*i.p.*) 30 min before each JCP challenge. Frequency of sneezing was counted for 20 min after each nasal challenge. On days 18 and 25, the histamine (Hist, *i.n.*)-induced sneezing was also counted. In mice that were sensitized and repeatedly challenged with JCP, both serum levels of IgG and IgE specific for Cry J1, a major allergen of JCP, were significantly increased. In the JCP-sensitized mice, JCP challenge caused a significant increase in sneezing, indicating that nasal allergic response was induced. The *i.n.* application of Hist also caused an increase in sneezing. The Hist-induced sneezing was further increased significantly on day 25, indicating that nasal hyperresponsiveness (NHR) had occurred after the repeated JCP challenges. Both the nasal allergic response and NHR induced by JCP were inhibited by pretreatments with Dex. Thus, the murine AR model used might be useful for making clear the mechanisms of the AR pathogenesis and the action of corticosteroid effects.

Protective effect of VEGFR1 signaling on LPS-induced lung injury in mice

LPS誘導急性肺障害に対するVEGFR1シグナルの保護的作用

○長田 真由子[1]、伊藤 義也[1]、山下 敦[1]、田邉 美奈[1]、古江 明子[1]、細野 加奈子[1]、畑中 公[1]、澁谷 正史[2]、馬嶋 正隆[1,3]、天野 英樹[1]

[1]北里大・医・薬理、[2]上武大・医学生理学研究所、[3]神奈川工科大・健康医療・病態治療

Acute respiratory distress syndrome (ARDS) is characterized by increased permeability of pulmonary blood vessels, leading to respiratory failure. Although VEGF is responsible for the vascular permeability, it remains unknown about the involvement of signaling for VEGFR1, a receptor for VEGF. We examined the role of VEGFR1 in pathology of ARDS. ARDS was created by an intra-tracheal injection of LPS to wild type (WT) mice and VEGFR1 tyrosine kinase deficient mice (TKKO). Compared with WT mice, TKKO mice displayed lower survival rates, increases in lung injury score, total protein concentrations, and pro-inflammatory cytokines including TNF and IL-6 in bronchial alveolar lavage fluids. Alveolar macrophages were diminished in both types of mice after LPS injection. Instead, neutrophils were extensively accumulated, and the number of neutrophils in TKKO mice was higher than that in WT mice. The same was true for macrophages recruited into the lung tissues. VEGFR1 was expressed in alveolar and recruited macrophages. These results suggested that VEGFR1 signaling attenuated LPS-induced acute lung injury by suppressing vascular permeability, cytokines production and neutrophil accumulation.

ACE2-like carboxypeptidase B38-CAP suppresses severe acute lung injury induced by aspiration pneumonia and abdominal sepsis as well as SARS-CoV-2 infection

ACE2様カルボキシペプチダーゼB38-CAPは、誤嚥性肺炎、腹部敗血症、およびSARS-CoV-2感染によって誘発される急性肺損傷を抑制する

○山口 智和[1]、湊 隆文[1]、星崎 みどり[2]、韮澤 悟[3]、安 健博[1]、高橋 砂織[4]、今井 由美子[2]、久場 敬司[1]
[1]秋田大・院医・分子機能学・代謝機能学、[2]医薬基盤・健康・栄養研感染病態制御、[3]国際農研生物資源・利用領域、[4]秋田県総合研

Angiotensin-converting enzyme 2 (ACE2) is the carboxypeptidase to degrade angiotensin II (Ang II) to angiotensin 1–7 (Ang 1–7) and improves the pathologies of cardiovascular disease and acute respiratory distress syndrome (ARDS) / acute lung injury. B38-CAP is a bacteria-derived ACE2-like carboxypeptidase as potent as human ACE2, recombinant B38-CAP is prepared with *E. coli* protein expression system more efficiently than recombinant soluble human ACE2. We have demonstrated that B38-CAP ameliorates hypertension, heart failure (*Nat Commun*. 2020) and SARS-CoV-2-induced lung injury in mice (*Nat Commun.* 2021). We show therapeutic effects of B38-CAP on abdominal sepsis- or acid aspiration-induced acute lung injury. ACE2 expression was downregulated in the lungs of mice with cecal ligation puncture (CLP)-induced sepsis or acid-induced lung injury thereby leading to upregulation of Ang II levels. Intraperitoneal injection of B38-CAP significantly decreased Ang II levels while upregulated angiotensin 1–7 levels. B38-CAP improved survival rate of the mice under sepsis. B38-CAP suppressed the pathologies of lung inflammation, improved lung dysfunction and downregulated elevated cytokine mRNA levels in the mice with acute lung injury. Thus, systemic treatment with an ACE2-like enzyme might be a potential therapeutic strategy for the patients with severe sepsis or ARDS (*PLos One.* 2022).

Molecular basis of sex difference in sepsis

骨格筋炎症応答に着目した性転換マウス敗血症性差因子の探索

○岩鶴 果奈、坂本 多穂、竹下 舜也、山下 ほのか、黒川 洵子
静岡県立大・薬・生体情報

Male sex is more prone to septic death. However, its molecular mechanism remains unknown. Our studies revealed that inflammatory responses in skeletal muscle play a pivotal role in sepsis. Furthermore, recent findings suggested that septic responses in skeletal muscle is a key of the sex differences in septic death. To elucidate if the sex differences in sepsis is from gonadal and sex chromosomal differences, cecal ligation and puncture (CLP)-induced septic symptoms in Four Core Genotypes (FCGs) mice (XX gonadal males or females, and XY gonadal males or females) were investigated in this study. Our survival analysis showed that XX female mice were significantly resistant among FCGs to septic death. Furthermore, our RNA-seq analysis in skeletal muscle of septic FCGs revealed that different activity of inflammatory pathways and four inflammation related genes (*Ifi205*, *Mmp3*, *Prg4*, *Saa3*) were overexpressed specifically in XX females. In vitro analysis using C2C12 myotubes revealed that estradiol-treatment but not testosterone-treatment enhance mRNA expressions of these genes. Our study suggests the involvement of interactive effects of gonadal and sex chromosomal differences in sex differences in sepsis. Four genes were identified as candidate genes involved in sex difference of sepsis.

OpTER: a low-cost method for measuring the transepithelial/endothelial electrical resistance TER of cell layers, which reduces research costs and the burden on us all

OpTER：低コストな上皮・内皮バリア機能評価法の開発 ー研究費削減，及び研究者負担軽減を目指してー

○吉川 慧、原田 佳奈、田中 茂、秀 和泉、酒井 規雄

広島大・大学院医系科学研究科・医歯薬学専攻　神経薬理学

Transepithelial/endothelial resistance (TER) measurement is a non-invasive method to assess the integrity of tight junctions in model cells such as the intestinal epithelium and the blood-brain barrier. This technique is essential for experiments on drug kinetics and tissue damage, but commercial devices have many limitations. Additionally, the high-grade analyzers for long-term measurements are prohibitively expensive, raising barriers to entry into this research field.

The open source-based experimental equipment has advantages such as cost reduction and high versatility. There have been reports of TER instruments using 'open-source way' methods, but some of these have reproducibility problems.

We propose OpTER, a reproducible, and inexpensive TER measurement method. An Arduino-based measuring circuit can be created for less than 10,000 yen. Our method enables the recording of results equivalent to those of a commercially available product. Along with homemade electrodes made of biocompatible metals, this enables continuous measurement of TER in an incubator.

The circuits and program will be available, and its simple mechanism, which can be assembled by non-experts in electrical engineering, can easily be modified to suit the researcher's objectives. This idea is a new 'OpTion' for both amateurs and professionals.

Development of a novel mouse model of immune checkpoint inhibitor-associated myocarditis

免疫チェックポイント阻害剤関連心筋炎の新規病態モデル開発

○新村 貴博[1,2]、運天 拡人[2]、濱野 裕章[1,3]、内田 和志[2]、友近 七海[2]、宮田 晃志[2]、合田 光寛[2,4]、八木 健太[1,2]、相澤 風花[4]、石澤 有紀[5]、座間味 義人[2,3]、石澤 啓介[1,2,4]

[1]徳島大学病院・総合臨床研究センター、[2]徳島大・院医歯薬・臨床薬理学分野、[3]岡山大学病院・薬剤部、[4]徳島大学病院・薬剤部、[5]徳島大・院医歯薬・薬理学分野

Immune Checkpoint Inhibitors (ICI) show anti-tumor activity against various types of cancer, but they also disrupt the balance of the immune system and cause autoimmune-like adverse events. ICI-related myocarditis, in particular, has a fatality rate of over 40%, making the development of preventive and therapeutic agents an urgent priority. In present study, we developed a simple and reproducible experimental model for ICI-associated myocarditis.

Myocardial myosin peptide (50 μg) was administered subcutaneously to male, 8-week-old BALB/c wild-type and PD-1KO mice at day 0 and 7. Three weeks after the initial myosin administration, the development of myocarditis was evaluated. HE staining and Masson trichrome staining showed inflammatory cell infiltration and fibrosis in myocardial tissue in myosin peptide-treated PD-1 KO mice. Next, the involvement of $CD4^+$ and $CD8^+$ cells was examined by immunostaining, and the infiltration of $CD4^+$ and $CD8^+$ cells was confirmed in the hearts of myosin-treated PD-1KO mice. Finally, the results of real-time PCR showed that myosin administration tended to increase gene expression of inflammatory cytokines and fibrosis markers in the hearts of PD-1KO mice.

It is expected that this model will be used to develop new prophylactic and therapeutic agents for ICI-associated myocarditis.

Identification of the therapeutic target for tendinopathy through the combination of real world data analysis and pharmacological experiments

リアルワールドデータ解析と薬理学実験の統合による腱障害の創薬標的の導出

○古田 晴香[1]、山田 麻莉[1]、長島 卓也[1]、松田 秀一[2]、永安 一樹[1]、白川 久志[1]、金子 周司[1]

[1]京都大・院薬・生体機能解析学、[2]京都大・院医・整形外科学

Tendinopathy is a degenerative disease characterized by rupture, pain or loss of strength at tendon tissues. Multiple intrinsic and extrinsic factors, such as aging and fluoroquinolones, are involved in the development of tendinopathy. However, despite much work, the exact pathophysiological mechanism remains unclear. In this study, we analyzed databases of self-reported adverse events and IBM MarketScan insurance claims database to explore a coexisting drug that reduced the incidence of tendinopathy, and found that dexamethasone prevented fluoroquinolone-induced tendinopathy. In experimental validation of the hypothesis, chronic treatment of pefloxacin to rats caused mechanical fragility and histological changes in tendon, which were both mitigated by the cotreatment of dexamethasone. For its molecular mechanism, in vitro studies revealed that oxidative stress was increased in pefloxacin-treated tenocytes, which was suppressed with the cotreatment of dexamethasone. Also, the increase in the gene expression level of glutathione peroxidase 3 (GPX3) was observed in dexamethasone-treated tenocytes. In fact, the overexpression of GPX3 mitigated pefloxacin-induced oxidative stress in tenocytes. These results suggest that dexamethasone reduces risk of tendinopathy by suppressing oxidative stress through the upregulation of GPX3. This data-driven approach based on clinical evidence will pave the way for the identification of therapeutic target for tendinopathy with high clinical predictability.

Investigating molecules that promote well-being through the brain - locomotor system interaction

脳と運動器を連関してWell-beingを促進する分子の検討

○稲田 祐奈、東田 千尋

富山大・和漢研・神経機能学領域

Subjective well-being (SWB) has been shown to correlate with longevity and healthy state. Several studies suggested that factors influencing SWB were at least physical activity, cognitive function, and social resource. The fact that SWB, which is an emotional issue, is influenced by physical activity and cognitive activity, and vice versa SWB affects physical activity and cognitive activity, suggests that the locomotor system and cognitive function are closely related to mental health. However, the molecular basis of these interactions has not been clarified. We hypothesized that some molecules responsible for these interactions circulate the brain and the locomotor system. This clinical study aimed to find molecules responsible for controlling SWB from the blood circulation.

Subjects were healthy elderly people over 65 years old who have no functional troubles in daily life. Evaluation items were SWB, lifestyle, cognitive function (CF), motor function (MF) and daily activity (DA). To elucidate features of elder people with high SWB, subjects were divided by their SWB scores into 4 groups. High SWB was associated with high CF, MF and DA. Comprehensive analysis of responsible molecules in plasma for controlling high SWB are under investigation.

There has been no molecular explanation of why physical activity, cognitive activity, and social activity affect SWB. The present study has the potential to answer the question and to provide a new perspective for health and longevity research with significant impact for medical, psychological, and social sciences.

Development of a novel anti-EpCAM monoclonal antibody and its application for cancer diagnosis and therapy

新規抗EpCAMモノクローナル抗体の開発とがん診断・治療への応用

○李 冠傑[1]、鈴木 裕之[1]、金子 美華[2]、加藤 幸成[1,2]

[1]東北大・院医・分子薬理学、[2]東北大・院医・抗体創薬研究分野

The epithelial cell adhesion molecule (EpCAM) is a cell surface glycoprotein, which is highly expressed on carcinoma cells. Because EpCAM is involved in cell adhesion, proliferation, survival, stemness, and tumorigenesis, it is thought to be a promising target for cancer diagnosis and therapy. Herein, we developed anti-EpCAM monoclonal antibodies (mAbs) using the Cell-Based Immunization and Screening (CBIS) method. One of the established anti-EpCAM mAb, EpMab-37 (mouse IgG_1, kappa), reacted with EpCAM-overexpressed Chinese hamster ovary-K1 cells (CHO/EpCAM) or a colorectal carcinoma cell line (Caco-2) in flow cytometry. In contrast, EpMab-37 did not react with EpCAM-knocked out Caco-2 (BINDS-16) cells in both flow cytometry and Western blot analysis. EpMab-37 could stain formalin-fixed paraffin-embedded colorectal carcinoma tissues by immunohistochemistry. Furthermore, we converted the subclass of EpMab-37 from mouse IgG_1 into IgG_{2a} (named as EpMab-37-mG_{2a}), and further produced a defucosylated version (EpMab-37-mG_{2a}-f), using FUT8-deficient ExpiCHO-S (BINDS-09) cells. The EpMab-37-mG_{2a}-f administration significantly suppressed the development of Caco-2 xenograft tumors in mice compared with the control IgG. In contrast, EpMab-37-mG_{2a}-f did not suppress the development of BINDS-16 xenograft tumors. These results indicated that EpMab-37 is useful for detecting EpCAM in tumors, and EpMab-37-mG_{2a}-f could contribute to the antibody therapy for EpCAM-positive tumors.

The role of PGD_2/CRTH2 signaling in allergic reaction

アレルギー反応におけるPGD_2/CRTH2シグナルの機能解明

○木田 美聖、中村 達朗、永田 奈々恵、村田 幸久

東京大・院農学生命科学

【Background & Aim】 We previously showed that prostaglandin D_2 (PGD_2) promotes allergic reaction by increasing antigen-specific IgE production. In the present study, we investigated the mechanism underlying the promotion of IgE production by PGD_2 focusing on the role of its receptor, chemoattractant receptor-homologous molecule on Th2 cells (CRTH2).

【Methods & Results】 We intradermally sensitized wild type (WT) and CRTH2 deficient mice (*Crth2*$^{-/-}$) with ovalbumin (OVA). The serum OVA specific IgE level and the allergic reaction against OVA stimulation were lower in *Crth2*$^{-/-}$ than those of WT. Immunostaining of lymph nodes showed that dendritic cells (DCs) expressed PGD_2 synthase. Consistently, bone marrow derived DCs released PGD_2 in response to OVA stimulation in vitro. The OVA-sensitization increased immune cell number in lymph node and Th2 cytokine productions from lymphocytes in WT. CRTH2 deficiency significantly decreased the immune cell number and cytokine productions. We finally revealed that intravenous transplantation of WT DCs but not T cells or B cells restored the serum levels of OVA specific IgE production and allergic reaction in *Crth2*$^{-/-}$.

【Conclusion】 In summary, antigen invasion stimulates PGD_2 production from DCs which promotes Th2 cytokine production in lymph node through CRTH2 signaling. These phenomena result in promoting antigen specific IgE production.

Regulation of STING signal by advanced glycation end products

終末糖化産物によるSTINGシグナルの調節

○西中 崇[1]、ハティポール オメル ファルク[1]、和氣 秀徳[1]、渡邊 政博[2]、豊村 隆男[2]、森 秀治[2]、西堀 正洋[3]、高橋 英夫[1]
[1]近畿大・医、[2]就実大・薬・薬理、[3]岡山大・院医歯薬・創薬研究推進室

Cyclic GMP-AMP (cGAMP) synthase (cGAS) and stimulator of interferon genes (STING) composes signal pathway which initiates innate immunity. 2'3'-cyclic GMP-AMP (cGAMP) is enzymatically produced by cGAS and activates several signaling pathway through binding to STING. Accumulating evidence indicates that cGAS-STING signaling play an important role in cancer, inflammatory disease, and senescence. Advanced glycation end products (AGEs) are biologically reactive compounds produced by prolonged exposure of proteins to carbonyl compounds. Chemical and physiological properties of AGEs depend on type of carbonyl compound. Accumulation of AGEs are observed in organs and tissues according to aging and leads to induction of proinflammatory effect. Therefore, AGEs are associated with the development of inflammatory and age-related diseases. However, relationship between cGAS-STING signal and AGEs remains unclear. In the present study, we investigate the effect of different types of AGEs on STING signal in macrophage. In THP-1 cells which is a human monocytic leukemia cell line, cGAMP transfection increased phosphorylation of TANK-binding kinase 1 (TBK1) and interferon regulatory factor 3 (IRF3), resulting in upregulation of IFNβ and CXCL10. Glycolaldehyde-derived AGEs dose-dependently suppressed cGAMP-induced the phosphorylation of TBK1 and IRF3. In contrast, ribose-derived AGEs enhanced the phosphorylation of TBK1 and IRF3. These results may suggest that different types of AGEs contribute to the regulation of STING signal in macrophage.

Inhibitory effects of Ninjinyoeito and Juzentaihoto on myeloid-derived suppressor cells (MDSC) stimulated by cancer cells

がん細胞によって促進される骨髄由来免疫抑制細胞(MDSC)の遊走に対する人参養栄湯および十全大補湯の抑制作用

○近藤 雄太[1]、村上 一仁[1]、韓 立坤[2]、高橋 隆二[2]、礒濱 洋一郎[1]

[1]東京理科大・薬・応用薬理、[2]クラシエ製薬・漢方研究所

Myeloid-derived suppressor cells (MDSC) are heterogeneous population of immature myeloid cells, that support tumor growth, by reducing T cell activity. Therefore, drugs which can inhibit MDSC are new predictive immunotherapeutic medicines. We have previously shown that Kampo medicines, Ninjinyoeito (NYT) and Juzentaihoto (JTT), suppress the differentiation of MDSC. In the tumor-bearing state, MDSC migrate to the tumor microenvironment (TME). In the present study, we have investigated the effects of NYT and JTT on the migration to TME. MDSC were isolated from C57BL/6J mice and differentiated into MDSC by the treatment with IL-6 and GM-CSF, after which the migration activity was assessed with transwell assay. The migration of MDSC was stimulated by treatment of 4T1 cancer cells or 4T1-conditioned media, NYT and JTT significantly inhibited the migration. Inaddition, NYT inhibits the phosphorylation of ERK1/2 in MDSC induced by 4T1-conditioned media. Furthermore, NYT considerably suppressed the expression of CCR2 in MDSC. These data indicated that, NYT and JTT suppress not only differentiation, but also the migration of MDSC to TME. This multi-step approach in cancer treatment may be important in the immunomodulatory effects of these Kampo medicines.

Single cell multiomic analysis of PBMCs from SARS-COV-2 infected patients

SARS-COV-2感染患者のPBMCの単一細胞マルチオーム解析

○Llamas Covarrubias Mara Anais[1]、衣笠 泰葉[1]、鈴木 穣[2]、今井 由美子[1]

[1]医薬基盤・健康・栄養研・感染病態制御ワクチンプロジェクト、[2]東京大・院新領域創成科学

Recent advances in single cell technology now allow researchers to simultaneously profile the transcriptional program and chromatin accessibility from individual cells, providing access to both the characterization of cell types and states, and the exploration of gene regulatory programs at the same time and in the same cells. Here, we present an analysis of single cell transcriptomes and chromatin accessibility profiles in PBMCs from a group of SARS-COV-2 infected subjects with a range of disease severities. We were able to identify several immune cell types at a coarse level based on transcriptomic profiles. By additional subclustering, we found 4 clusters of CD8T cells which show differential distribution across COVID-19 severities. Analysis of DEGs revealed higher expression of genes associated with CD8 T cell terminal differentiation and effector functions in the cluster enriched in mild patients. Chromatin accessibility analysis of the selected DEGs in CD8T cells confirms higher accessibility in patients with mild disease vs severe patients. Interestingly, the transcripion factor ZEB2 was identified as one of the top markers of the mild-severity cluster. Further motif analysis will clarify the importance of this TF in CD8T cell differentiation and outcome in SARS-COV-2 infected subjects.

The development of new therapeutic method by gene analysis using feline mammary tumor organoids

猫乳腺腫瘍オルガノイドの遺伝子解析による新規治療法の開発

○山本 晴、臼井 達哉、佐々木 一昭

東京農工大学・農・獣医薬理学研究室

【Background and Purpose】Feline mammary tumor is known to be the third most common tumors in cats and high grade tumors but more effective treatment has not been established. The culture method of organoids is also familiar with a method which can maintain the epithelial tissue structures and the characteristic like stem cell by three-dimensional culture using a special culture medium. In previous study, we created the feline mammary tumor organoids from tissue extracted patients and revealed the suitable culture condition. Then, we compared original tissue and organoid, and clarified the relation with the pathological characteristics or the expressions of hormone receptor, the differences of anti-cancer drug sensitivity in each case. In this study, we compared the gene expression of feline mammary tumor organoids with normal mammary organoids and searched for new therapeutic targets.

【Method】RNA was extracted from organoids produced from normal mammary glands 3 cases and feline mammary tumor 6 cases, and RNA sequencing was performed using next generation sequencer.

【Result】In RNA sequencing, there were 112 significant differentially genes. 81 genes were markedly upregulated in feline mammary tumor, and 31 genes were in normal mammary glands. In addition, the expression of X genes related with estrogen receptor was significantly upregulated in feline mammary tumor. In GESA analysis, the pathways related to apoptosis, epithelial-mesenchymal transition, and estrogen were activated.

【Conclusion】In feline mammary tumor and normal mammary glands, genetic differences was cleared and a strong association with estrogen was observed. We will plan to analysis the relationship between the gene identified in this study and feline mammary tumor, and the metabolite activity by metabolome analysis using feline serum, search for biomarkers lead to more earlier diagnosis.

Development and application of a novel probe that realize the imaging analysis of oxytocin dynamics in brain tissue

オキシトシンの脳組織内動態解析を実現する新規プローブの開発と応用

○中村 花穂

横浜国立大学大学院・環境情報学府・自然環境

Oxytocin is a peptide hormone known for its strong central actions regulating a variety of behaviors including parenthood and social bonding to others, in addition to the long-known peripheral actions. Despite increasing realization of its importance, dynamics and sites of action of oxytocin in the brain are poorly understood due to a lack of appropriate probe; it is too small to be tagged with bulky fluorophores for visualization. Therefore, to overcome the current technical limitation and to facilitate the understandings of oxytocin‘s action in the brain, we tried to develop and apply a new probe. To this end, we conjugated oxytocin with “alkyne-tag” via a widely applicable simple coupling reaction. The alkyne-tag is far smaller than oxytocin, so it is expected that its tagging will be possible without significantly changing the original properties of oxytocin molecule. After incubation with the living brain tissues where alkyne-oxytocin behaves similarly to endogenous oxytocin, the tagged-oxytocin can be specifically visualized by a click chemistry reaction. Using this probe, we conducted various experiments to characterize the spatiotemporal dynamics of oxytocin in brain tissues. Here, I will introduce our novel strategy and findings brought by this probe including the region-specific binding sites and dynamics of oxytocin.

PDGF-BB mediates phosphate regulation in the central nervous system

中枢神経系におけるPDGF−BBのリン酸輸送調節機構の解析

○大内 一輝、高瀬 奈央子、三島 彩音、村山 佑斗、栗田 尚佳、保住 功、位田 雅俊
岐阜薬科大・薬・薬物治療学研究室

【Background】

Idiopathic Basal Ganglia Calcification (IBGC) is a progressive neurodegenerative disease characterized by calcification of the basal ganglia and cerebellar dentate nucleus. Several gene mutations of IBGC are reported, including *SLC20A2* which are involved in phosphate transport. IBGC is thought to be caused by abnormal phosphate transport. However, its mechanism is not yet clear in central nervous system. Therefore, we elucidate the regulation of phosphate transport in neuronal cells, focusing on PDGF-BB, which is one of the causative gene of IBGC and activates phosphate transport in vascular smooth muscle cells.

【Methods】

The effect of PDGF-BB on phosphate uptake was evaluated with *SLC20A2-KD* (knockdown) SH-SY5Y cells, which is the human neuroblastoma cell type. To determine whether the effect of PDGF-BB is mediated by *SLC20A1* or *SLC20A2,* knockdown assays were conducted. To investigate the mechanism of PDGF-BB on the phosphate uptake, the expression and membrane translocation of phosphate transporters, PiT1 and PiT2 (encoded by *SLC20A1* or *SLC20A2*) was evaluated when PDGF-BB was treated.

【Results】

PDGF-BB enhanced the phosphate uptake by *SLC20A1*, not *SLC20A2*. Interestingly, the activation of phosphate uptake by PDGF-BB was not due to increase in the expression of phosphate transporters but to activation of membrane translocation. The activation of membrane translocation by PDGF-BB was canceled when Akt inhibitor was treated.

【Conclusion】

PDGF-BB enhanced the phosphate uptake by the membrane translocation of PiT1, the mechanism of which is thought to be Akt signaling.

GPR143, an L-DOPA receptor, in cholinergic interneurons, modulates haloperidol-induced extrapyramidal symptoms through coupling between GPR143 and dopamine D2 receptor

コリン介在性神経L−DOPA受容体GPR143は、ドパミン D2 受容体との機能連関を介してハロペリドールによる錐体外路様症状を修飾する

○荒井 柾美、増川 太輝、北村 慧、大瀧 百々代、五嶋 良郎

横浜市立大・院医・分子薬理神経生物学

We propose that L-DOPA by itself is a neurotransmitter. Recently, GPR143 was identified as an L-DOPA receptor. We previously showed non-effective dose of L-DOPA potentiates behavioral response to quinpirole, a dopamine D2 receptor (D2R) agonist. However, it remains undetermined whether and how GPR143 regulates D2R-mediated behaviors. We analyzed behavioral responses to several D2R ligands using *Gpr143* gene-deficient (GPR143-KO) mice. We found that haloperidol, a D2R antagonist (0.5mg/kg)-induced catalepsy was attenuated in GPR143-KO mice compared to wild-type (WT) mice. To clarify which neural circuits that are responsible for this phenotype, we investigated haloperidol-induced catalepsy using conditional KO mice that expressing cre recombinase in D2R-, adenosine A2A receptor (indirect pathway)-, and in choline acetyltransferase (cholinergic interneuron)-positive neurons. Haloperidol-induced catalepsy was attenuated in D2R-cre (+); $Gpr143^{flox/y}$ and ChAT-cre; $Gpr143^{flox/y}$ mice. Furthermore, we found that a synthetic peptide, which inhibited the interaction between GPR143 and D2R, attenuated haloperidol-induced catalepsy. These results suggest that GPR143 expressed in the striatal cholinergic interneurons modulates haloperidol-induced extrapyramidal symptoms through coupling GPR143 and D2R.

Development of a mutant allele-specific transcriptional repressive agent in CAG/CTG triplet repeat diseases

CAG/CTGトリプレットリピート疾患における変異アレル特異的転写抑制剤の開発

○松尾 和哉[1]、池ノ下 侑[1]、矢吹 悌[1]、川久保 厚佑[1]、朝光 世煌[1,2]、杉山 弘[3]、塩田 倫史[1]

[1]熊本大・発生研・ゲノム神経、[2]理研BDR・脳エピトランスクリプトミクス、[3]京都大・院理・生物化学

Expansion of CAG and CTG (CWG) triplet repeats is causal in a number of inherited neurological diseases. The CWG triplet repeat diseases can be broadly classified into coding and non-coding types based on their location in the genome, such as Huntington's disease (HD) by expanded CAG repeat in a coding region and myotonic dystrophy type-1 (DM1) by expanded CTG repeat in a 3'-untranslated region. The CWG repeat diseases are thought to induce complex pathogenic mechanisms through expanded CWG repeat-derived RNAs and polypeptides. Here we show a CWG repeat DNA targeting compound, cyclic Pyrrole-Imidazole Polyamide (CWG-cPIP) suppresses the pathogenesis of coding and non-coding CWG repeat diseases. CWG-cPIP binds to hairpin form of the mismatched CWG DNA, interfering with transcriptional elongation of RNA polymerase in a repeat length-dependent manner. CWG-cPIP inhibits pathogenic mRNA transcripts from expanded CWG repeat, result in reduction of CUG RNA foci and polyglutamine accumulations in DM1 and HD patient cells and mouse models, respectively. Treatment with CWG-cPIP also ameliorates learning and memory deficits and synaptic dysfunction in DM1 and HD mouse models with less off-target effects. Taken together, we present a novel candidate compound that targets expanded CWG repeat DNA independent of genomic location, demonstrating the concept of reducing the levels of pathogenic RNAs and proteins.

Inhibition of heterogeneous nuclear ribonucleoprotein U suppresses astrocyte proliferation in astroglial scar formation after spinal cord injury

Heterogeneous nuclear ribonucleoprotein Uの阻害による脊髄損傷後のアストログリア瘢痕形成におけるアストロサイト増殖の抑制

○全 麗麗、村松 里衣子

国立精神・神経医療研究セ・神経研究所・神経薬理研究部

Spinal cord injury (SCI) is a devastating trauma that results in a severe disability and irreversible motor and sensory dysfunction, whereas efficient therapies have not been fully developed. After SCI, astrocytes are the predominant cellular component that proliferates around the lesion core and contributes to the glial scar formation, which has long been considered one of the primary causes of spinal cord regeneration failure. However, the molecular mechanisms underlying the proliferation of astrocytes in response to central nervous system (CNS) injury remain unclear. In this study, we found that heterogeneous nuclear ribonucleoprotein U (Hnrnpu), a DNA/RNA binding protein, regulated astrocyte proliferation after SCI. siRNA-mediated knockdown of Hnrnpu suppressed the primary astrocyte proliferation without affecting the cell viability *in vitro*. Moreover, *in vivo*, inhibition of Hnrnpu expression by intraspinal injection of AAV5-Hnrnpu shRNA under the control of the astrocytic glial fibrillary acidic protein (GFAP) promoter inhibited astrocyte proliferation, increased lesion size, and suppressed functional recovery of mice after SCI. Taken together, Hnrnpu exerts a crucial role in astrocyte proliferation, where its changes would be regarded as a hallmark of CNS diseases and injuries in which astrocytes are involved.

Developmental intracerebral hemorrhage induces microglial heterogeneity

発達期脳内出血はマイクログリアに不均一性をもたらす

○河野 玲奈[1]、池谷 裕二[1,2]、小山 隆太[1,2]

[1]東京大・院薬・薬品作用、[2]東京大・Beyond AI研究推進機構

Microglia arise from embryonic yolk sac and colonize the brain parenchyma, followed by the maturation of microglia-specific gene expression. Single-cell transcriptional analysis has revealed that microglia are transcriptionally heterogeneous. However, the environmental factors that induce microglial heterogeneity are largely unknown. Here we found that developmental intracerebral hemorrhage induces microglial heterogeneity. In neonatal mice, we found that a portion of microglia phagocytose the red blood cells (RBCs) and expressed *Hmox1*, which encodes Heme Oxygenase 1, significantly higher than non-RBC-phagocytic microglia. To examine the effect of RBC phagocytosis on the transcriptional property of microglia, we labeled Hmox1-expressing microglia with red fluorescent protein by developing a transgenic mouse line, finding that microglia that underwent RBC phagocytosis expressed genes typical for yolk sac microglia in the second postnatal week. Thus, this study reveals that neonatal environmental factors induce microglial heterogeneity.

Pharmacological analysis of behavioral addiction by quantifying the motivation for wheel-running in mice

ランニングホイール回転行動に対する動機づけを指標とした行動嗜癖の行動薬理学的解析

○西谷 直也[1,2]、谷口 遥海[2]、小久米 泰祐[2]、金田 勝幸[1,2]

[1]金沢大・院薬・薬理、[2]金沢大・薬・薬理

In behavioral addiction, addicts repeat certain behaviors, such as internet gaming and gambling, despite negative consequences. No cure has been established due to the lack of animal models to explore its neuronal mechanisms. Here, by focusing on voluntary wheel-running in rodents, we have developed a novel operant conditioning task as a model of behavioral addiction, in which male C57BL/6J mice (> 7 weeks old) were allowed for wheel-running after certain numbers of nose pokes. In this task, the magnitude of motivation for wheel-running is quantifiable by evaluating the number of nose pokes.

We first measured dopamine (DA) release using fiber photometry with GRAB-DA sensors and found that DA was increased in the nucleus accumbens (NAc) immediately after nose-poking. Systemic administration of antagonists for DA D1 receptor (SCH23390; 0.025–0.1 mg/kg), D2 receptor (raclopride; 0.1–0.6 mg/kg), or an agonist for adenosine A2A receptor (CGS21680; 0.05-0.1 mg/kg) dose-dependently decreased the number of nose pokes. Intra-NAc infusion of these drugs (SCH23390; 0.05 μg/side, raclopride; 0.3 μg/side, CGS21680; 1 ng/side) also reduced the number of nose pokes. Additionally, systemic administration of serotonin (5-HT)2A receptor antagonist (volinanserin; 0.01–0.1 mg/kg) or 5-HT2C receptor antagonist (SB242084; 0.3–1.0 mg/kg) dose-dependently decreased the number of nose pokes. These results suggest that neurotransmission via D1, D2, and A2A receptors in the NAc and 5-HT2A and 2C receptors are involved in the motivation for wheel-running.

The mechanisms of which microglia are related to the pathology in the "primary astrocytic disease" Alexander disease.

「一次性アストロサイト病」アレキサンダー病におけるミクログリアの病態関連機序の解明

○齋藤 光象[1,2]、繁冨 英治[1,2]、篠崎 陽一[1,2]、パラジュリ ビジェイ[1,2]、小泉 修一[1,2]

[1]山梨大・院医・薬理、[2]山梨大・GLIAセンター

Alexander disease (AxD) is an intractable neurodegenerative disorder caused by *GFAP* mutations. AxD astrocytes show several abnormal phenotypes. Our previous study has shown that astrocytes in AxD model mice show aberrant Ca^{2+} signal that was a cause of etiology of AxD. In addition, using 2 photon imaging and Iba1-GCaMP6-60TM, an AxD model for microglial Ca^{2+} imaging, we recently found that microglial Ca^{2+} signals were also dramatically enhanced in the AxD model with more frequent Ca^{2+} signals in both the processes and cell bodies. Such increases in Ca^{2+} signals were inhibited by $P2Y_{12}R$ antagonist but not by TTX, suggesting that these enhancement should be independent of neuronal activity, but dependent on extracellular ATP-mediated signals. Thus, we hypothesized that these microglial abnormal Ca^{2+} signals would be caused by increase of ATP amount released from astrocytes. Our analysis data of scRNAseq suggested that some astrocyte subclusters unique to the AxD model exhibit the lower expression level of the gene of astrocyte-specific ectonucleotidase subtype. In *in situ* ATP imaging using *in vivo* injection of AAV GfaABC1D ATP1.0, the signals of locally puffed ATP persisted longer in acute slice in AxD model than control WT mice, indicating a delay of ATP degradation in AxD brain that could cause the hyperactive Ca^{2+} signals in microglia. To study if these $P2Y_{12}R$-mediated Ca^{2+} signals in AxD microglia play any significant roles in the mechanism of pathology, $P2Y_{12}R$ antagonist was administered. AxD model with treatment of $P2Y_{12}R$ antagonist showed an exacerbation of pathological markers. This suggested that microglia play a protective role in AxD pathology via $P2Y_{12}R$. Our findings hold promise for the future development of therapies based on microglial manipulation.

Effects of fluoxetine on stress-induced neuronal activity in the ventral hippocampus

ストレスによるうつ様行動発現に関わる神経回路に対する抗うつ薬フルオキセチンの作用検討

○塩崎 裕美[1]、久我 奈穂子[1]、池谷 裕二[2,3]、佐々木 拓哉[1,2]

[1]東北大・院薬・薬理学分野、[2]東京大・院薬、[3]国立研究開発法人　情報通信研究機構　情報通信融合研究センター

Fluoxetine is a selective serotonin reuptake inhibitor (SSRI) and exerts antidepressant effects, which has been considered to be mediated by increasing serotonin concentration in the inter-synaptic cleft. However, the monoamine hypothesis has not been fully supported as there is a time lag between transient increases in brain serotonin levels after SSRI treatment and the expression of antidepressant effects. The temporal inconsistency may be partly reconciled by memory mechanisms, especially in the hippocampus. A theory of memory suggests that learned memory needs to be consolidated into neuronal circuits by repeated reactivation of memory-encoding neuronal activity. Our previous study demonstrated that the inhibition of the ventral hippocampus after stress experiences inhibits subsequent depression-like behavior in mice. Especially, we found that sharp wave ripples (SWRs), which represent synchronized neuronal spikes in the ventral hippocampus, are a primary neuronal activity pattern to mediate this effect. Here, we tested whether fluoxetine affects SWRs in the ventral hippocampus in resting and depression model mice and found that fluoxetine administration significantly reduced ventral hippocampal SWRs in both resting mice and mice that received social defeat stress. These results demonstrate that fluoxetine inhibits SWR-induced neuronal reactivation, suggesting an antidepressant effect mediated by suppressing memory consolidation.

Neuronal activity of claustral populations during anxiety-related behaviors is altered by exposure to stress

ストレス誘発不安応答を制御する前障神経細胞の in vivo カルシウムイメージング

○田沼 将人[1]、宮司 渓汰[1]、大久保 仁[1]、野村 洋[2]、南 雅文[3]、橋本 均[1,4,5,6,7]、笠井 淳司[1]

[1]大阪大・院薬・神経薬理学分野、[2]名古屋市立大・院医・認知機能病態、[3]北海道大・院薬・薬理、[4]大阪大・院連合小児発達・子どものこころセンター、[5]大阪大・先導的学術研究機構、[6]大阪大・データビリティフロンティア機構、[7]大阪大・院医・分子医薬

We have recently reported that excitatory neurons in the claustrum mediate anxiety responses to acute psychological stressors that induce negative emotional states. However, it is unclear how claustral neurons represent information related to anxiety responses to a stressor. To address this question, here we performed calcium imaging of GCaMP6f-expressing claustral neurons in freely moving mice during three behavioral tests; the elevated plus maze, the open field test, and a second open field test after an exposure to a ten-minute single social defeat stress. Prior to exposure to a stressor, we found that a subset of claustral neurons displayed an increase in calcium levels upon transitioning to areas associated with increased anxiety in the elevated plus maze and the open field. In the open field test after exposure to social defeat stress, a different subset of neurons, including neurons that were activated by of stress, exhibited sustained high levels of calcium when entering and exiting the less anxiogenic corner zones of the open field. These results suggest that stress-related anxiety information is represented in a claustral neuronal population that is different from the population representing anxiety under non-stressed conditions.

Role of ER stress-regulated high-temperature requirement A1 (HTRA1) in the function of placental cells in hypertensive disorder of pregnancy

妊娠高血圧症候群における胎盤細胞の機能における小胞体ストレス誘導性high-temperature requirement A1 (HTRA1)の役割

○吉田 佳乃子[1]、草間 和哉[1]、安曇 麻奈[1]、小島 淳哉[2]、吉江 幹浩[1]、加藤 聖子[3]、田村 和広[1]

[1]東京薬科大・薬・薬理学、[2]東京医科大・医・産科婦人科学、[3]九州大・医・産科婦人科学

Hypertensive disorder of pregnancy (HDP) affects about 10% of pregnant women, which may be caused by dysfunction of placental trophoblasts following impaired uterine spiral arterioles. Our previous analysis revealed that endoplasmic reticulum (ER) stress signaling may be altered in HDP, compared to normal pregnancy. In addition, the expression of high-temperature requirement A1 (HTRA1), a serine protease was decreased in the HDP placenta. However, a role of ER stress and HTRAs in HDP pathophysiology remains unknown. The relationship between of ER stress and HTRAs was explored in vitro human trophoblast cells. Treatment with ER stress inducers thapsigargin or tunicamycin increased the expression of HTRA1 and its subtype HTRA3, but did not alter HTRA2 and HTRA4 in trophoblasts. In vitro invasion assay revealed that either thapsigargin or tunicamycin treatment and the knockdown of HTRA1 or HTRA3 inhibited trophoblast invasion. In addition, ER stress inducers or knockdown of HTRA1 altered the ratio of soluble fms-like tyrosine kinase-1 (sFLT1) to placental growth factor (PGF), a severity index of HDP. The expression of HTRA1 was lower in HDP placenta compared to the normal placenta tissues. These results suggest that ER stress may regulate trophoblast invasion partly via HTRA1 and HTRA3 and is involved in the pathogenic mechanism of HDP. It might be possible to develop a therapeutic means that targets HTRA1 to improve pregnancy complications such as HDP.

Identifying antidepressants less likely to cause hyponatremia: triangulation of retrospective cohort, disproportionality, and pharmacodynamic studies

低ナトリウム血症を起こしにくい抗うつ薬の同定：後方視コホート・不均衡・薬力学解析のトライアンギュレーション研究

○長島 卓也[1]、早川 隆[1]、秋元 勇人[1]、皆川 貴美乃[2]、高橋 泰夫[2]、浅井 聰[1,2]

[1]日本大・医・生体機能医学系薬理、[2]日本大・医・臨床試験研究セ

Antidepressants are known to cause hyponatremia, but conflicting evidence exists regarding specific antidepressants. To identify antidepressants less likely to cause hyponatremia, we conducted a triangulation study integrating retrospective cohort, disproportionality, and pharmacodynamic studies. In the retrospective cohort study, a significant decrease in serum sodium levels was observed for selective serotonin reuptake inhibitors (SSRIs) and serotonin-noradrenaline reuptake inhibitors (SNRIs), whereas no decrease was found for a noradrenergic and specific serotonergic antidepressant (mirtazapine). Within-class comparison revealed no decrease in serum sodium levels for fluvoxamine among SSRIs and milnacipran among SNRIs. In the disproportionality analysis, a significant increase in hyponatremia reports was observed for SSRIs and SNRIs, but not for mirtazapine, fluvoxamine, and milnacipran. Finally, pharmacoepidemiological–pharmacodynamic analysis revealed a significant correlation between the decrease in serum sodium levels and binding affinity for serotonin transporter (SERT), suggesting that lower binding affinity of mirtazapine, fluvoxamine, and milnacipran against SERT is responsible for the above difference. These data suggest that mirtazapine, fluvoxamine, and milnacipran are less likely to cause hyponatremia.

Bidirectional regulation of tumor progression by the endogenous μ-opioidergic system

μ オピオイドシステムのがんの進行に対する双方向性制御機構の解析

○濱田 祐輔[1,2]、吉田 小莉[1]、成田 道子[2,3]、須田 雪明[1,2]、葛巻 直子[1,2]、森 友久[1]、成田 年[1,2]

[1]星薬科大・薬・薬理、[2]国立がん研セ・研・がん患者病態生理、[3]東京医科大・医学総合研・分子細胞治療

Opioid analgesics are widely used to manage moderate to severe pain in cancer patients. The μ opioid receptor (MOR) is expressed in not only the brain and spinal cord, but also a wide range of peripheral sites, including the gastrointestinal tract and immune cells. It has been considered that the central μ-opioidergic system is closely associated with analgesia and euphoric effects, whereas the peripheral μ-opioidergic system is responsible for side effects such as constipation and nausea. However, little is known about the role of the endogenous μ-opioidergic system in the control of the innate immune response. In this study, we investigated the functional role of central and peripheral μ-opioidergic systems in tumor progression using a pharmacological and genetics approach. First, we found that treatment with peripheral MOR antagonists significantly decreased Lewis lung carcinoma (LLC)-graft compared to that in control mice. On the other hand, activation of the hypothalamic μ-opioidergic system using the Gq-DREADD technique significantly decreased the tumor volume compared to that in control mice. Taken together, these findings suggest that the central and peripheral μ-opioidergic systems may play a bidirectional role in the control of tumor progression.

Bidirectional pain control by spinal noradrenaline via astrocyte-neuron interactions

アストロサイト―神経相互作用を介した脊髄ノルアドレナリンによる双方向性痛覚制御

○川邉 陸[1]、吉原 康平[1]、古賀 啓祐[1,2]、津田 誠[1]

[1]九州大・院薬・薬理、[2]兵庫医科大・神経生理

Pain transmission in the spinal dorsal horn (SDH) is regulated by descending neuronal pathways from the brain, such as noradrenergic (NAergic) neurons from the locus coeruleus. While it is known that spinal NA produces an antinociceptive effect, we have recently shown that NA has an ability to produce pain hypersensitivity via Hes5-expressing SDH astrocytes. However, the mechanism underlying the bidirectional effect of spinal NA remains unknown. In this study, we showed that while intrathecal injection of NA at a low dose (NA^{low}) induced pain hypersensitivity via α_{1A}-adrenergic receptors (α_{1A}-ARs) in $Hes5^{+}$ astrocytes, the hypersensitivity was not observed by intrathecal high-dose NA (NA^{high}). The effect of NA^{high} was also mediated by activation of inhibitory interneurons via α_{1A}-ARs. We found that NA^{high} also activated β_1-ARs in astrocytes that suppressed the astrocytic α_{1A}-ARs-mediated effect. However, if α_{1A}-ARs are expressed in inhibitory interneurons, why does NA^{low} produce pain hypersensitivity? We further found that activation of astrocytic α_{1A}-ARs increased release of adenosine, a factor that suppresses inhibitory interneurons. Therefore, our findings indicate that NA bidirectionally modulates pain transmission via astrocyte-neuron interactions in a concentration-dependent manner.

Regulatory mechanism of fatty acid-binding protein 3 expression via docosahexaenoic acid during pain

疼痛時におけるドコサヘキサエン酸を介した FABP3 発現調節機構

○橘 男、中本 賀寿夫、徳山 尚吾

神戸学院大・薬・臨床薬学

Fatty acid-binding protein (FABP) regulates polyunsaturated fatty acids intracellular trafficking and functions as a signal transduction via modulation of gene expression. We have demonstrated that FABP3 protein was observed in microglia of the median eminence (ME) of hypothalamus and this protein was increased in the ME of pain model mice. These changes were correlated with the increment of hypothalamic docosahexaenoic acid (DHA) levels. Here, we assessed the effect of DHA on FABP3 expression using MG6 cell, a microglia cell line. Also, we tested the effect of FABP inhibitor on the mechanical allodynia in postoperative pain model mice. MG6 cells were cultured in Dulbecco's modified eagle medium with or without 10% fetal bovine serum (FBS) as a cell stress. FABP3 was measured by qPCR. Mechanical allodynia was assessed by von Frey test. FABP3 mRNA was expressed on the MG6 cell. Under the condition of serum-free media, FABP3 mRNA was also significantly increased compared to the media with 10% FBS. This increment was suppressed by DHA (300 μM). Repeated intraventricular injection of FABP inhibitor was significantly suppressed mechanical allodynia in postoperative pain mice. These results indicated that DHA might be involved in the regulation of microglial FABP3, and brain FABP might work as a regulator of pain.

Mirtazapine suppresses dopamine neurodegeneration by inducing metallothionein expression via stimulation on serotonin 1A receptor of astrocyte.

ミルタザピンはアストロサイトのセロトニン1A受容体を介したメタロチオネイン発現によりドパミン神経保護作用を示す

○菊岡 亮[1,3]、宮崎 育子[1]、久保田 菜月[1]、前田 恵実[1]、香川 大樹[1]、守山 雅晃[1]、佐藤 明日香[1]、村上 真樹[1]、北村 佳久[2]、千堂 年昭[2]、座間味 義人[3]、浅沼 幹人[1]

[1]岡山大・院医歯薬・脳神経機構学、[2]岡山大・院医歯・臨床薬剤学、[3]岡山大学病院・薬剤部

Parkinson's disease (PD) is one of the most prevalent neurodegenerative diseases and disease-modifying treatment is required to inhibit the disease progression. We have previously reported that 8-OH-DPAT, serotonin (5-HT)1A full agonist, induced expression of antioxidant metallothionein (MT) in astrocyte and exhibited protective effects against 6-OHDA-induced neurodegeneration of nigrostriatal dopamine (DA) neuron. In this study, we investigated neuroprotective effect of anti-depressant mirtazapine, as an indirect 5-HT1A agonist, against dopaminergic neuronal death. Mirtazapine administration to 6-OHDA-injected hemi-parkinsonian mice significantly increased MT expression in striatal astrocytes and inhibited the reduction of nigrostriatal DA neuron. These effects were cancelled by simultaneous administration of 5-HT1A antagonist. To explore the precise neuroprotective mechanism, we examined effects of mirtazapine using primary cultured mesencephalic neurons and striatal astrocytes from rat fetus. Neuroprotection by mirtazapine was observed only in neuron-astrocyte co-cultured condition. Furthermore, MT expression in astrocyte was significantly increased when astrocytes were treated with mirtazapine-pretreated neuronal conditioned medium (Mir-NCM). Treatment with medium from Mir-NCM-treated astrocytes (Mir-NCM-ACM) showed dopaminergic protection against 6-OHDA. These effects were cancelled when astrocytes were treated Mir-NCM and 5-HT1A antagonist. Moreover, MT antibody completely cancelled the neuroprotective effects of Mir-NCM-ACM. These results suggested that mirtazapine protected DA neurons by inducing expression and secretion in/from astrocytes via indirect stimulation on astrocytic 5-HT1A receptor.

Histamine promotes tube formation of vascular endothelial cells

ヒスタミンは血管内皮細胞の管腔形成を促進する

ハティポール オメル ファルク[1]、西中 崇[1]、西堀 正洋[2]、渡邊 政博[3]、豊村 隆男[3]、森 秀治[3]、和氣 秀徳[1]、高橋 英夫[1]
[1]近畿大・医・薬理学教室、[2]岡山大・院医歯薬、[3]就実大・薬・生体情報学

【Aim】
Histamine is well known as an inflammatory mediator whereas little is demonstrated about the mechanism of histamine-induced angiogenesis. The role of histamine in angiogenesis was first reported in 1983 in the chorioallantoic membrane (CAM) assay. Still, the histamine-induced tube formation mechanism has not been investigated with histamine single-acting in vitro study. In the present study, we demonstrated the effects of histamine on the tube formation processes of human endothelial cells.

【Method】
Histamine-induced tube formation was analyzed by the matrigel assay using the human-derived vascular endothelial cell line EA.hy926. To investigate the effects of histamine H1 receptor (H1R) antagonist and protein kinase C (PKC) inhibitors vascular endothelial cells were stained with Calcein-AM and observed under a microscope. In addition, the expression of angiogenesis-related factors such as vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) was analyzed by the RT-PCR method.

【Result】
Histamine concentration-dependently remarkably enhanced tube formation of EA.hy926 cells in a matrigel assay at 16 hours. In addition, histamine-induced tube formation was entirely blocked by inhibitors of H1R and PKC. This result suggests that H1R-PKC signaling is involved in histamine-induced tube formation. We have also shown treatment of EA.hy926 cells with 10 μM histamine resulted in a marked upregulation of VEGF mRNA expression with a peak at 3 hours and an inhibitor of VEGF Receptor (VEGFR) -2 suppressed histamine-induced tube formation. Additionally, histamine stimulation induced the expression of MMP-9 and MMP-14, which play important roles in the regulation of angiogenesis, and MMP inhibitors blocked histamine-induced tube formation.

【Conclusion】
In this study, we have shown a remarkable tube formation in vitro model induced by histamine in endothelial cells through the H1 receptor. In addition, this action was linked to the activation of PKC, VEGF, VEGFR2, and MMPs.

Epithelial BLT1 Plays an Important Role in Colonic Mucosal Wound Repair

腸管上皮BLT1は腸管粘膜の創傷治癒において重要な役割を担う

○林 周作[1]、Quiros Miguel[2]、奥 牧人[1]、Parkos Charles A.[2]、Nusrat Asma[2]

[1]富山大・和漢研・未病、[2]ミシガン大・医・病理

Acute and chronic intestinal inflammation is associated with epithelial damage, resulting in mucosal wounds in the forms of erosions and ulcers in the intestinal tract. Intestinal epithelial cells (IECs) and immune cells in the wound milieu secrete cytokines and lipid mediators to influence wound repair. Leukotriene B_4 (LTB_4), a lipid chemokine, binds to its receptor BLT1 and promotes migration of immune cells to sites of active inflammation, however a role for intestinal epithelial BLT1 during mucosal wound repair is not known. Intestinal epithelial BLT1 expression is increased when epithelial cells are exposed to an inflammatory microenvironment. Using human and murine primary colonic epithelial cells, we reveal that LTB_4-BLT1 pathway promotes epithelial migration and proliferation leading to accelerated epithelial wound repair. Furthermore, *in vivo* intestinal wound repair experiments in BLT1-deficient mice and bone marrow chimeras demonstrate an important contribution of epithelial BLT1 during colonic mucosal wound repair. Taken together, our findings show a novel pro-repair mechanism in IECs mediated by BLT1 signaling.

The prediction of therapeutic targets and microRNA network in the coronavirus pathogenesis pathway

コロナウィルス病態パスウェイのmicroRNAネットワークと治療ターゲット予測

○田邊 思帆里[1]、カデール サビーナ[2]、小野 竜一[3]、カブラル オラシオ[4]、青柳 一彦[5]、広瀬 明彦[1]、横崎 宏[6]、佐々木 博己[7]

[1]国立医薬品食品衛研・安セ・安全性予測評価、[2]ナノ医療イノベーションセンター、[3]国立医薬品食品衛研・安セ・毒性、[4]東京大・院工、[5]国立がん研・基セ・臨床ゲノム解析、[6]神戸大・院医・病理、[7]国立がん研・基セ・シーズ探索

The coronavirus pathogenesis pathway is activated in coronavirus infection. To reveal the therapeutic targets of the coronavirus pathogenesis, public gene expression data were analyzed in Ingenuity Pathway Analysis (IPA). Among more than 100,000 analyses and datasets, 106 analyses and 106 datasets were related to SARS coronavirus 2. The 49 analyses were involved in SARS coronavirus 2 and human, which comprise of 27 analyses including 9 analyses on tissue “skin” GSE156754 and 22 analyses on lung adenocarcinoma. FOS and JUN in the coronavirus pathogenesis pathway were activated in SARS-CoV-2 infected lung adenocarcinoma. Coronavirus pathogenesis pathway was activated in SARS-CoV-2 infected iPS cell-derived cardiomyocytes. The molecular networks and gene expression in diffuse- and intestinal-type gastric cancer (GC) have been analyzed as well. Coronavirus pathogenesis pathway was activated in diffuse-type GC and inactivated in intestinal-type GC. Telmisartan, acetaminophen and arsenic trioxide were found to interact with the coronavirus pathogenesis pathway. NFkappaB, a target of thalidomide, was activated in diffuse-type GC. The coronavirus pathogenesis pathway had direct relationships between microRNAs including let-7, mir-10, mir-15, and mir-155. The molecules identified have potential to be the therapeutic targets.

Upregulation of neuregulin-1 in the ventricle of diabetic cardiomyopathy model mice and its functional significance

糖尿病性心筋症モデルマウス心室におけるニューレグリン−1の発現増加とその機能的意義

○三上 義礼、岩瀬 奎輝、冨田 太一郎、大島 大輔、赤羽 悟美

東邦大・医・生理・統合生理

Diabetic cardiomyopathy (DMCM) is myocardium disorder in diabetic patients independent of diabetic vascular dysfunction. It is characterized by an early diastolic dysfunction and subsequent progression to systolic dysfunction. The underlying mechanism of the DMCM development has not yet been fully understood. We aimed to elucidate the molecular mechanism of DMCM progression. In the streptozotocin (STZ)-induced DM model mice 4 weeks after STZ injection (STZ-4W), diastolic function was impaired without systolic dysfunction. In the ventricles of STZ-4W mice, the mRNA and protein expression levels of neuregulin1 (NRG -1) turned out to be significantly higher than that of control mice. Chronic insulin administration restored the blood glucose, left ventricular diastolic function, and NRG-1 expression to the control levels. NRG-1 was localized in the epicardium, endocardium, and endothelial cells of blood vessels in the ventricle. To clarify the role of up-regulated NRG-1 in the early stage of DMCM, we examined the effects of trastuzumab (TRZ), antibody against NRG-1 receptor ErbB2. Not only diastolic function but also systolic function was significantly impaired in the TRZ-injected STZ-4W mice. These results suggest that a compensatory increase in NRG-1 prevents the progression to systolic dysfunction during the early stage of DMCM.

Effects of esaxerenone on blood pressure and sodium balance in Dahl salt-sensitive hypertensive rats

エサキセレノンによって食塩感受性高血圧ラットにおいて生じる血圧とナトリウムバランスの変化

○西山 成、服部 真依、北田 研人、ラフマン アサダ

香川大・医

The nonsteroidal mineralocorticoid receptor blocker, esaxerenone, is effective in reducing blood pressure (BP) in hypertensive patients. However, the mechanism responsible for anti-hypertensive effect of esaxerenone is not clear. Therefore, we investigated esaxerenone-driven sodium homeostasis and its association with changes in BP in Dahl salt-sensitive (DSS) hypertensive rats. BP was measured by a radiotelemetry system, and sodium homeostasis was determined by an approach of sodium intake (food intake) and excretion (urinary excretion) in DSS rats with a low-salt diet (0.3% NaCl), high-salt diet (HSD, 8% NaCl), HSD plus 0.001% esaxerenone (w/w), and HSD plus 0.05% furosemide. HSD-fed DSS rats showed a dramatic increase in BP with a non-dipper pattern, while esaxerenone treatment, but not furosemide, significantly reduced BP with a dipper pattern. The cumulative sodium excretion in the active period was significantly elevated in esaxerenone- and furosemide-treated rats compared with their HSD-fed counterparts. However, a significant increase in the sodium/potassium ratio was only observed in esaxerenone-treated rats. Sodium content in the skin, skinned carcass, and total body tended to be lower in esaxerenone-treated rats than in their HSD-fed counterparts, while these values were unchanged in furosemide-treated rats. Consistently, sodium balance tended to be reduced in esaxerenone-treated rats during the active period. These data indicate that esaxerenone-induced reduction in BP is associated with improvement of body sodium homeostasis in salt-dependent hypertension.

mPGES-1/PGE2 axis induces recovery from ischemia via SDF-1/CXCR4 axis-mediated accumulation of Tregs in ischemic muscle

mPGES-1/PGE2 はSDF-1/CXCR4 axis によりTregsを虚血部位に集積することで虚血改善を促進する

○天野 英樹[1]、江島 耕二[2]、伊藤 義也[1]、細野 加奈子[1]、畑中 公[1]、植松 智[3]、審良 静男[4]、成宮 周[5]、馬嶋 正隆[6]
[1]北里大・院医療、[2]北里大・理・免疫学、[3]大阪市立大・医・ゲノム免疫学、[4]大阪大・生体防御部門・自然免疫学分野、[5]京都大・院医・創薬医学講座、[6]神奈川工科大学・臨床工学科・病態治療研究室

We have reported that mPGES-1/PGE2 induced blood flow recovery from ischemia by promoting accumulation of Tregs. Expression of chemokines and the SDF-1/CXCR4 axis has been implicated in Tregs migration in angiogenesis. Therefore, we examined whether the SDF-1/CXCR4 axis plays a role in the accumulation of Tregs in angiogenesis.

Male 6-8 week-old wild-type mice (WT), mPGES-1-deficient mice (mPges-1-/-) were used. Recovery from ischemia was estimated by laser Doppler imaging. Contribution of Tregs was estimated by immunohistichemical study against FoxP3.The expression of Sdf-1 and Cxcr4 in ischemic muscle and accumulated Tregs were estimated by real time RT-PCR. The function of Tregs was estimated by in vitro suppression assay.

In WT, recovery from ischemia was significantly suppressed in CXCR4 antibody treated mice compared to Control IgG. In contrast, there was no significant changes in mPges-1-/-. In ischemic muscle tissue, expression of Sdf-1 and Cxcr4 mRNA was significantly decreased in mPges-1-/- mice compared with WT mice. In accumulated CD4+CD25+ Tregs in ischemic muscle tissue, expression of Cxcr4 mRNA was significantly decreased in mPges-1-/- mice compared with WT mice. Furthermore, in vitro analysis revealed that expression of Cxcr4 mRNA was induced in Tregs from WT mice upon anti-CD3 stimulation but not in Tregs from mPges -1-/- mice. In vitro suppression assay showed there was no difference in Treg function between WT and mPges -1-/- mice.

These results suggested that SDF-1/CXCR4 axis induces ischemic recovery by the accumulation of Tregs in ischemic muscle which was depended on mPGES-1/PGE2 axis.

Metalloprotease Nardilysin regulates sinus node automaticity through modulating ion channel transcription.

多機能プロテアーゼによる洞機能制御機構

○大野 美紀子[1]、松浦 博[2]、牧山 武[3]、糀谷 泰彦[3]、西 清人[1]、西 英一郎[1]

[1]滋賀医科大・医・薬理、[2]滋賀医科大・医・生理、[3]京都大・医・循環器内科

Nardilysin (NRDC) is a metalloprotease of the M16 family, which has multiple functions such as enhancing the ectodomain shedding of membrane proteins in the extracellular space and regulating transcription in the nucleus. NRDC-deficient mice (*Nrdc*-/-) show various phenotypes such as hypomyelination, hypothermia, and bradycardia. In the present study, we have focused on the role of NRDC in regulating heart rate and obtained the following results: (1) The intrinsic heart rate, determined by pharmacological blocking of the autonomic nervous system, was significantly reduced in *Nrdc*-/-; (2) Funny (If) current and T-type calcium current were significantly reduced in isolated Nrdc-/- sinus node cells; (3) Messenger RNA levels of Cav3.1 and HCN1/4, ion channels involved in sinus automaticity, were markedly decreased in *Nrdc*-/- hearts; (4) Gene knockdown of NRDC in primary rat cardiomyocytes reduced HCN1/4 mRNA levels; (5) Chromatin immunoprecipitation PCR showed NRDC binding to the promoter regions of Cav3.1 and HCN1/4; (6) Reintroduction of wild-type NRDC, but not the enzymatically inactive mutant of NRDC (E>A mutant), into NRDC-deficient cells restored HCN1 mRNA expression; (7) NRDC-E>A mutant knock in mice showed bradycardia and significantly reduced intrinsic heart rate, suggesting that NRDC enzyme activity is important for the control of heart rate. Together, our results indicate that NRDC in cardiomyocyte controls heart rate through the transcriptional regulation of ion channels critical for sinus automaticity.

Regulation of cardiac robustness and mitochondrial quality by sulfur metabolism

硫黄代謝によるミトコンドリア品質制御を介した心筋頑健性機構の解析

○西村 明幸[1]、湯 肖康[1]、下田 翔[2]、加藤 百合[2]、西山 和宏[2]、西田 基宏[1,2]

[1]生理研・心循環、[2]九州大・院薬・生理学

Proper mitochondrial quality control is indispensable for cardiac homeostasis and defects in mitochondrial dynamics are implicated in the development of cardiac diseases. Our group has investigated the molecular mechanism underlying the development of maladaptive cardiac remodeling, especially myocardial senescence, and found that mitochondrial hyperfission induced by aberrant activation of Dynamin-related protein 1 (Drp1), a mitochondrial fission-accelerating protein, is a key determinant of cardiac remodeling and fragility. Supersulfides have been recently recognized as a key molecule to regulate redox homeostasis and are abundantly discovered in both prokaryotes and eukaryotes. We found that Drp1 activity is negatively regulated by supersulfide-mediated polysulfidation of Drp1 at Cys^{624}. Ischemic stress induced by myocardial infarction converted supersulfides into hydrogen sulfide, and reduced supersulfides promoted Drp1 hyperactivation via depolysulfidation of Cys^{624}, causing myocardial senescence and cardiac fragility. Exposure of cardiomyocytes to environmental electrophiles such as methylmercury also induced supersulfide depletion and triggered mitochondrial hyperfission-associated myocardial senescence.

加齢による心臓組織マクロファージのケモカインとケモカインセプターの発現変化

Aging induces alterations in expression pattern of chemokines and chemokine receptors in cardiac tissue macrophages

○工藤 藤美[1]、金 恵理[2]、眞鍋 一郎[1]

[1]千葉大・院医・疾患システム医学、[2]千葉大・院薬・分子心血管薬理学

The number of patients with heart failure has increased due to the aging of society. We previously showed that cardiac Ly6C^{lo} macrophages from aged mice lost the cardioprotective function observed in those from young mice. Notably, our single-cell RNA-sequencing (scRNA-seq) analysis demonstrated that tissue-resident macrophage subpopulations were replaced with aged mice-specific subpopulations. In young mice, cardiac macrophages were constitutively expressed various chemokines, although there was little or no expression of cytokines in the steady state. Our scRNA-seq data revealed lower expression levels of *Ccl2, Ccl3, Ccl4* and *Cxcl2* and higher expression of *CCR5* in aged mice-specific cardiac macrophages. These results suggest that alteration of the expression of chemokines in cardiac macrophages may result in changes in the proportion of each immune cells in the heart of aged mice, triggering age-associated disease induction. We confirm the pathophysiology of macrophage-specific *Cxcl2*-deficient mice in a transverse aortic constriction (TAC) model to elucidate the regulatory mechanism of progression of tissue fibrosis by macrophage-derived chemokines. Furthermore, we assessed the effects of CCR5 inhibitor on tissue fibrosis in TAC mice. Thus, we clarify the relationship between aging-associated heart failure and phenotypic changes in cardiac macrophages.

Preceding inhibition regulates hippocampal spikes sequences

海馬リップル直前の抑制性入力によるCA1野錐体細胞の発火タイミング制御機構の解明

○野口 朝子[1]、池谷 裕二[1,2]

[1]東京大・院薬・薬品作用、[2]東京大・Beyond AI研究推進機構

Neuronal spike times are essential for coding information. A subset of neurons often emits a series of action potentials and generate sequences of spikes. The spike times of neurons during a sequence are regulated at the millisecond level while preserving flexibility to generate diverse patterns in different sequences. Spike sequences are exemplified by memory replays during sharp wave–ripples (SWRs). Hippocampal pyramidal cells that are sequentially activated during behavior are reactivated in time-compressed manner during SWRs while animals are immobile or asleep. This replay of sequential activity has been believed to contribute to memory consolidation and navigational planning. However, the mechanism for such flexible modification of spike times remains unclear. In this study, we conducted in vivo whole-cell recordings simultaneously from up to three CA1 pyramidal cells and examined the membrane potential dynamics at the single-cell level. Neurons were transiently hyperpolarized tens of milliseconds before SWRs. The pre-SWR hyperpolarizations varied in magnitude across SWR events and individual neurons, and larger pre-SWR hyperpolarizations induced later spike times during SWRs. Thus, pre-SWR inhibition coordinates the sequential spike times of CA1 pyramidal cells and diversifies the repertoire of sequence patterns.

Single-molecule imaging of synaptic molecules within the brain tissue

脳組織内部におけるシナプス分子動態の1分子イメージング

○大久保 洋平[1]、並木 繁行[2]、浅沼 大祐[2]、櫻井 隆[1]、廣瀬 謙造[2]

[1]順天堂大・医・薬理学、[2]東京大・院医・細胞分子薬理学

Accumulating evidence suggests that molecular dynamics at nanometer scale is crucial for synaptic functions. Single-molecule fluorescence imaging is a super-resolution live imaging method that enables direct tracking of movement of individual molecules. However, conventional single-molecule imaging has been applicable only to dissociated cells on coverslips due to technical limitations, preventing the analysis of events that occur only in the intact brain tissue. In this study, we set out to develop a method for single-molecule imaging within brain slices and the brain *in vivo*. We developed and employed a novel chemical tag technology named De-QODE. This technology consists of a small-molecular QODE probe and DeQODE protein tag. Non-fluorescent QODE becomes highly fluorescent upon reversible binding to DeQODE. These properties realize fluorescent labeling of proteins of interest with extremely low-background fluorescence even within tissue samples. Furthermore, De-QODE-based labeling is repeatable after photobleaching. Owing to De-QODE-based single-molecule imaging, we succeeded in high-density tracking of synaptic molecules in pyramidal neurons deep within acute cortical slices.

Mechanism of aggregation of α-Synuclein initiated by RNA phase transition

RNA 相転移による α−シヌクレイン凝集体誘導メカニズム

○矢吹 悌[1,2]、松尾 和哉[1]、酒井 勇輔[1,2]、塩田 倫史[1,2]

[1]熊大・発生研・ゲノム神経、[2]熊本大・薬

The mechanism underling dysfunction of cellular proteostasis on α-synuclein (α-Syn) leading to pathogenesis of synucleinopathy remains unclear. Recently, we reported that the binding of an RNA secondary structure G-quadruplex (G4RNA) to a prion-like protein FMRpolyG causes its liquid-to-solid phase transition, leading to neurodegeneration in a hereditary neurodegenerative disease, Fragile X-associated Tremor/Ataxia Syndrome (FXTAS) (*Sci Adv.* 2021). Here, we introduce the possibility that G4RNA is a key pathogen on the phase transition of α-Syn. Purified α-Syn protein binds to G4 structure formed RNA specifically, and that the addition of G4RNA promoted the liquid-to-solid phase transition on α-Syn under molecular crowding *in vitro*. In mouse primary neurons, G4RNA assembly was immediately observed under cellular stress conditions, thereafter co-aggregation of α-Syn with G4RNA was occurred. Artificial assembly of G4RNA using an optogenetic approach initiated α-Syn aggregation, thereby elicits neuronal dysfunction in mouse primary neurons. These results suggest that G4RNA assembly evoked by various cellular stress triggers to develop aggregation of α-Syn, which may be a cellular mechanism underlying onset of sporadic synucleinopathy. We now analyze relationship between G4RNA and α-Syn aggregation *in vivo*.

Clarification of molecular mechanisms for axonal regeneration in the brains of Alzheimer's disease model mouse

アルツハイマー病モデルマウスの脳内で軸索が再伸長する分子メカニズムの解明

○楊 熙蒙、東田 千尋

富山大・和漢研・神経機能学

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by Aβ deposition and disruption of neural networks in the brain. We previously found that diosgenin, a constituent of Dioscorea Rhizoma, restored Aβ -induced axonal atrophy in neurons (*in vitro*) and recovered memory deficits in a mouse model of AD, 5XFAD. Importantly, we were the first to discover that diosgenin administration promoted long-distance axonal regeneration in 5XFAD mice brains. In the present study, we aimed to clarify molecular mechanisms for controlling accurate pathfinding of injured axons in AD brains.

Axon-regenerated neurons (after diosgenin administration) in the neural circuits contributing memory formation; from the hippocampus to the prefrontal cortex, were selectively visualized by retrograde tracings. Naïve neurons and axon-regenerated neurons in the brain slices were separately captured by laser microdissection to serve DNA microarray. Overexpression of the gene, whose expression level was drastically elevated in axon-regenerated neurons, to the hippocampal neurons promoted axonal regeneration in the brain and recovered memory deficits in 5XFAD mice.

Our study identified key molecules for promoting axonal regeneration toward long distance away target area in AD brains. This finding proposes a novel therapeutic strategy for AD treatment.

Regulation of antinociceptive tolerance to morphine by RTP4, an endogenous chaperone protein

内因性シャペロン蛋白質 RTP4 によるモルヒネ鎮痛耐性形成の制御

○藤田 和歌子[1]、内田 仁司[2,3]、川西 雅史[4]、黒岩 祐介[4]、阿部 学[2,5]、崎村 建司[2,5]

[1]長崎大・院医歯薬・医薬理、[2]新潟大、[3]新潟大、[4]長崎大・薬・創薬薬理学分野、[5]新潟大

Previous studies have shown that receptor transporter protein 4 (RTP4), one of the receptor chaperone proteins, contributes to maturation and membrane trafficking of novel opioid receptor heterodimers consisting of mu (MOPr) and delta (DOPr) opioid receptors (MOPr-DOPr). Although MOPr-DOPr is shown to contribute to the mechanism of development of antinociceptive tolerance to morphine, the role of RTP4 in such mechanism has not been elucidated yet. Since *rtp4* will be upregulated by repeated administration of morphine especially in the hypothalamus, here we determined the effect of knockdown of RTP4 in the selective brain region on the development of antinociceptive tolerance to morphine by using $Rtp4^{flox/flox}$ mice. In this study, $Rtp4^{flox/flox}$ mice were generated and infected with AAV expressing Cre recombinase. Knockdown of *rtp4* levels in hypothalamus partially but significantly suppressed the development of morphine tolerance. In addition, we found that the induction of *rtp4* gene by MOPr-activation was reversed by inhibitors of Gi and MAPK pathways in neuronal cells. These results indicate that RTP4 in hypothalamus partly but significantly contributes to the mechanism of development of morphine tolerance after repeated administration of morphine. Also the upregulation of *rtp4* by morphine may be mediated by MAPK pathways.

Electrophysiological properties of a mutation in the human Cav3.1 T-type channel associated with neuropsychiatry.

神経疾患関連 Cav 3.1 T 型カルシウムチャネル変異体の電気生理学的機能解析

○酒井 勇輔[1,2]、矢吹 悌[1,2]、塩田 倫史[1,2]

[1]熊本大・発生医学研究所・ゲノム神経学分野、[2]熊本大・薬

Low-threshold T-type calcium channels (approximately at -60 mV) show unique electrophysiological features with the fast inactivation and slow deactivation kinetics. T-type calcium channels are expressed in the mammalian brain and involved in the pathophysiology of epilepsy, pain and sleep. Recently, a novel de novo mutant Cav3.1 T-type calcium channel at V1330E have been reported by meta-analyses of the exome sequences of patients with schizophrenia (Iyegbe CO et al., 2022. *Nature*), however, its electrophysiological properties are still unknown. In this study, we aimed to compare the electrophysiological properties of mutant Cav3.1 at between the A961T associated with cerebellar ataxia (gain-of-function) and the V1330E. Each mutant Cav3.1 at A961T and V1330E was generated and transiently transfected in Neuro2A cells. Using the whole-cell patch-clamp technique, we successfully demonstrated that mutant Cav3.1 (A961T) displays very slow inactivation kinetics and unique changes in the steady state kinetics compared with wild-type Cav3.1 channel as well as previously observation. We currently analyze the property of mutant Cav3.1 (V1330E) and would like to present the data and discuss the significance of the mutation at V1330E on site.

Protective effect of lansoprazole against cisplatin-induced ototoxicity

シスプラチン誘発性聴覚障害に対するランソプラゾールの保護効果

○若井 恵里[1]、弓削 瑞葵[2]、池村 健治[3]、奥田 真弘[3]、西村 有平[2]

[1]大阪大・院医・統合薬理学、[2]三重大・院医・統合薬理学、[3]大阪大学医学部附属病院・薬剤部

Cisplatin accumulates in the inner ear cochlea via organic cation transporter 2 (OCT2) and causes ototoxicity. Lansoprazole, a proton pump inhibitor, ameliorated cisplatin-induced nephrotoxicity via inhibiting OCT2. In the present study, we investigated the protective effect of lansoprazole against cisplatin-induced ototoxicity. In the zebrafish study, we compared the effect of lansoprazole on cisplatin induced ototoxicity using in vivo fluorescence imaging of the hair cells stained with YO-PRO1. The fluorescence signals in hair cells in zebrafish treated with cisplatin dose-dependently decreased. Co-treatment with lansoprazole significantly suppressed the decrease of fluorescence signals in zebrafish treated with cisplatin. Knockout of a zebrafish homolog of OCT2 also ameliorated the reduction of fluorescence signals in hair cells in zebrafish treated with cisplatin. We then retrospectively analyzed the medical records of Mie University Hospital to examine the otoprotective effect of lansoprazole. The incidence rate of ototoxicity was significantly lower in patients co-treated with LPZ compared to those without lansoprazole. These results suggest that lansoprazole should suppress cisplatin-induced ototoxicity by inhibition of OCT2.

Upregulation of Galectin-7 in specific tumor microenvironment contributes to squamous cell carcinoma metastasis

特異的がん微小環境にて誘導されるGalectin-7は扁平上皮癌の転移促進因子である

○安 健博[1]、長岐 雄志[2]、星崎 みどり[1,3]、山口 智和[1]、久世 裕太[4]、今井 由美子[3]、本山 悟[2]、鈴木 穣[4]、久場 敬司[1]

[1]秋田大・院医・分子機能学・代謝機能学講座、[2]秋田大・院医・地域がん医療学講座、[3]医薬基盤・健康・栄養研、[4]東京大・大学院新領域創成科学研究科・メディカル情報生命専攻生命システム観測分野

Metastasis predicts poor prognosis in patients with esophageal squamous cell carcinoma, whereas the regulatory mechanism of metastasis remains largely unknown. In this study, by using murine tumor metastasis model and spatial transcriptome analysis, we aimed to decipher the molecular basis of metastasis. In a syngeneic mouse squamous cell carcinoma (SCC) model of NR-S1M cells, we isolated metastasized NR-S1M cells from lymph nodes in tumor-bearing mice and established metastatic NR-S1M cells in in vitro culture. RNA-seq analysis revealed that interferon gene signature was markedly downregulated in metastatic NR-S1M cells compared with parental cells, and in vivo NR-S1M tumors heterogeneously developed focal immunosuppressive areas featured by deficiency of anti-tumor immune cells. Spatial transcriptome analysis (Visium) for the NR-S1M tumors revealed that various pro-metastatic genes were significantly upregulated in immunosuppressive areas when compared to immunocompetent areas. Notably, Galectin-7 was identified as a novel metastasis-driving factor. Galectin-7 expression was induced during tumorigenesis particularly in the microenvironment of immunosuppression, and extracellularly released at later stage of tumors progression. Deletion of Galectin-7 in NR-S1M cells significantly suppressed lymph node and lung metastasis without affecting primary tumor growth. Therefore, Galectin-7 plays a crucial role in tumor metastasis of SCC as a pro-metastatic factor in the immune-suppressed tumor areas and may be a potential target of cancer immunotherapy.

Effectiveness of proton pump inhibitors on obsessive-compulsive disease discovered in real-world data and the molecular mechanism

リアルワールドデータで見い出された強迫性障害に対するプロトンポンプ阻害薬の有効性と治療メカニズムの解明

○幡鎌 輝[1]、浅岡 希美[2]、永安 一樹[1]、白川 久志[1]、金子 周司[1]

[1]京都大・院薬・生体機能解析学、[2]京都大・院医・システム神経薬理学

Obsessive-compulsive disorder (OCD) is a psychiatric syndrome accompanied with inadequate repetitive and/or habitual actions, which often appears as an adverse effect of dopamine receptor stimulants. In this study, to find a new therapeutic target for OCD, we analyzed human real-world data including adverse events self-reports and insurance claims and found that concomitant use of proton pump inhibitors suppressed the incidence of OCD symptoms induced by the use of pramipexole or ropinirole. In an in vivo validation of the hypothesis, OCD-like abnormalities, such as spontaneous repetitive behavior and facilitation of habit formation were observed in mice received repetitive injections of quinpirole (QNP; a dopamine D_2 receptor agonist; 1 mg/kg; 8 days). In this OCD model, a systemic vonoprazan (Vpz; a proton pump inhibitor; 100 mg/kg i.p.) or an i.c.v. injection of Vpz (3 μg) suppressed the QNP-induced OCD-like behaviors. In ex vivo cortical slices prepared from OCD model mice, an increase in the firing rate was observed in pyramidal neurons of lateral orbitofrontal area, which was reduced in the presence of Vpz (10 μM) or by intracellular acidification from pH 7.3 to 7.0. In primary cultured cortical neurons in which Atp4a gene (encoding proton pump) was knocked down, a decrease in pH by Vpz was mitigated in parallel with a reduction of firing rate. These results demonstrate that regulation of acid-base balance of orbitofrontal neurons will be a novel therapeutic target for OCD.

TRAb-IgM induced by Epstein-Barr virus reactivation did not inhibit TSH binding to the receptor

Epstein-Barr virus再活性化に誘導されるIgM型のTSHレセプター抗体はTSHのレセプター結合を阻害しない

○長田 佳子[1]、林 一彦[2]、市原 克則[1]、澤野 達哉[1]、プリヨノ アグン[1]、三明 淳一郎[1]、今村 武史[1]

[1]鳥取大・医・薬理学・薬物療法学、[2]鳥取大・医・病理学

Objective

Thyrotropin receptor antibody (TRAb) is a causative antibody of Graves' disease. Epstein-Barr virus (EBV) persists in human B cells and occasionally reactivates. During EBV reactivation, the host B cells differentiate to be plasma cells and produce IgM-dominant antibodies. We have previously observed that TRAb-IgM disruptes thyroid follicular epithelial cells and does not transduce thyroid hormone-producing signals. However, it is still unclear how it works on receptor-binding of TSH. We aimed to investigate this.

Methods

TRAb-IgM were separated from sera or EBV-reactivated culture media of peripheral blood mononuclear cells from Graves' disease patients. TSH binding-inhibitory activity of TRAb-IgM was assessed by a commercial radio-receptor assay kit.

Results & Discussion

All TRAb-IgM samples showed gamma-ray counts that were almost twice that of the 0 standard. This meant that two molecules of ^{125}I-TSH bound to one TRAb-IgM binding complex because TRAb-IgM kept a TSH receptor for the separation procedure. This result indicated that TRAb-IgM bound to almost all TSH receptors coated in the test tubes, and did not inhibit TSH binding to TSH receptors. Although thyroid-stimulating TRAb is IgG type, TRAb-IgM may has particular role on Graves' disease.

Conclusions

TRAb-IgM did not inhibit TSH binding to the TSH receptor. TRAb-IgM does not function as an antagonist.

Effect of high testosterone levels on endothelial function in aorta and erectile function in rats

ラットへの高用量のテストステロン投与がもたらす血管内皮機能および勃起機能への影響

○片岡 智哉[1,2,3]、深本 絢子[2]、堀田 祐志[2]、真川 明将[2]、前田 康博[2]、日比 陽子[2,3]、木村 和哲[2,3]

[1]千葉科学大・院薬・薬理学分野、[2]名古屋市立大・院薬・病院薬剤学、[3]名古屋市立大・院医・臨床薬剤学

Objectives: Testosterone is an important hormone for the physical and mental health of men. However, testosterone administration has also been suggested to adversely affect the cardiovascular system. We investigated the effects of excessive testosterone administration on vascular endothelial and erectile function in rats.

Methods: 12-week-old rats were divided into the following groups: Sham, castrated (Cast), castrated with subcutaneous administration of 100 mg/kg/month testosterone (Cast+T1), and castrated with subcutaneous administration of 100 mg/kg/week testosterone (Cast+T4). To observe the changes in testosterone level after the administration, rats were further divided into the following groups: control; T(6.25); T(25); and T(100), wherein the rats were subcutaneously injected with 6.25, 25 or 100 mg/kg testosterone per week. Erectile and endothelial functions were measured using intracavernosal pressure (ICP) and isometric tension.

Results: The ICP/MAP ratio in the Cast group (0.42 ± 0.04) was significantly lower than that in the Sham group (0.79 ± 0.07). The ICP/MAP ratio in the Cast+T1 group (0.73 ± 0.06) was significantly higher than that in the Cast group ($P < 0.01$) and that of the Cast+T4 (0.38 ± 0.01) group was unchanged ($P > 0.05$). The T(25) and T(100) groups exhibited significantly lower responses to ACh than the control group at four weeks ($P < 0.01$). Meanwhile, the ICP/MAP ratios in the T(25) group (0.44 ± 0.07) and T(100) group (0.47 ± 0.03) were significantly lower than that in the control group (0.67 ± 0.05) at stimulation frequencies of 16 Hz ($P < 0.05$).

Conclusion: Excessive testosterone may cause endothelial dysfunction in the aorta and erectile dysfunction in rats and that the blood concentration should be monitored after testosterone administration.

Image-based obesogenic screening using young zebrafish.

ゼブラフィッシュ稚魚を用いた内臓脂肪評価スクリーニング技術

○島田 康人[1,2,3]、中山 寛子[3,4]、臧 黎清[2,3]、松岡 いづみ[4]、幡 佳苗[4]、塚本 恒星[5]、深田 一剛[5]、湯浅 翔平[5]、熊沢 益徳[5]、勝崎 裕隆[6]、西村 訓弘[3,4]

[1]三重大・院医・薬理、[2]三重大・先端セ・バイオインフォ、[3]三重大・ゼブラセ、[4]三重大・院地域イノベ、[5]ロート製薬、[6]三重大・院生資

[Background] The obesity epidemic has been drastically progressing in both children and adults worldwide. Pharmacotherapy is considered necessary for its treatment. Because many anti-obesity drugs have been withdrawn from the market due to their adverse effects, the development of new drugs is still needed. Zebrafish are ideal model animals for *in vivo* testing of anti-obesity compounds, and disease models of several types of obesity have been developed.

[Methods] We developed a screening system using young zebrafish, "zebrafish obesogenic test (ZOT)", and performed screening using the focused natural product (NP) library. We then performed anti-adipogenic testing using the mouse 3T3-L1 preadipocytes to make comparison with ZOT outputs.

[Results] Seven and eleven NPs reduced lipid accumulation in zebrafish visceral fat tissues and mouse adipocytes, respectively. Of these, five NPs suppressed lipid accumulation in both zebrafish and 3T3-L1 adipocytes. We confirmed that these five NPs (globin-digested peptides (GD), green tea extract (GTE), red pepper extract, nobiletin, and Moringa (MO) leaf powder) exerted anti-obesity effects in diet-induced obese adult zebrafish, as a conventional model (Nakayama H, *et al.*, *Molecules*. 2020;25:5840). In addition, we validated that GD improved visceral adiposity in high-fat fed mice through UCP1 upregulation (Zang L, *et al.*, *Front Nutr*. 2021;8:650975). Based on the ZOT techniques, we further analyzed the gene expression profiles of the adipose tissue in GTE-fed zebrafish (Zang L, *et al.*, *Molecules*. 2021;26:2627) and tried to discover bioactive compounds in MO leaf using ZOT (Mastuoka I, *et al.*, *Food Sci Nurt*. 2022;00:1-9.).

[Conclusion] ZOT can be a high-throughput alternative to adult zebrafish models and can be applied for in vivo screening to discover novel therapeutics for visceral obesity and potentially also other metabolic disorders.

Effects of molecular hydrogen on dysbiosis and intestinal inflammation in high fat diet-loaded senescence-accelerated mice

高脂肪食負荷老化促進マウスのディスバイオシスおよび腸管炎症に対する分子状水素の効果

○高橋 さやか、中川 慶一、永田 亘、小泉 明穂、石塚 俊晶

防衛医科大

【Subject】It is suggested that aging and excessive intake of fat may induce dysbiosis and intestinal inflammatory damage additively. Previously, we revealed that the treatment with molecular hydrogen suppresses intestinal injury in high fat diet-loaded senescence-accelerated (SAMP8) mice.

【Method】SAMP8 mice were fed control diet or high fat diet (HFD) for 14 weeks, and then the each group was fed placebo jelly (PJ) or hydrogen-rich jelly (HRJ) for 4 weeks. After the treatment, small intestinal tissues were harvested for morphological examination. In addition, we performed TBARS assay by using homogenized small intestine to analyze peroxidation damage by measuring malondialdehyde (MDA) level. Moreover, we analyzed alterations of microbiota composition in cecal feces by 16S rRNA gene analysis of microbiota profiling.

【Result & Conclusion】The treatment with HRJ prevented the increases of CD11b expression and MDA level in HFD-loaded SAMP8 mice. The treatment with HRJ did not affect the abundance of Proteobacteria phylum in HFD-loaded SAMP8 mice. However, the expressions of Gracilibacter, Lactinobactor and Marvinbryantia were increased in the HRJ group.

These findings suggest that treatment with molecular hydrogen may affect the microbiota profiling and suppress intestinal inflammation and peroxidation damage in HFD-loaded SAMP8 mice.

D-Serine increases released acetylcholine levels in interstitial fluids in rat submandibular glands

D−セリンはラット顎下腺間質液中に遊離されるアセチルコリン量を増加する

○吉川 正信[1]、大久保 みぎわ[2]、川口 充[3]

[1]東海大・医、[2]川野歯科医院、[3]東京歯科大

Several D-amino acids have been observed in saliva, but their origin and function remain to be clarified. In the present study, large amounts of D-aspartate and small amounts of D-serine and D-alanine were detected in all three major salivary glands in rat. No other D-enantiomers were detected. Protein expression of D-amino acid oxidase and D-aspartate oxidase, the enzymes responsible for the oxidative deamination of neutral and dicarboxylic D-amino acids, respectively, and that of serine racemase, the enzyme converts L-serine to D-serine, were detected in all three major salivary glands in rat. The N-methyl-D-aspartate (NMDA) receptor subunit proteins NR1 and NR2D, but not NR2A, NR2B, or NR2C, were detected in all the three major salivary glands. Perfusion of D-serine with L-glutamate through rat submandibular artery increased salivary secretion during parasympathetic nerve stimulation in a D-serine dose-dependent manner. In vivo microdialysis applied to submandibular glands revealed that perfusion of L-glutamate with D-serine through the microdialysis probe increased acetylcholine contents in interstitial fluids in the glands of anesthetized rats as compared to that with L-glutamate alone in an NMDA receptor antagonist-sensitive manner. The present study suggests that D-amino acids play a physiological role in salivary glands.

TGF-β plays a role in platelet-mediated lymph-blood partitioning

TGF−βは血小板を介したリンパ管と血管の分離にはたらく

○椎谷 友博、劉 歆儀、杉山 彰、吉松 康裕、平島 正則

新潟大・院医歯

In the vascular system, lymphatic vessels play a cooperative role with blood vessels, but they form networks separated from each other. It has been shown that lymph-blood partitioning requires lymphatic endothelial cell (LEC)-induced platelet activation. However, it remains unclear whether platelet activation is involved in establishing a network of lymphatic vessels unconnected to blood vessels. In this study, we show that platelet activation blocks misconnection of lymphatic to blood vessels in peripheral tissues. Angiography detected lymph-blood misconnection in phospholipase C γ 2-deficient mice which lack LEC-induced platelet activation. LEC protrusion was detected inside the blood vessel lumen which can potentially activate platelets. LEC protrusion was immediately retracted by platelet-derived factors containing TGF-β in culture. Inhibition of TGF-β signaling induces the formation of lymph-blood misconnection in mouse embryonic dorsal skin. Our findings advance understanding of peripheral lymph-blood partitioning.

Regulation of remote spatial memory formation by neurosteroids and its diurnal change in mice

ニューロステロイドによるマウス空間記憶形成の制御とその日周変化

○清水 貴美子[1,2,3]、前畑 佳納子[2]、池野 知子[2]、Wang Qiuyi[4]、高尾 敏文[4]、深田 吉孝[2,3]

[1]東京医科歯科大・難治研・病態細胞生物、[2]東京大・院理・生科、[3]東大・院医・疾患生命工学・動物資源、[4]阪大・蛋白研・機能発現プロテオミクス

The biological clock regulates not only sleep or hormone secretion but also higher brain functions such as memory formation in mammals. We found that remote spatial memory was most efficient when learning occurred at the beginning of the light period (dawn) in mice, although there was no diurnal change in recent memory. We found that two 7 α -hydroxy-neurosteroids (7 α -hydroxypregnenolone [7 α -OH-Preg] and 7 α -hydroxydehydroepiandrosterone [7 α -OH-DHEA]) are involved in spatial memory maintenance. The neurosteroids are synthesized from cholesterol by a new member of P450 hydroxylase, CYP7B1. *Cyp7b1* mRNA was detected widely in the mouse brain with high levels in the hippocampus with diurnal change. We identified the occurrence of 7 α -OH-Preg and 7 α -OH-DHEA in the mouse hippocampus after Morris's water maze task at the beginning of the light period by using LC-MS/MS. *Cyp7b1* deficiency impaired remote spatial memory, with recent memory mostly unaffected. The hippocampal dendritic spine densities were reduced in *Cyp7b1*-KO mice and no more increased by the training in *Cyp7b1*-KO mice. Chronic intracerebroventricular administration of 7 α -OH-Preg and 7 α -OH-DHEA in *Cyp7b1*-KO mice improved the spine density and remote spatial memory performance. Notably, this improvement was more significant when the mixture of 7 α -hydroxylated steroids was administered than the single neurosteroid administration. We concluded that the 7 α -hydroxylated neurosteroids are required for synaptic remodeling for long-term maintenance of spatial memory in mice (iScience 2020).

Plasma clearance of intravenously infused human adrenomedullin in rats with renal dysfunction

腎障害ラットにおけるアドレノメデュリンの体内動態

○細田 洋司[1,2]、中村 任[3]、吉原 史樹[2]

[1]信州大・医・分子病態学、[2]国循・病院・腎臓高血圧内科、[3]大阪医科薬科大・薬・臨床薬学教育研究セ

Aim

Plasma adrenomedullin concentrations are reportedly elevated in patients with renal failure; however, the underlying mechanism is unclear. The aim of this study is therefore to investigate the pharmacokinetic changes of ADM in two renal dysfunction rats.

Methods and results

The pharmacokinetic parameters were calculated from individual plasma ADM concentration vs time curves during and after 1 h intravenous infusion of hADM in rats with acute renal dysfunction by mercury chloride treatment (RD-Ag) and bilateral renal blood flow blockage (RD-BL). At the end of hADM infusion, plasma ADM levels in RD-Ag rats were approximately three times as high as in RD-Bl and normal control rats. We measured a statistically significant positive correlation between ADM C60 and sCr. AUC0–60 during continuous hADM infusion was also significantly increased for RD-Ag than for normal controls. Plasma ADM disappearance after the end of infusion was similar among the three groups. The total systemic clearance of RD-Ag was significantly lower than that of normal rats. Pharmacokinetic analysis revealed that elevated plasma ADM in RD-Ag rats may be caused by a reduced volume of distribution.

Conclusion

These results suggest that decreased plasma ADM clearance in RD-Ag is not due to impaired renal excretion but to a decreased volume of distribution.

Highly sensitive detection of superoxide by the application of bortezomib

ボルテゾミブ添加によるスーパーオキシドの高感度な検出法

○松本 みさき[1]、矢部 千尋[2]、楳村 敦詩[1]

[1]京都府立医科大・院医・病態分子薬理、[2]京都工場保健会総合医学研・臨床薬理学研究セ

The specific detection of superoxide is challenging because of its higher reactivity and short-lived property. Among the various chemical probes for the detection of superoxide, L-012, a luminol-based chemiluminescent probe, enables the specific detection of extracellular superoxide. Here, we demonstrated that coapplication of the peptide boronic acid proteasome inhibitor, bortezomib, with L-012 significantly increased its luminescence without affecting the background. The increase in the ratio of L-012 luminescence by bortezomib was more than five-fold in both NADPH oxidase-expressing cells and the xanthine oxidase-dependent cell-free superoxide generation system. The application of MLN2238, another peptide boronic acid proteasome inhibitor, also enhanced the luminescence of L-012. In contrast, carfilzomib, an epoxyketone proteasome inhibitor, did not increase luminescence, suggesting that the effects of bortezomib depend on the chemical structure of the peptide boronic acid, but not on its pharmacological effects. The highly sensitive detection of superoxide by the application of bortezomib may become useful in the experimental assessment of oxidative stress and future diagnostic applications, particularly in limited amounts of samples.

Cholinergic suppression of Ca^{2+} signaling in pancreatic β-cells.

膵β細胞におけるコリン作動性カルシウム抑制

○太向 勇、金丸 和典、飯野 正光

日本大・医

Intracellular Ca^{2+} signal plays an essential role in insulin secretion from pancreatic β-cells. Recent reports suggest that Ca^{2+} release from the endoplasmic reticulum (ER) through cholinergic receptor stimulation mediated by parasympathetic nerves contributes to Ca^{2+} signals in β-cells. However, how the Ca^{2+} release from the ER shapes the intracellular Ca^{2+} signal remains elusive due to limitations in the methods for direct visualization analysis. We recently developed transgenic mouse lines expressing a genetically encoded cytosolic Ca^{2+} indicator, YC-Nano50, or an ER Ca^{2+} indicator, CEPIA specifically in β-cells. We successfully observed periodic oscillations of both cytosolic and ER Ca^{2+} signals evoked by high glucose in isolated pancreatic islets. We also confirmed a cholinergic agonist-induced decrease in ER Ca^{2+}, i.e. Ca^{2+} release from the ER. Surprisingly, during a high glucose condition, short-term cholinergic agonist application induced a transient suppression of cytosolic Ca^{2+} level to the extent comparable with the resting level, despite the release of Ca^{2+} from the ER. Our results suggest that parasympathetic nerves mediate suppressive regulation of Ca^{2+} signaling. Further analysis is required to reveal the physiological roles and underlying mechanisms of this unexpected Ca^{2+} suppression.

Cardiac glycosides inhibit GLUT1-mediated glucose uptake and glycolysis in human cancer cells by targeting intracellular Na^+,K^+-ATPase

強心配糖体は細胞内Na^+,K^+−ATPaseを標的としてヒトがん細胞におけるGLUT1依存性のグルコース取り込みおよび解糖系を抑制する

○藤井 拓人[1]、加藤 瑞希[1]、清水 貴浩[1]、田渕 圭章[2]、清水 康晴[3]、竹島 浩[4]、酒井 秀紀[1]

[1]富山大・薬・薬物生理学、[2]富山大・生命科学・ゲノム機能解析学、[3]救心製薬株式会社、[4]京都大・薬・生体分子認識学

Cardiac glycosides (CGs), potent inhibitors of Na^+,K^+-ATPase, have been used to treat congestive heart failure. Recently, the effectiveness of CGs for cancer therapy has been suggested. Here, we examined the effects of CGs on glucose metabolism in human cancer cells. Low concentrations (nM levels) of CGs (ouabain, oleandrin, and digoxin) significantly decreased the expression level of glucose transporter GLUT1 in the plasma membrane of the cancer cells. Ouabain (20-2000 nM) inhibited 2-deoxy-D-glucose uptake and lactate secretion of cancer cells. In intracellular vesicles of human cancer cells, Na^+,K^+-ATPase α3-isoform (α3NaK) is abnormally expressed. Interestingly, the knockdown of α3NaK significantly inhibited the ouabain-decreased GLUT1 expression, while the α1NaK knockdown did not. Ouabain (200 nM) inhibited the enzyme activity of α3NaK but not α1NaK. The ouabain-induced GLUT1 decrease was significantly inhibited by a Ca^{2+} chelator, a Ca^{2+}-ATPase inhibitor, an NAADP antagonist, a dynamin inhibitor, and PI3K inhibitors. These results suggest that CGs act on intracellular α3NaK and induce the NAADP-mediated Ca^{2+} mobilization and PI3K activation followed by dynamin-dependent GLUT1 endocytosis. This mechanism may explain why CGs inhibit glucose uptake and glycolysis in human cancer cells.

Cyclic AMP/PKA potentiates Ca^{2+}-dependent plasma membrane translocation of aquaporin 5

Cyclic AMP/PKAシグナルは Ca^{2+}により生じるアクアポリン5の細胞膜移行を亢進する

○服部 友万成、村上 一仁、礒濱 洋一郎

東京理科大・薬・応用薬理

Aquaporin-5 (AQP5) is selectively expressed in the apical membrane of exocrine glands, such as salivary and lacrimal, and plays important roles to maintain their secretory functions. Because AQP5 is not regulated by structural gating, translocation between plasma membrane and intracellular space is important for its water-permeable function. We and other groups have been shown that intracellular Ca^{2+}-dependent signaling increases AQP5 translocation to plasma membrane. On the other hand, the role of cAMP/PKA-dependent signaling on AQP5 translocation is still unclear, although phosphorylation of AQP5 by PKA has been suggested. In several secretory cells, interaction between cAMP and Ca^{2+} signals regulate their function. In this study, therefore, we examined the combined effect of Ca^{2+} ionophore and PKA activator on subcellular localization of AQP5. In MLE -12 cells, ionomycin alone increased AQP5 translocation to plasma membrane, whereas forskolin alone did not. The combined effect of ionomycin and forskolin was considerably greater than that of ionomycin alone. This enhancement of the effect of ionomycin by forskolin was inhibited by H-89, a PKA inhibitor. Now we are investigating whether PKA-dependent phosphorylation of AQP5 is involved in this potentiated translocation.

Na^+/Ca^{2+} exchanger type 1 functions as a “brake” of hyperactivation in hamster sperm.

Na^+/Ca^{2+} 交換体タイプ1がハムスター精子の超活性化運動のブレーキとして機能する。

○竹井 元[1]、小椋 悠平[2]、氏原 嘉洋[2]、外山 史[3]、林 啓太郎[1]、藤田 朋恵[1]

[1]獨協医科大・医・薬理学講座、[2]名古屋工業大学・大学院工学研究科、[3]宇都宮大学・工

Mammalian sperm including human have to undergo several physiological and biochemical changes, collectively called capacitation, to be fertilization-competent. Capacitated sperm show specialized vigorous motility called hyperactivation. Hyperactivation is necessary to progress in the viscous oviductal environment.

We found that the fluid of oviduct, where fertilization occurs, contains higher concentration of Na^+ than in the media used for *in vitro* fertilization (IVF). Therefore, we first investigated if this difference in the concentration of Na^+ affects hyperactivation or not. We found that increase in the Na^+ concentration delays the hyperactivation by lowering intracellular Ca^{2+} levels ($[Ca^{2+}]_i$). The Na^+/Ca^{2+} exchanger (NCX)-specific inhibitor SEA0400 increased $[Ca^{2+}]_i$, and canceled the delay of hyperactivation by Na^+. These results suggest that NCX is involved in the regulation of hyperactivation. Next, we searched for the NCX isoforms expressed in hamster sperm, and found that NCX1 mRNA and protein are expressed in the hamster testis and sperm. Lastly, we tried to detect NCX1 activity by measuring Na^+-dependent Ca^{2+} influx by Fura2. The Na^+-dependent Ca^{2+} influx was detected in hamster sperm, and was inhibited by SEA0400 at NCX1 specific concentration. Moreover, NCX1 activity was declined in the capacitated sperm. These results showed that NCX1 functions as a “brake” of hyperactivation in the hamster sperm, and its downregulation triggers hyperactivation. An inhibitor of NCX1 is a possible candidate drug to facilitate IVF.

Identification of Ryanodine receptor 1-selective agonists

リアノジン受容体1選択的アゴニストの同定

○金谷 啓之[1]、桑島 謙[1]、大出 晃士[1]、上田 泰己[1,2]

[1]東京大・院医・システムズ薬理学、[2]理研・生命機能科学研究セ・合成生物学

Ryanodine receptors (RyRs) are large-conductance Ca^{2+} channels located at the membrane of endoplasmic reticulum (ER) or the muscle equivalent, sarcoplasmic reticulum (SR). Three isoforms of mammalian RyRs are expressed across tissues and regulate diverse cellular physiology. Although various chemicals have been identified as agonists and antagonists of RyRs, the isoform-selective pharmacological manipulation is still challenging. In this study, we screened selective agonists of type 1 Ryanodine receptor (RyR1) and investigated the structure-activity relationships. The ortho/meta/para-orientation and the length of alkyl chains affect their potency. Furthermore, experiments with RyR1 mutants revealed the amino acid residues responsible for the channel activation by agonists. The novel agonists identified in this study are prospective tools for the isoform-selective pharmacology of RyRs.

Clarification of pharmacological features of water-soluble components isolated from Qing-dai (Sei-tai), as a nuclear receptor AhR activator

青黛由来の水溶性成分の核内受容体AhR活性化薬としての薬理学的特徴

○坪井 妙惠、村上 一仁、礒濱 洋一郎

東京理科大・薬・応用薬理

Aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor, which can suppress the inflammatory response. Recently, several studies indicated that activating AhR can inhibits inflammatory processes in the gut, and that AhR ligand may be a predicted drug to treat inflammatory bowel disease (IBD). In our previous study, *indigo naturalis*, a herbal medicine, considerably inhibited LPS-induced cytokine expression in cultured gastric epithelial cells and in intestine tissue of dextran sodium sulfate (DSS)-induced ulcerative colitis mice. Indigo, a major component of this medicine, is known to activate AhR. However, after the fractionation of *Indigo naturalis*, potent inhibitory effect on cytokine expression was obtained from water soluble fraction, which doesn't contain indigo. This new active component of *Indigo naturalis* inhibited cytokine expression both in vitro and in vivo, as well as indigo, whereas it did not activate AhR with the same strength. Now, we are investigating the characteristics of the pharmacological effect of this new AhR ligand isolated from the water-soluble fraction. It might be a new AhR ligand, which can stimulate transrepressional activity of this receptor.

The involvements of sulfatide-selectin signaling in the spinal cord on inflammatory pain

炎症性疼痛における脊髄スルファチド−セレクチンシグナルの関与

○渡辺 俊[1]、森田 元樹[1,2]、野村 夏実[1,2]、尾山 実砂[1,2]、岩井 孝志[1,2]、田辺 光男[1,2]

[1]北里大・薬、[2]北里大・薬・附属医薬研究施設

The altered levels of sulfatide are observed in various kinds of diseases in the nervous system. In our previous reports, sulfatide synthase gene expression in the spinal cord was increased one day after intraplantar injection of complete Freund's adjuvant (CFA). On the other hand, intrathecal injection of sulfatide led to mechanical allodynia. Sulfatide is not only the major glycosphingolipid in the myelin sheath but also the potent selectin activator. Thus, we investigated the effect of a selectin inhibitor, bimosiamose, on the sulfatide-induced mechanical allodynia.

Intrathecal sulfatide injection produced an increase in mRNA expression of inflammatory cytokines such as TNF-α and interleukin-1β in the spinal cord. Bimosiamose inhibited the increase of these cytokine gene expressions and the mechanical allodynia produced by sulfatide, suggesting that sulfatide induced inflammatory cytokine expression via selectin activation. Furthermore, bimosiamose also attenuated the CFA-induced mechanical allodynia and lipid analysis using thin-layer chromatography revealed the sulfatide accumulation in the spinal cord during inflammatory pain.

These results indicated that the accumulated sulfatide in the spinal cord enhanced selectin activation during inflammatory pain, which resulted in mechanical allodynia.

Orofacial neuropathic pain is elicited by structural changes in NAergic fibers

ミクログリアのMHCクラスIが口腔領域の神経障害性疼痛に寄与する

○林 良憲、篠田 雅路

日本大・歯

Microglia are known to be involved in the structural changes of synapses and axons. Microglia in the spinal cord are critical for the development of neuropathic pain. However, it remains unclear whether microglia involve structural changes of neurons during neuropathic pain. Here, we investigated the above possibilities using an orofacial neuropathic pain model. After infraorbital nerve injury, the density of DbH immunofluorescence in the trigeminal spinal subnucleus caudalis (Vc) was decreased, while DbH positive particles increased within microglia. DbH-positive particles in microglia were positive for MHC-I. Surprisingly, MHC-I immunofluorescence was also observed in microglial processes. To clarify whether MHC-I secretion from microglia induces the uptake of DbH-positive axons, exosomes were isolated from primary cultured microglia. Intracisternal administration of exosomes from IFNgannma-stimulated microglia elicited mechanical allodynia in the whisker pad and downregulation of DbH expression in the Vc. In contrast, exosomes from IFNgannma-stimulated MHC-I knockdown microglia unchanged pain sensitivity and DbH expression in the Vc. These results suggest that activated microglia-derived MHC-I causes the reduction of NAergic axons, culminating in enhanced neuronal activity in the Vc.

Androgens determine sex differences of spinal microglia

脊髄ミクログリアの性差はアンドロゲンによって決定される

○木口 倫一[1]、雑賀 史浩[1]、波多野 裕[2]、日野 信次朗[3]、鈴木 堅太郎[2]

[1]和歌山県医大・薬・生体機能解析、[2]山梨大・生命環境・器官形成ダイナミクス、[3]熊本大・発生医研・細胞医

Several lines of evidence indicate that spinal microglia exacerbate abnormal pain processing. Recent findings demonstrated there are significant functional differences of microglia in pain hypersensitivity between male and female animals, but underlying mechanisms are poorly understood. Here, we investigated whether androgens affect sex differences of microglia in neuropathic pain model mice. Peripheral nerve injury-induced mechanical allodynia was suppressed by the treatment of PLX3397, a microglial inhibitor, in male but not in female mice, and the effects of PLX3397 in the spinal dorsal horn of male mice was significantly greater than that of female mice. Gonadectomy (GDX) decreased in serum testosterone concentration and mechanical pain threshold in male mice. Susceptibility of spinal microglia for PLX3397 in GDX-treated male mice was similar to that of normal female mice. Moreover, intrathecal administration of colony-stimulating factor 1 (CSF1) elicited mechanical allodynia in male mice, but not GDX-treated male mice or normal female mice. Collectively, functional roles of spinal microglia contributing pain hypersensitivity are different between male and female, and sex-dependent characters of spinal microglia might be determined through androgen actions.

Involvement of platelet-derived HMGB1 in oxaliplatin-induced peripheral neuropathy (OIPN): OIPN prevention by antiplatelet agents

オキサリプラチン誘起末梢神経障害（OIPN）への血小板由来HMGB1の関与：抗血小板薬のOIPN予防効果について

岸本 彩野[1]、堂本 莉紗[1]、松永 浩明[1]、松本 亜紗菜[1]、坪田 真帆[1]、関口 富美子[1]、王 登莉[2]、西堀 正洋[3]、○川畑 篤史[1]

[1]近畿大・薬、[2]岡山大・院医歯薬・薬理、[3]岡山大・院医歯薬・創薬研究推進

HMGB1, a nuclear protein, once released extracellularly, promotes inflammation and pain. We have shown the involvement of macrophage-derived HMGB1 in chemotherapy-induced peripheral neuropathy (CIPN) caused by paclitaxel, bortezomib or vincristine, and of non-macrophage cell-derived HMGB1 in CIPN caused by oxaliplatin (OIPN). Given the involvement of platelet-derived HMGB1 in thrombosis, we asked whether the pathogenesis of OIPN would involve platelet-derived HMGB1 in rodents. In rat platelet-rich plasma (PRP), thrombin induced HMGB1 release from platelets. An anti-CD42b platelet-depleting antibody dramatically decreased platelet count and increased plasma HMGB1 levels in mice, leading to OIPN development after subsequent oxaliplatin treatment at a subeffective dose. Splenectomy almost doubled platelet count and accelerated OIPN development. Repeated oral administration of different antiplatelet agents, aspirin, clopidogrel, cilostazol and ozagrel, prevented OIPN development in mice and/or rats. In rats subjected to repeated treatment with oxaliplatin, HMGB1 levels dramatically decreased in PRP, but tended to increase in platelet-poor plasma. Together, our study provides novel evidence for a critical role of platelet-derived HMGB1 in OIPN development, and suggests the usefulness of antiplatelet agents to prevent severe OIPN.

Mirogabalin and pregabalin, $\alpha_2\delta$ subunit ligands of voltage-gated Ca^{2+} channels, suppress acute and chronic itch

電位依存性Ca^{2+}チャネル$\alpha_2\delta$サブユニットリガンドのミロガバリンとプレガバリンは急性と慢性掻痒を抑制する

○尾山 実砂、星山 歩海、三宅 綾乃、別宮 洸亮、山田 望美、渡辺 俊、岩井 孝志、田辺 光男
北里大・薬

Antihistamines and steroids, targeting peripheral tissues, have been used as first-line drugs in the treatment of chronic pruritus. Here, we focused on mirogabalin (MGB) and pregabalin (PGB), gabapentinoids that are widely used to treat neuropathic pain, to explore their potential antipruritic effects in several acute and chronic itch models. MGB and PGB, when injected i.p., i.c.v., or i.t., inhibited scratching behavior induced by chloroquine (CQ; histamine-independent pruritogen) in the neck model of acute itch. MGB (i.p., i.c.v., or i.t.) also reduced biting and licking behaviors reflecting acute itch and pain sensation, respectively, after CQ injection into the front of the left calf, while PGB produced apparent antipruritic effects only when it was injected i.t. in this calf model. The antipruritic effects of MGB and PGB were also examined under chronic itch conditions induced by applying 1-fluoro-2,4-dinitrobenzene (DNFB) repeatedly to the nape of the neck. I.p. injection of MGB and PGB, when used therapeutically or prophylactically, suppressed spontaneous scratching behavior and the development of chronic itch, respectively. Therefore, gabapentinoids are considered to be effective as the therapeutic agents acting on the central nervous system including the spinal cord for antihistamine-refractory chronic itch.

Possibilities of statins in oxaliplatin-induced chronic peripheral neuropathy

オキサリプラチン誘発性慢性末梢神経障害におけるスタチン系薬剤の可能性

○相澤 風花[1]、岡林 亜美[2]、森山 大嗣[2]、薗田 悠平[1,2]、高橋 志門[1]、合田 光寛[1,2]、八木 健太[3]、新村 貴博[3]、座間味 義人[4]、石澤 有紀[5]、石澤 啓介[1,2,3]

[1]徳島大病・薬剤部、[2]徳島大・院医歯薬・臨床薬理学、[3]徳島大病・総合臨床研究セ、[4]岡山大病・薬剤部、[5]徳島大・院医歯薬・薬理学

Many patients treated with oxaliplatin experience sensory deficits in the distal extremities. This impairment may persist for several months after treatment is discontinued, resulting in a negative impact on quality of life. Statins, which are used to treat dyslipidemia, have a pleiotropic action, such as anti-inflammatory effect. Here we show that statins can be a therapeutic agent for oxaliplatin-induced peripheral neuropathy via the regulation of glutathione S-transferase (GST). The mechanical hypersensitivity induced by oxaliplatin was ameliorated on day 7 in mice repeated orally administered statins and lasted for 21 days. Furthermore, mechanical hypersensitivity was suppressed even when statins were administered after day 7 of oxaliplatin exposure. On the other hand, statins were not effective against cold hyperalgesia. Uptake of oxaliplatin in the DRG was not inhibited by statins. Analysis of gene association databases revealed that the expression of GST family members is regulated by statins. Co-administration of the GST inhibitor, ethacrynic acid, reversed the statin-induced suppression of oxaliplatin-induced mechanical allodynia. Statins might be potential therapeutic agents for the treatment of anticancer drug-induced chronic peripheral neuropathy that do not suppress the effects of oxaliplatin.

Visualization of water dynamics in brain tissue using multiphoton multimodal imaging

多光子マルチモダルイメージングを使った脳組織内の水動態の可視化

○篠塚 崇徳[1]、宮澤 剛史[1]、唐澤 啓子[1]、小関 泰之[2]、安井 正人[1]、塗谷 睦生[1]

[1]慶應義塾大・医・薬理、[2]東京大・工・電気系工学

The flow of cerebrospinal fluid is considered to be a critical factor in the clearance of wastes in the brain. The dynamics of fluid has been investigated by several experimental methods such as fluorescence microscopy and magnetic resonance imaging. However, because probes used for these imagings are much larger compared to water itself, the dynamics of the fluid have been poorly understood. Here, we applied a multimodal multiphoton imaging system to the living brain tissue. Combining stimulated Raman scattering and two-photon fluorescent imaging, the system enables us to visualize spatiotemporal dynamics of deuterated water and fluorescent dyes simultaneously at a cellular level. We demonstrate that deuterated water diffuses faster than fluorescent dyes in the brain tissue. Detailed analysis reveals deuterated water rapidly exchanges inside and outside of cells, whereas fluorescent dyes only diffuse through extracellular spaces. Furthermore, we find that the dynamics of deuterated water is robust to changes under physiological and pathophysiological conditions; there is little change in the spatiotemporal dynamics of deuterated water during development and ischemia whereas fluorescent dyes are severely affected. Thus, our new approach reveals unique properties of the dynamics of the fluid in the living brain tissue.

Electrical activity imaging and drug response with super spatiotemporal resolution in *in vitro*, organoid and *ex vivo* neural networks

In vitro, organoid, ex vivo神経ネットワークの超時空間分解を有する電気活動イメージングと薬剤応答

○鈴木 郁郎

東北工業大

Electrical activities of neuronal network with high-spatio-temporal resolution is useful for understanding brain functions and elucidating neurological disorders, and for drug screening and safety assessment of drugs. Complementary metal-oxide semiconductor micro-electrode array (CMOS-MEA) is excellent in detecting detailed electrical activity patterns of neural networks due to the large number of electrodes. In this study, we investigated the electrical activity characteristics of brain slices, brain organoids, and cultured neural networks using CMOS-MEA with 236,880 electrodes, which have the highest specifications in the world. In the measurement of brain slices, we succeeded in measuring the interregional propagation of the hippocampus and cerebral cortex area in detail, and detected changes of propagation patterns due to drug administration. In sensory neuron measurement, calculation of axon conduction velocity in single neuron and drug responses based on firing pattern of each neuron were detected. In human iPS cell-derived central nervous system networks and human cerebral organoids, network activity was detected on a cell-by-cell basis, and changes in propagation patterns due to drug administration were detected. It was found that CMOS-MEA with 236,880 electrodes and a large measurement area can measure the electrical activity characteristics of ex vivo and in vitro neural networks and single neuron in detail. It was suggested that big data with high temporal resolution is effective for elucidation of neural circuit function and drug evaluation based on new neural activity information.

The potentiation of photic response in the suprachiasmatic nucleus by lactoferrin

ラクトフェリンによる視交叉上核の光同調刺激反応の増強作用の解析

高津 麻衣、佐藤 可那江、○守屋 孝洋

奥羽大・薬

Lactoferrin (LF) is reported to have various bioactivity such as bone growth stimulation and analgesic activity. Recently, LF has been reported to recover the fatigue in humans after the shift-work. In this study, we examined the effect of administration of LF on the functions of the circadian clock such as the free-running rhythm as well as the light pulse-induced phase shift of the behavioral rhythm using mouse model. Since an orally-administered LF is known to be largely degraded in stomach, we used the enteric microcapsule bovine lactoferrin (eMC-LF) which protects from stomach digestion, but can be absorbed in the small intestine. The wheel-running activity of mice at 6 min-bin was automatically recorded in PC and was analyzed by CLOCK Lab. The level of mRNA of several clock-related genes was quantified by in situ hybridization method. We found that oral single administration of eMC-LF at ZT24 promoted the re-entrainment of wheel-running rhythm to 8 hr advanced LD cycle in mice. Both acute and chronic administration of eMC-LF potentiated the light pulse-induced phase shift of the wheel-running rhythm. Furthermore, eMC-LF increased the light pulse-induced expression of *Per1* mRNA in the restricted area of the suprachiasmatic nucleus (SCN). We also found that eMC-LF potentiated the *Per1* mRNA upregulation by i.c.v. administration of gastrin-releasing peptide (GRP), a neuropeptide involved in the photic signaling in the SCN. These results suggest that LF promotes the light entrainment of the mouse circadian clock.

Engulfment of Amyloid β-protein in neurons and astrocytes mediated by MEGF10

神経細胞およびアストロサイトによるMEGF10を介したAβの貪食除去機構の解析

○藤田 融[1]、前田 智司[2]、駒野 宏人[3]、田邉 由幸[1]

[1]横浜薬科大・薬・薬理学研究室、[2]日本薬科大・薬・生命医療薬学分野、[3]北海道大・院医薬・認知症先進予防・解析分野

Amyloid-β proteins (Aβ), including Aβ42 and Aβ 43, are known pathogenesis factors of Alzheimer's disease (AD). Unwanted substances in the brain, including Aβ, are generally removed by microglia, astrocytes, or neurons via a phagocytosis receptor. We observed that neurons and astrocytes engulfed Aβ 42 and Aβ 43, which are more neurotoxic than Aβ 40. We previously showed that multiple-EGF like domains 10 (MEGF10) that is the mammalian homologue of Draper, a phagocytosis receptor of apoptotic cells in *Drosophola*, and is the type I transmembrane protein plays an important role in apoptotic cell elimination and is expressed in mammalian neurons and astrocytes. Therefore, we assessed whether MEGF10 is involved in Aβ42 and Aβ43 engulfment in MEGF10-expressing neurons and astrocytes. We found that MEGF10-expressing astrocytes and neurons engulfed Aβ42 and Aβ43 but not Aβ40. Furthermore, incubation of the neurons and astrocytes with Aβ42 and Aβ43 augmented MEGF10 phosphorylation; however, incubation with Aβ40 did not have this augmenting effect. Our findings suggest that MEGF10 plays a phagocytosis receptor function for Aβ42 and Aβ43 in neurons and astrocytes.

Evaluation of acute toxicity of oxaliplatin and therapeutic candidates by extracellular potential measurement using rat primary dorsal root ganglion cells

ラット初代神経後根節細胞を用いた細胞外電位計測によるオキサリプラチン急性毒性の評価

○有馬 太陽[1,2]、寺薗 英之[1,2]、栗原 崇[3]、小八重 薫子[2]、武田 泰生[1,2]

[1]鹿児島大学病院・薬剤部、[2]鹿児島大・院医歯薬・薬物動態制御学、[3]鹿児島大・院医歯薬・生体情報薬理学

It is known that peripheral neuropathy as an unwanted side effect of the anticancer drug oxaliplatin, especially cold allodynia, which reacts hypersensitively to cold stimuli, greatly impairs the QOL of patients. Therefore, it is desired to develop preventive or therapeutic medicines for cold allodynia. Previous studies have shown that pituitary adenylate cyclase-activating polypeptide (PACAP) antagonists have potent effects in preventing cold allodynia in in vivo studies. In this study, we evaluated the adverse effects of oxaliplatin and their protective effects using an extracellular potential measurement system using rat E14 primary dorsal root ganglion cells (DRG). The effect of oxaliplatin and antagonist of PAC1, that is a PACAP receptor, on extracellular action potential was verified. The firing frequency of extracellular action potential increased in a concentration-dependent manner with oxaliplatin. On the other hand, the addition of PAC1 antagonists abolished the firing of the extracellular potential. Moreover, the firing of PAC1-treated DRG neuron decreased compared to before addition of oxaliplatin. The evaluation system for peripheral neuropathy caused by anticancer drugs by measuring the extracellular potential of DRG was able to show the usefulness as a system that can evaluate side effects in the acute phase.

Effects of Monosulfide and Trisulfide on Pathological Events in Mouse Model of Intracerebral Hemorrhage

マウス脳内出血病態に対するモノスルフィドおよびトリスルフィドの作用

Atef Yara[1]、木下 慶大[1]、市原 侑晟[1]、牛田 啓介[2]、倉内 祐樹[1]、関 貴弘[1]、香月 博志[1]

[1]熊本大・院生命・薬物活性、[2]熊本大・薬・薬物活性

Intracranial hemorrhage (ICH) is a type of hemorrhagic strokes that is caused due to bleeding in the brain parenchyma. Here we investigated the effects of Na_2S and Na_2S_3 on ICH in mice. Na_2S and Na_2S_3 were injected into two separate groups of mice (25 μmol/kg i.p.) 30 min before induction of ICH by collagenase injection into the striatum. Both agents attenuated the neutrophil infiltration (MPO), inhibited the upregulation of monocyte/macrophage chemokine (CCL-2) expression and maintained the axonal fibers transport function (APP). Moreover, pretreatment with Na_2S improved the motor function, prevented the decrease in the neuronal count (NeuN), maintained the integrity of axonal fiber structures (neurofilament-H) and lowered the upregulation of neutrophil chemokine (CXCL-2) expression after induction of ICH. On the other hand, pretreatment with Na_2S_3 significantly attenuated the activation of microglia/macrophages (Iba-1) in the perihematomal area. Both agents failed to prevent ICH-induced increase in the brain vascular permeability or to lower the pro-inflammatory cytokine (IL-6) expression significantly. In conclusion, these results suggest that Na_2S exhibits more potent neuroprotective effect than Na_2S_3 and promotes recovery of neurological functions after ICH.

Involvement of Protein tyrosine phosphatase delta (PTPd) in the cortical pyramidal dendritic growth of through the regulation of Signal Regulatory Protein alpha (SIRPa) phosphorylation.

チロシンホスファターゼδのSIRPαリン酸化制御による皮質錐体細胞の樹状突起伸長

○中村 史雄、若槻 実祐、瀧澤 光太郎、實木・高橋 葵

東京女子医科大・医

We previously reported that protein tyrosine phosphatase delta (PTPd), one of type IIa receptor type protein tyrosine phosphatases, mediates Sema3A-induced dendritic growth of cortical pyramidal neurons. However, its endogenous substrates involved in cortical dendritic arborization have been yet identified. Phosphotyrosine-proteome analysis of PTPd knockout brains revealed the hyperphosphorylation of Signal Regulatory Protein alpha (SIRPa) at Tyr501 (Y501) residue. Immunohistochemistry with anti-phospho-Y501 SIRPa antibody showed that olfactory epithelium, cortical II to V layers, thalamic nuclei and axon-bundles including corpus callosum, fimbria, and pyramidal tract were hyperphosphorylated in PTPd knockout brains. Knockdown of SIRPa by siRNA transfection or the overexpression of cytoplasmic deletion mutant of SIRPa suppressed Sema3A-induced growth cone collapse response of mouse dorsal root ganglion neurons. Primary culture of mouse cortical neurons revealed that Sema3A-stimulation induced the dephosphorylation of SIRPa in the dendritic growth cones of wild-type but not in those of PTPd knockouts. Overexpression of non-phosphorylated SIRPa mutant Y501F in cultured cortical neurons attenuated Sema3A-induced dendritic growth. In utero electroporation of SIRPa-Y501F to mouse brains showed that the apical dendrites of cortical layer II/III pyramidal neurons were disoriented. Similar irregular projection of cortical apical dendrites was also observed in PTPd knockout brains. These results suggest that PTPd may regulate the phosphorylation of SIRPa in cortical dendritic growth.

Development and application of label-free Ca^{2+} Image Sensor to visualize extracellular Ca^{2+} dynamics in hippocampus.

Ca2+イメージセンサーを用いた海馬の細胞外Ca^{2+}の可視化

○パラジュリ ビージェイ[1,2]、土井 英生[3]、繁冨 英治[1,2]、鈴木 秀明[1,2]、野田 俊彦[3]、高橋 一浩[3]、澤田 和明[3]、小泉 修一[1,2]
[1]山梨大・院医・薬理、[2]山梨大・GLIAセンター、[3]豊橋技科大・電気・電子情報工学

Extracellular calcium ion ($[Ca^{2+}]o$) change occurring during physiological and pathological conditions play important roles in regulation of brain functions, but has received limited attention due to the lack of easy to use device. Here, we show the development and application of highly selective calcium image sensor for the imaging of glutamate-induced $[Ca^{2+}]o$ change in hippocampal slices. Using this sensor, we found that glutamate decreased $[Ca^{2+}]o$ by more than 50 % with different spatiotemporal patterns. The glutamate-evoked decrease in $[Ca^{2+}]o$ was inhibited by a NMDA receptor antagonist D-AP5 but not AMPA receptor antagonist CNQX, and mimicked by NMDA. The spatial pattern of the glutamate-evoked $[Ca^{2+}]o$ decrease was associated with that of distribution of NMDA receptors in the hippocampus. Moreover, using an ATP-imaging fluorescent probe, we found that the stimulation with NMDA triggered increase in ATP release from astrocytes via either connexin hemichannels or chloride channels. The NMDA-evoked ATP release was mediated by decrease in $[Ca^{2+}]o$ because the astrocytic ATP release was mimicked by Ca^{2+}-free medium. Taken together, using the newly developed Ca^{2+} image sensor, we demonstrated that $[Ca^{2+}]o$ is dramatically decreased during excitatory synaptic transmission by glutamate, and $[Ca^{2+}]o$ decrease act as a signal that transmits neuronal excitation to astrocytes via ATP release. The application of this Ca^{2+} sensor is expected to clarify the physiological and pathophysiological roles of $[Ca^{2+}]o$, which have received limited attention so far.

Analysis of synaptic structural changes induced by chronic social stress and their molecular mechanisms

慢性社会ストレスによるシナプス構造変化とその分子機序の解析

○永井 裕崇[1]、永井 碧[1]、沼 知里[1]、大田 康平[1]、山下 朋美[2]、川島 祐介[3]、大野 伸彦[4,5]、片岡 洋祐[6,7]、新間 秀一[8,9]、清末 優子[10]、加藤 太朗[2]、曽我 朋義[11]、古屋敷 智之[1]

[1]神戸大・院医、[2]住友ファーマ、[3]かずさDNA研究所、[4]自治医科大・医・組織、[5]生理研・超微形態、[6]理研―JEOL連携センター、[7]理研・生命機能科学研究セ・細胞機能評価、[8]大阪大・院工・生物工学、[9]大阪大・島津分析イノベーション協働研、[10]理研・生命機能科学研究セ・分子細胞動態研究チーム、[11]慶應義塾大・先端生命科学研

Excessive or chronic social stress induces emotional and cognitive disturbances and precipitates mental illness. Altered neuronal morphology and functions in the medial prefrontal cortex (mPFC) underlie these behavioral abnormalities. However, its subcellular mechanisms remain elusive. Here we examined ultrastructural and multi-omics changes in the mPFC after social stress in mice. Social stress caused the loss of dendritic branches with morphological alterations of subcellular mitochondria and induced synaptic shrinkage selectively at the synapses with mitochondria. Multi-omics and functional analyses revealed that social stress deteriorated mitochondrial functions with altered mitochondrial proteome at synapses and dysregulated central metabolic pathways in the mPFC. Molecular biological and pharmacological manipulation targeting central metabolism and mitochondria attenuated the synaptic shrinkage and depression-related behaviors. These findings demonstrate that chronic social stress alters the central metabolism at mPFC synapses, leading to neuronal pathology and depression-related behaviors.

Analysis of neural circuit alterations caused by chronic social stress

慢性社会ストレスによる神経回路変容の解析

○篠原 亮太[1]、奥田 裕己[1]、山口 真広[2]、小坂田 文隆[2]、古屋敷 智之[1]

[1]神戸大・院医・薬理、[2]名古屋大・院創薬科学・細胞薬効解析

Stress caused by aversive and psychological stimuli induces sustained emotional and cognitive abnormalities and precipitates the onset and relapse of symptoms in psychiatric illnesses, such as depression. We have demonstrated that chronic social stress causes dendritic atrophy of pyramidal neurons in the medial prefrontal cortex (mPFC) only in mice susceptible to chronic social stress, whereas acute social stress leads to dendritic growth of these neurons concomitant with suppression of behavioral changes. However, it remains unknown how stress-induced dendritic remodeling of mPFC pyramidal neurons affects neural circuits associated with depressive behaviors. Here we utilized monosynaptic retrograde tracing with a G-deficient rabies viral vector to identify neural projections to the mPFC that can be anatomically altered by chronic social stress. A G-deficient rabies viral vector encoding a red fluorescent protein (RFP) was unilaterally infused into the mPFC to visualize neurons that directly input to the mPFC. Among 90 brain regions where RFP-positive cells were observed in mice subjected to chronic social stress, some particular regions showed a decrease or increase in RFP-positive cells. This finding suggests that chronic social stress anatomically alters neural projections to the mPFC, leading to sustained behavioral changes.

Focus on the diabetic brain: Upregulation of orexin receptor and plasma orexin level in obese diabetic rats

肥満を伴う糖尿病ラットにおける脳内オレキシン受容体発現と血漿中オレキシン濃度の増大

○幸田 祐佳[1]、福石 信之[2]、松村 人志[1]

[1]大阪医科薬科大・薬・薬物治療、[2]金城学院大・薬・薬理学

Diabetes mellitus and brain toxicity are closely linked. Oxidative stress, obesity, insulin resistance, and glucose toxicity can affect the brain. Orexin-A, also known as hypocretin-1, participates in many physiological processes through its activated receptor. Orexin-A has been associated with feeding behavior, obesity, and pathogenesis of Alzheimer's disease. We reported that high-dose thiamine in obese diabetic Otsuka Long–Evans Tokushima Fatty (OLETF) rats leads to reduced obesity and metabolic disorders. In addition, we found that plasma orexin-A levels in OLETF rats can be modulated by thiamine supplementation under conditions of oxidative stress. Herein, we focused on orexin-A in obese diabetic OLETF rats. At 58 weeks of age, the rats showed an increase in body weight and blood glucose levels. Plasma orexin-A was measured by ELISA and tended to be higher in obese diabetic OLETF rats than in non-obese diabetic control rats. We evaluated hypocretin receptor 1 (Hcrtr1, also orexin-A receptor) gene expression in the brain of diabetic OLETF rats by reverse transcription-polymerase chain reaction and found that diabetic OLETF rats exhibited higher orexin-A receptor gene expression in the brain than controls. The results presented here are expected to provide a better understanding of the role of orexin-A and its contribution to diabetic brain.

Changes in enteric cellular environment of the rotenone-induced parkinsonian mice that reproduce central and enteric neurodegenerative features

パーキンソン病の脳・腸神経変性を再現できるロテノン曝露モデルマウスにおける腸管細胞環境の変化

○浅沼 幹人、宮崎 育子、進 浩太郎、都 明希、正井 加織、小林 壮太朗、津田 光希、小野 鈴香

岡山大・院医歯薬・脳神経機構

We previously established an animal model of Parkinson's disease (PD) induced by chronic low-dose rotenone as an environmental neurotoxic pesticide that reproducible central and enteric neurodegenerative features of PD, and also found that rotenone-induced enteric neurodegeneration is caused by dysfunction of enteric glia using primary cultured enteric cells. However, the mechanism of enteric neurodegeneration and inflammation are still obscure. In this study, we examined changes in enteric cellular environment in the enteric epithelium and myenteric plexus of the rotenone-induced PD model mice. Chronic subcutaneous administration with low-dose rotenone (2.5 mg/kg/day) for 4 weeks using an osmotic mini pump reduced the number of dopamine neurons in the substantia nigra and the intestinal myenteric neurons and glial cells of mice. Furthermore, it produced disruption of mucosal epithelial barrier and marked translocation of HMGB1 to the cytosol beside nuclear membrane towards the apical lumen side. These results suggest that the rotenone-induced dysfunctions of epithelial barrier and HMGB1 transportation are involved in the inflammatory reactions and dysfunction of enteric glia and consequent enteric neurodegeneration.

Role of microsomal prostaglandin E synthase-1 in hippocampal inflammation after repetitive febrile seizures in mouse pups

マウス幼児での反復熱性けいれん後の海馬炎症における膜結合型プロスタグランジンE合成酵素−1の役割

○松尾 由理[1]、友利 徳志[1]、竹本 陽祐[1]、植松 智[2]、審良 静男[3]、髙橋 達雄[1]

[1]北陸大・薬・薬理、[2]大阪市立大・院医・ゲノム免疫、[3]大阪大・院医・免疫フロンティア研セ

Febrile seizures (FSs) are the most common convulsive seizure in early childhood. About 30% of them are “complex”, with repetitive seizures, and related to adult temporal lobe epilepsy (TLE). In this study, we investigated the role of membrane-associated prostaglandin E synthase-1 (mPGES-1), an inducible terminal enzyme for PGE_2 synthesis, in hippocampal inflammation induced by repetitive FSs (RFSs) in mouse pups as a model of complex FSs.

Wild-type (WT) and mPGES-1 knockout (ES1KO) mice at P9-11 were given intraperitoneal injections of lipopolysaccharide (100 μg/kg) and exposed to heat lamp to induce hyperthermia and FSs. The induction of FSs was repeated twice at 4 h-interval (RFSs).

In WT mice, mPGES-1 mRNA was significantly up-regulated in hippocampus after RFSs. The production of hippocampal PGE_2 observed after RFSs in WT mice was completely absent in ES1KO mice. The seizure score and increase in rectal temperature during the hyperthermia induction in ES1KO mice were slightly but significantly lower than those in WT mice. The inductions of IL-1β, TNF-α and GFAP observed significantly in WT mice were less in ES1KO mice even in which the seizure scores were almost the same level.

These results suggest that mPGES-1 contributes to inflammatory hyperthermia, convulsive events, glial activation and production of inflammatory cytokines through PGE_2 production in hippocampus. Thus, mPGES-1 may contribute to the complex FSs-induced adulthood TLE and may be a potential therapeutic target for the development of epilepsy after RFSs.

Microvesicles are released from microglia in mouse models of both peripheral acute inflammation and chronic skin inflammation.

末梢の急性および慢性炎症モデルマウスにおけるミクログリア由来のマイクロベシクル分泌の観察

○坂井 謙斗[1]、小泉 修一[1,2]

[1]山梨大・院医・山梨GLIAセンター、[2]山梨大・院医・薬理

My research focuses on the relationship between peripheral inflammation and microglia. Lipopolysaccharide (LPS) is used to induce peripheral acute inflammation. It has been reported that LPS application induces microvesicle (MV) release from microglia *in vitro,* and these MVs contain inflammatory cytokines such as IL-1b. These data indicate that microglia detect peripheral inflammatory signals and react by releasing MVs. However, MVs released from microglia had not previously been observed *in vivo*. To observe microglial MVs, we made cranial windows into the heads of Iba1-GFP mice and performed *in vivo* live imaging using 2 photon-microscopy. GFP+ MVs were observed 24h after LPS injection (1 mg/kg, ip), and the peak of the increase in GFP+ MVs was at 48h after LPS injection. Because Iba1 is expressed in not only microglia but also some macrophages, we treated mice with clodronate (33 mg/kg, ip) to deplete peripheral macrophages. Clodronate treatment did not affect the increase of LPS-induced GFP+ MVs. This indicates that the GFP+ MVs were released from Iba1+ cells like microglia, but not macrophages. Interestingly, the GFP+ MVs were also observed in contact dermatitis model mice with chronic skin inflammation. These results provide a framework to study the role of microglial MVs in peripheral inflammatory mouse models.

Effects of KNT-127, a delta opioid receptor agonist, on non-REM sleep in mice.

δオピオイド受容体作動薬KNT−127のノンレム睡眠に対する作用

○岩井 孝志[1]、藤田 芽生[1]、渡辺 理紗子[1]、平山 重人[2]、尾山 実砂[1]、渡辺 俊[1]、藤井 秀明[2]、田辺 光男[1]

[1]北里大・薬・薬理学、[2]北里大・薬・生命薬化学

Recently we have shown that delta opioid receptor agonists (DORs) shorten sleep time during the light period. However, the impact of DOR agonists on sleep quality has not been determined. In the present study, we investigated the effects of the DOR agonist KNT-127 on delta wave power (1-4 Hz), which is an indicator of non-rapid eye movement (REM) sleep depth. The vigilance states (e.g., wakefulness, REM and non-REM sleep) of the ddY-mice (6-10 weeks) were classified based on the hippocampal local field potential (LFP) and neck muscle electromyogram. KNT-127 (10-30 mg/kg, i.p.) significantly decreased the mean REM and non-REM sleep periods, and prolonged the mean wakefulness period during 5 hr after its injection. KNT-127 significantly increased delta wave power during non-REM sleep compared to saline, and this effect was also observed at 3 mg/kg without the arousal. KNT-127 (3 mg/kg), when administered in the urethane-anesthetized mice, increased delta wave power, indicating that the action is not a rebound due to sleep suppression. Together, KNT -127 promotes deeper non-REM sleep independently of its arousal effects.

Propolis ameliorates cognitive decline in Alzheimer‘s disease model mice

プロポリスによる細胞内カルシウムシグナル賦活化を介した認知機能改善効果

○稲垣 良[1]、山國 徹[2,3]、森口 茂樹[1]

[1]東北大・院薬・医薬品開発研究センター、[2]東北大・院工・附属超臨界溶媒工学研究センター、[3]東北大・未来科学技術共同研究センター

Propolis (Brazilian green propolis) is a chemically complex resinous substance that is expected to be beneficial to therapy for Alzheimer‘s disease (AD). To reveal its beneficial effect on impaired cognition, we first performed three memory-related behavior tasks in mice aged 4 and 12 months: Y-maze task, novel object recognition task, and passive avoidance task. Oral dosages of 300-1000 mg/ kg once daily for 8 weeks, did significantly prevent the cognitive decline in the APP-KI mice aged 4 months, but not 12 months. Consistent with the observations from behavioral tasks, impaired hippocampal long-term potentiation (LTP) was markedly ameliorated in the acute brain slices prepared from the mice that underwent the repeated propolis administration. In addition, increased phosphorylation of CaMKII and AMPAR subunit (GluA1) was simultaneously observed in the CA1 of the mice. Similar to CaMKII activation, the propolis administration also increased CaMKIV and CREB phosphorylation and BDNF production in the CA1 of the mice. Finally, we confirmed that the presence of 30 μg/ mL propolis significantly elevated intracellular Ca^{2+} concentration in Neuro2A cells. These findings suggest that propolis is capable of rescuing the cognitive dysfunction via both upregulated activities of CaMKII and CaMKIV in the CA1 of the APP-KI mice.

Inhibitory role of small leucine-rich proteoglycans regulated by NOX1/NADPH oxidase in cardiac fibrosis

NOX1/NADPHオキシダーゼによって調節される小型ロイシンリッチプロテオグリカンの心線維化における役割

○岩田 和実[1]、矢部 千尋[2]、楳村 敦詩[1]

[1]京府医大・院医・病態分子薬理、[2]京都工場保健会総合医学研・臨床薬理学研究セ

Cardiac fibrosis is a leading cause of heart failure, particularly heart failure with preserved ejection fraction (HFpEF). Currently, there is no effective treatment for HFpEF. We previously reported that doxorubicin-induced cardiac fibrosis was suppressed in mice deficient in *Nox1*, a non-phagocytic isoform of superoxide-producing NADPH oxidase. In this study, the role of NOX1 in the development of cardiac fibrosis was investigated in cultured cells using a rat cardiomyoblast cell line H9c2 and cardiac fibroblasts isolated from adult male mice. Increased proliferation was demonstrated when cardiac fibroblasts were exposed to homogenates from wild-type H9c2. On the other hand, increased proliferation was significantly attenuated in cardiac fibroblasts exposed to homogenates from *Nox1*-disrupted H9c2. In *Nox1*-disrupted H9c2 cells, the expression of osteoglycin (Ogn), and podocan (Podn), which are small leucine-rich proteoglycans and known to regulate cardiac remodeling, were up-regulated. When homogenates from *Nox1*-disrupted H9c2 with disruption of Ogn or Podn exposed to cardiac fibroblasts, the proliferation of fibroblasts was significantly restored compared to those exposed to *Nox1*-disrupted H9c2. These findings suggest that NOX1 promotes cardiac fibrosis via down-regulation of Ogn or Podn in cardiomyocytes.

SGLT2 inhibitor reduces the inducibility and duration of atrial fibrillation in the diet-induced obese mouse model.

SGLT2阻害薬は肥満における心房細動誘発性および持続時間を低下させる。

○澤野 達哉[1]、三明 淳一朗[1]、クルニアワン　プリヨノ アグン[1]、岡村 昌宏[2]、友森 匠也[2]、高見 亜衣子[2]、長田 佳子[1]、今村 武史[1]

[1]鳥取大・医・薬理学・薬物療法学分野、[2]鳥取大・医・循環器・内分泌代謝内科学分野

Background: Atrial fibrillation (AF) is the most common arrhythmia. AF is highly correlated with multiple risk factors including heart failure, age, obesity, and type 2 diabetes. Among risk factors, the incidence in obesity is increasing worldwide. Recently, it was reported that SGLT2 inhibitors reduced the incidence of atrial fibrillation. However, it is unclear how the treatment with SGLT2 inhibitors has effects on vulnerability to AF. In this study, we examined the effects on the inducibility and duration of AF by treatment with SGLT2 inhibitors in diet-induced obese mice.

Methods: Mice were fed a normal chow diet (NCD) or high-fat diet (HFD). Following diet-loading, we randomly divided the animals into groups: NCD+vehicle, HFD+vehicle, and HFD+ SGLT2 treatments. Induction of AF was performed by transesophageal atrial burst pacing. Furthermore, we evaluated cardiac function, blood pressure, atrial fibrosis, and glucose tolerance at the end of the treatments.

Results: The results showed that HFD-fed mice increased the inducibility of AF compared to NCD mice. In addition, treatment with the SGLT2 inhibitor in HFD-fed mice dose-dependently reduced the inducibility and duration of AF. There were no significant differences in cardiac function, blood pressure, and fibrosis among all groups. Impairment of glucose tolerance in HFD-induced obesity was improved by treatment with the SGLT2 inhibitor.

Conclusion: Treatment with the SGLT2 inhibitor reduced the inducibility of AF and shortened the duration of AF without affecting atrial structural remodeling, suggesting that the SGLT2 inhibitor effectively prevents AF in obesity.

Evaluation of antiarrhythmic agents for catecholaminergic polymorphic ventricular tachycardia (CPVT) using multiple lines of RyR2-mutant mouse models

複数のRyR2変異マウスモデル系統を用いたカテコラミン誘発性多型性心室頻拍（CPVT）に対する抗不整脈薬の評価

○呉林 なごみ[1]、児玉 昌美[1]、村山 尚[1]、杉原 匡美[2]、石井 光一郎[1]、岡部 雄太[3]、村越 伸行[3]、三浦 綾[4]、西尾 元[4]、井上−上野 由紀子[5]、井上 高良[5]、野口 悟[5]、中村 衣里[6]、金井 富三夫[6]、多田 昇弘[6]、櫻井 隆[1]

[1]順天堂大・医・薬理、[2]筑波大・医・循内、[3]順天堂大・医・臨床検査医学、[4]兵庫医大・医・法医、[5]国立精神・神経医療研究セ、[6]順天堂大・院医・疾患モデル研究セ

Gain-of-function mutations in type 2 ryanodine receptors (RyR2) are known to cause severe arrhythmias such as catecholaminergic polymorphic ventricular tachycardia (CPVT). Conventional antiarrhythmic drugs are sometimes insufficient to suppress these arrhythmias. To seek more effective drugs, we aimed to provide a basis for quantitative evaluation of the two important effects of antiarrhythmic drugs, i.e., prevention and treatment/stopping of arrhythmia, using multiple RyR2 mutant mouse lines (R420W, I4093V and K4750Q) with varying degrees of enhanced Ca^{2+} release activity. Short term ECG was recorded from the limb leads under isoflurane anesthesia, and arrhythmia was induced by administration of adrenaline alone or adrenaline/caffeine mixture. For monitoring basal arrhythmia during everyday activities, long range ECG was recorded by telemetry system. The R420W mice having moderately activated channels showed little basal arrhythmia but exhibited severe ventricular arrhythmias by adrenaline/caffeine induction. On the contrary, the RyR2-I4093V and K4750Q strains having highly activated channels showed frequent basal arrhythmia without adrenergic induction. Na channel blockers, Ca channel blockers and beta blockers suppressed the induced arrhythmia and basal arrhythmia to varying degrees. Interestingly, the preventive effect and the stopping effect seemed to differ depending on their mechanism of action. The usage of multiple lines of mice with different degrees of activity are useful for the evaluation of therapies for CPVT.

Methylglyoxal-induced enhancement of uridine diphosphate-mediated contraction in rat femoral artery was due to activation of p38 MAPK and Syk tyrosine kinase

ラット大腿動脈におけるウリジン二リン酸誘発収縮反応のメチルグリオキサールによる増強には p38 MAPK と Syk tyrosine kinase が関与する

○松本 貴之、吉岡 円、野上 あかね、工藤 美代、長田 友恵、小笠原 悠、田口 夏芽、遠藤 彩香、中村 圭吾、山田 愛子、田口 久美子、小林 恒雄

星薬科大

Although abnormalities of function in femoral artery, including aberrant vascular reactivity to various vasoactive mediators, are common in many chronic disorders including hypertension and diabetes, their inducible and/or progressive factors remain unclear. On the other hand, methylglyoxal (MGO), a highly reactive dicarbonyl compound, has been implicated in the pathogenesis of several chronic disorders. Although our previous studies demonstrated that uridine 5'-diphosphate (UDP)-induced contraction in the femoral artery is increased in hypertensive rat model, the direct relationship between MGO and UDP-mediated contraction is currently unknown in rat femoral artery. We therefore investigated the acute effect of MGO (4.2×10^{-4} M for 60 min) on UDP-induced contraction in the rat femoral artery. MGO amplified the UDP-induced contraction in the Wistar rat femoral artery. This augmented response was not abolished in all conditions, including nitric oxide synthase inhibition by L-NNA (10^{-4} M), cyclooxygenase inhibition by indomethacin (10^{-5} M), or endothelial denudation. Moreover, in the endothelium-denuded arteries, the p38 mitogen-activated protein kinase (MAPK) inhibitor SB203580 (10^{-5} M) reduced the UDP-induced contraction in both control and MGO-treated groups, while MGO enhanced the p38 MAPK activation regardless of the UDP presence. Moreover, in the endothelium-denuded arteries, the Syk tyrosine kinase inhibitor piceatannol (10^{-5} M) reduced the UDP-induced contraction in both control and MGO-treated groups. These results suggest that MGO enhances UDP-induced contraction in rat femoral arteries and that this enhancement may be partly due to increases in the activities of Syk tyrosine kinase and p38 MAPK in femoral arterial smooth muscle.

Anti-spasmolytic effects of BAY 60-2770, a soluble guanylate cyclase activator, in isolated coronary arteries

摘出冠動脈における可溶性グアニル酸シクラーゼ活性化薬BAY 60−2770の抗攣縮作用

○田和 正志、中川 恵輔、大喜多 守

大阪医科薬科大学・薬・病態分子薬理

This study investigated whether soluble guanylate cyclase (sGC) activators (activating the heme-oxidized and heme-free sGC) have potential as therapeutic drugs for coronary artery spasm. Isolated canine and porcine coronary arteries were suspended in organ chambers for isometric tension recording. The contractile responses of canine coronary arteries to potassium chloride (3×10^{-2} M), endothelin-1 (3×10^{-11} to 3×10^{-8} M), prostaglandin $F_{2\alpha}$ (10^{-8} to 10^{-5} M), and 5-hydroxytryptamine (10^{-9} to 10^{-6} M) were suppressed by previous exposure to the sGC activator BAY 60-2770 (10^{-10}, 10^{-9} and 10^{-8} M). In porcine coronary arteries, the addition of BAY 60-2770 (10^{-10}, 10^{-9} and 10^{-8} M) concentration-dependently prolonged the cycle length of 3,4-diaminopyridine (10^{-2} M)-induced phasic contractions and reduced the peak tension. As for vessel-size-dependent difference in vasoreactivity, BAY 60-2770 (10^{12} to 10^{-7} M) caused a greater relaxation of porcine coronary arteries precontracted with endothelin-1 (3×10^{-8} M) in small arteries (#9 in AHA classification) than in large arteries (#6 in AHA classification). These findings suggest that sGC activators are beneficial for the treatment of vasospastic angina. In addition, anti-spasmolytic efficacy of sGC activators may be expected to be observed even in microvascular angina.

Electropharmacological analysis of vernakalant as an anti-atrial fibrillatory drug using the isoflurane-anesthetized beagle dogs and the rat aorta

抗心房細動薬vernakalantの電気薬理学的作用：イソフルラン麻酔犬およびラット大動脈を用いた評価

○後藤 愛[1]、神林 隆一[1]、中瀬古（泉） 寛子[1]、武井 義則[1]、松本 明郎[2]、杉山 篤[1,2]

[1]東邦大・医・薬理、[2]東邦大・医・加齢薬理

Introduction: Vernakalant was approved as an anti-atrial fibrillatory drug by EMA and Health Canada but not by FDA due to its cardiovascular adverse events including bradycardia and hypotension. We characterized its electropharmacological profile *in vivo* with the isoflurane-anesthetized beagle dogs and *in vitro* with the rat aorta.

Methods: Vernakalant (0.3 and 3 mg/kg/10 min) was intravenously administered to the dogs in the absence (n=5) and presence (n=4) of α-adrenoceptor blocker phentolamine. Next, the *in vitro* vascular effect of vernakalant on the rat aorta was assessed by its cumulative application in concentrations of 0.001-100 μmol/L (n=13 preparations).

Results: Vernakalant suppressed the sinus automaticity, ventricular contractility and atrioventricular nodal as well as intraventricular conduction, whereas it increased the total peripheral vascular resistance, preload to the left ventricle and mean blood pressure. It delayed the ventricular repolarization in a reverse frequency-dependent manner; the extent of prolongation of early and late repolarization was similar. It also prolonged the atrial and ventricular effective refractory period similarly. Pretreatment of phentolamine hardly affected those results. Meanwhile, vernakalant did not induce the contraction of aorta *in vitro*.

Conclusion: Vernakalant exerted α-adrenodceptor-independent vasoconstrictor action only *in vivo*. It also showed electrophysiological effects on the atria and ventricles to a similar extent, which resembles those of *dl*-sotalol and bepridil.

Runx1 is upregulated by STAT3 and promotes cell proliferation in neonatal rat cardiomyocytes.

新生児ラット心筋細胞において、Runx1はSTAT3によって発現制御され、細胞増殖を促進する

○鈴木 翔大[1]、亀谷 祐介[1]、梅田 綾香[1]、西中 康介[1]、田中 翔大[1]、尾花 理徳[1,2]、岡田 欣晃[1]、藤尾 慈[1,2]

[1]大阪大・院薬・臨床薬効解析学分野、[2]大阪大・先導的学際研究機構生命科学融合フロンティア研究部

【Background】

Mammalian cardiomyocytes (CMs) retain proliferative capacity shortly after birth, though these cells are differentiated with contractile activity. By using Runx1 as a dedifferentiation marker, recent studies have demonstrated that CMs are dedifferentiated prior to cell proliferation; however, biological significance of Runx1 remains to be fully elucidated. The aim of this research is to clarify the regulatory mechanisms of Runx1 expression and its biological functions in CM proliferation.

【Method/Result】

CMs were prepared from neonatal rats. Cell proliferative activity was estimated by immune-fluorescent microscopic analysis with anti-Ki-67 antibody. CM exhibited proliferative activity in response to fetal bovine serum (FBS). Previously, since we demonstrated that STAT3 plays an important role in CM proliferation, the effects of STAT3 on CM proliferation was analyzed by using siRNA. STAT3 knockdown reduced the frequency of Ki-67^{+} CM, accompanied by the decrease in Runx1 expression. Importantly, Runx1 knockdown also suppressed CM proliferation in response to FBS.

【Conclusion】

Runx1 expression is regulated by STAT3, and promotes CM proliferation, indicating the functional importance of Runx1 in cardiac proliferation.

2,5-Dimethylcelecoxib prevents cardiac remodeling associated with myocardial ischemia/reperfusion injury in mice

2,5−ジメチルセレコキシブは心虚血再灌流障害に伴う心臓リモデリングを抑制する

○石兼 真[1]、幾島 栄悟[1]、岸上 赳大[1]、井川 和宣[2]、友岡 克彦[2]、有岡 将基[1]、高橋 富美[1]

[1]産業医科大・医・薬理、[2]九州大・先導物質化学研・分子集積化学

Myocardial ischemia/reperfusion (MI/R) injury leads to aggravated cardiac remodeling and heart failure. Previously, we reported that 2,5-dimethylelcelecoxib (DMC), a derivative of celecoxib without cyclooxygenase-2 inhibition, prevents cardiac remodeling in a non-ischemic cardiac fibrosis model. In this study, we examined whether DMC inhibited myocardial remodeling associated with MI/R injury. The left anterior descending coronary artery was ligatured for 0.5 hours and subsequently subjected to reperfusion for MI/R injury in male C57 BL/6 mice. Vehicle or DMC was administered orally (DMC: 150 mg/kg) immediately after awakening and followed by feeding (DMC: 1000 ppm). Echocardiographic evaluation showed significant improvement of left ventricular ejection fraction in the DMC-treated group compared to the Vehicle-treated group at 1-4 weeks after MI/R injury. In MI/R-injured hearts, protein expression of alpha-smooth muscle actin (myofibroblast marker) was significantly reduced by DMC treatment, as were mRNA expressions of fibronectin, connective tissue growth factor, and matrix metalloproteinase-9, 3 days after injury. Masson trichrome staining indicated that DMC significantly reduced cardiac fibrosis area 4 weeks after MI/R injury. This study revealed that DMC decreased myofibroblast appearance, and suppressed fibrosis and cardiac dysfunction associated with MI/R injury. DMC might be useful for preventing the development of heart failure associated with reperfusion therapy for acute myocardial infarction.

Establishment of a novel method for analysis of biological functions of myeloid cell subpopulation in cardiovascular diseases using TRECK system.

TRECKシステムを用いた、心血管疾患におけるミエロイド系細胞集団の機能解析法の構築

○横田 洸成[1]、冨松 聖史[1]、小南 春祐[1]、梶浦 僚太[1]、田中 翔大[1]、尾花 理徳[1,2]、岡田 欣晃[1]、藤尾 慈[1,2]

[1]大阪大・院薬・臨床薬効解析学分野、[2]大阪大・先導的学際研究機構生命医科学融合フロンティア研究部

［Background］ Though the importance of myeloid cells in the cardiac remodeling after myocardial infarction (MI) is widely accepted, it remains to be fully elucidated how myeloid cells regulate post-infarct inflammation, at least partially, because subpopulation-specific cell knock-out methods are not available.

［Methods and Results］ We generated transgenic mice expressing diphtheria toxin receptor (DTR)/GFP fusion protein under the control of CD11b promoter in a Cre recombinase-expressing cell-specific manner (CD11b-DTR TG mice). Double TG mice (DTG mice) were generated by crossing CD11b-DTR TG mice with LysM-Cre mice that express Cre recombinase preferentially in monocytes/macrophages. The MI model was created in DTG mice by ligation of the left anterior descending branch. Flow cytometry analysis revealed that monocytes were labeled with GFP in the peripheral blood 4 days after MI. Consistently, immunofluorescent microscopic analysis showed that GFP^+ cells infiltrated into the infarcted heart. Importantly, the administration of diphtheria toxin resulted in the depletion of GFP^+ cells in peripheral blood and post-infarct myocardium.

［Conclusion］ CD11b-DTR TG mice are useful for labeling and/or depleting subpopulation of myeloid cells in MI model.

Gas phase extract of mainstream smoke derived from heated tobacco products causes iron-dependent, ferroptosis-independent cell injury in vascular endothelial cells.

血管内皮細胞において，加熱式たばこ主流煙ガス相水抽出物は，鉄依存性，フェロトーシス非依存性の細胞傷害を引き起こす

○堀之内 孝広[1]、三輪 聡一[2]

[1]北海道大・院医・細胞薬理、[2]公立豊岡病院

Ferroptosis is defined as an iron-dependent regulated necrosis that is caused by massive lipid peroxidation-mediated cell membrane damage. The present study examined whether ferroptosis is involved in endothelial dysfunction by nicotine- and tar-free cigarette smoke extract (CSE) prepared from heated tobacco products (HTPs). The CSE of HTPs (Ploom X, IQOS 3, and IQOS ILUMA) and a combustion cigarette (1R6F) was prepared according to Health Canada Intense smoking method (55 mL puff volume, 2 sec puff duration, and 1 puff every 30 sec) using an analytical vaping machine LM5E (Borgwaldt KC GmbH). The cytotoxicity of CSE of HTPs and 1R6F to human umbilical vein endothelial EA.hy926 cells was evaluated by measuring mitochondrial metabolic activity and lactate dehydrogenase (LDH) leakage. CSE from cigarettes except for Ploom X, and erastin, a ferroptosis inducer by inhibiting cystine-glutamate exchange transporter (system X_C^-), triggered a decrease in mitochondrial metabolic activity and an increase in LDH leakage. The cytotoxic effects of CSE of IQOS 3 and 1R6F were reduced by an iron chelator deferoxamine mesylate (DFO), but not by a ferroptosis inhibitor UAMC-3203, which scavenges lipid reactive oxygen species (ROS). On the other hand, erastin cytotoxicity was inhibited by both DFO and UAMC-3203. These results suggest that erastin-induced, iron-dependent ferroptosis leads to cell damage characterized by a decrease in mitochondrial metabolic activity and an increase in LDH leakage, in EA.hy926 cells. CSE of IQOS 3 and 1R6F causes iron-dependent mitochondrial and cell membrane damage, both of which are independent of lipid peroxidation by ROS.

Glucocorticoid first increases urinary sodium excretion and urine volume, leading to skin sodium and water loss in mice

マウスに対するグルココルチコイド投与は、先ず尿中ナトリウム排泄量と尿量を増加させ、皮膚のナトリウムと水分喪失を惹起する

○北田 研人、中野 大介、西山 成

香川大・医・薬理学

We previously revealed that glucocorticoids such as cortisol and cortisone may also regulate sodium and fluid balance in healthy subjects. We also reported that dexamethasone administration to mice initially increased urinary sodium excretion and urine volume and reduced skin sodium and water content in the early phase of administration and that chronic dexamethasone injection only decreased skin sodium and water content. However, it remains to be clarified which organ, kidney or skin, is responsible for the initial sodium and water loss at initial dexamethasone injection. In the present study, we examined the effects of dexamethasone on skin sodium and water content in bilateral nephrectomized mice. In the sham-operated group, dexamethasone (1 mg/kg/day, s.c.) significantly increased urinary sodium excretion and urine volume and decreased skin sodium and water content 24 hours after the injection. Dexamethasone did not affect plasma sodium concentration and osmolarity. In bilateral nephrectomy groups, dexamethasone did not alter skin sodium and water content. These findings suggest that glucocorticoid originally increases urinary sodium excretion and urine volume, which decreases skin sodium and water loss to compensate for renal sodium and water loss. In order to elucidate the mechanisms of sodium and fluid homeostasis, it may be necessary to examine skin and glucocorticoids in addition to the known hormones and kidney systems.

Aquaporin-5 in airway epithelial cells enhance LPS-induced cytokine expression in vivo experiment.

気道上皮細胞のaquaporin-5はin vivo 実験系でLPSによって誘発される炎症性サイトカイン発現を亢進する.

○石井 慎也[1]、岡谷 拓海[2]、内山 雄太[2]、礒濱 洋一郎[2]、村上 一仁[2]

[1]東京理科大・院薬・応用薬理、[2]東京理科大・薬・応用薬理

Aquaporins (AQPs) are the water channel that facilitate water transport through plasma membrane. Among 13 AQP isoforms, AQP5 is selectively expressed in apical membrane of exocrine glands, airway and alveolar epithelial cells, and plays important role to maintain water secretion and clearance in airway tracts. Recently, it is revealed that AQPs have new functions, as well as water transport. In our previous study, AQP5 enhances TNF- α -induced chemokine expression in vitro experiments. It has also known that the expression of AQP5 is markedly reduced in airway inflammation mouse model. Therefore, changes in AQP5 expression may be contribute to the pathogenesis of inflammatory respiratory diseases. In this study, we have established a transgenic (Tg) mouse in which AQP5 is highly expressed specifically in the airway epithelial cells, to confirm the significance of the AQP5-madiated regulation in cytokine expression. Intratracheal treatment of LPS increased the expression of inflammatory cytokines, such as KC, TNF- α and IL-6 in WT mice. In AQP5-Tg mice, the increase in the expression of these cytokines by LPS was considerably less than that in WT. These results indicated that AQP5 can enhance airway inflammation not only in vitro, but also in vivo conditions.

移動

Effect of local IL-10 replacement therapy on severe asthma in mice

重症喘息モデルマウスに対するIL−10局所補充療法の効果

○松田 将也、大森 美侑、北谷 和之、奈邉 健

摂南大・薬・薬効薬理

Subgroups of severe asthma patients with marked increases in sputum eosinophils and neutrophils are insensitive to corticosteroids. Exogenous administration of IL-10 negatively regulates both migration of eosinophilic and neutrophilic into tissues. This study evaluated whether intratracheal IL-10 administration suppresses asthmatic responses in a severe model of mice. Ovalbumin (OVA)-sensitized mice were intratracheally challenged with OVA. Dexamethasone (DEX, 1 mg/kg, intraperitoneal) or IL-10 (25 ng/mouse, intratracheal) was administered during the challenges. The number of leukocytes, expressions of adhesion molecules and IL-10 receptor, and development of airway hyperresponsiveness (AHR) were evaluated after the challenges. Although DEX hardly suppressed the development of AHR, the infiltration of eosinophils and neutrophils, and the development of AHR were significantly inhibited by intratracheal IL-10 administration. Moreover, IL-10 administration markedly decreased the numbers of ICAM-1^+ and VCAM-1^+ pulmonary vascular endothelial cells, which express IL-10 receptor 1. IL-10 could suppress eosinophil and neutrophil infiltration by inhibiting the proliferation of ICAM-1^+ and VCAM-1^+ pulmonary vascular endothelial cells, resulting in inhibition of AHR in severe asthmatic mice.

Role of histone ubiquitination in SARS-CoV2 and influenza virus infection

SARS-CoV2及びインフルエンザウイルス感染におけるヒストンユビキチン化の役割

○衣笠 泰葉、星崎 みどり、Llamas Covarrubias Mara、今井 由美子

医薬基盤研・感染病態制御ワクチンPJ

Virus infection may affect the epigenetic regulations in host cells, including post-translational histone modifications. Ubiquitination of histone H2B, has been reported to be involved in transcription activation. However, it remains unknown the role of histone ubiquitination in the pathology of virus infection (e.g. influenza virus, SARS-CoV2). CNOT4 that is a component of the CCR4-NOT complex has a ubiquitin transferase activity at the RING domain (L16). Here we show that CNOT4 is responsible for histone H2B ubiquitination in the host cells, which was linked to H3K4 methylation. Upon influenza virus or SARS-CoV2 virus infection CNOT4 interacted to virus protein, resulting in the loss of H2B ubiquitination and H3K4 methylation, which suppress interferon-related gene expression. The cells with a ubiquitination activity site of L16 of CNOT4, have increased virus replication. These results suggest that the CNOT4 is involved in the virus replication through histone H2B ubiquitination.

Chemokine CCL28 suppresses liver fibrosis in a mouse model of carbon tetrachloride-induced chronic hepatitis

ケモカインCCL28は四塩化炭素誘発性の慢性肝炎モデルマウスにおいて線維化を抑制する

○原 雄大、細川 隼矢、奥村 遼平、松尾 一彦、中山 隆志

近畿大・薬・化学療法

The chemokine CCL28 is mainly expressed in mucosal tissues such as colon and salivary gland, and migrates IgA-secreting cells via its receptor CCR10. Because IgA protects mucosal tissues from pathogenic microorganisms, CCL28 is thought to play an important role in mucosal immunity. However, CCL28 has been shown to be also expressed in non-mucosal tissues. Although some previous studies reported that CCL28 was increased in liver of patients with chronic liver disease, the detailed involvement in the pathology remains unclear. In this study, we examined the influence of CCL28 deficiency on carbon tetrachloride (CCl_4)-induced chronic hepatitis in mice. Chronic treatment with CCl_4 increased CCL28 expression levels in the liver. CCL28-deficient mice showed increased serum ALT levels and fibrotic areas of the liver. CCl_4 treatment also increased IgA-secreting plasma cells (PC), which expressed CCR10, in the liver of wild-type mice, but not CCL28-deficient mice. The hepatic IgA-secreting PC, but not IgA-negative PC, expressed IL-10, FasL, and PD-L1. Furthermore, CCL28-deficient mice showed decreased apoptosis and activity of hepatic stellate cells which are a key player in the progression of liver fibrosis. These findings suggest that CCL28 would suppress the pathology of chronic hepatitis through the migration of IgA-secreting PC.

Intraperitoneal injection of the chemokine CX3CL1 improves aged recognition memory

ケモカインCX3CL1の腹腔投与による認知機能老化の改善

○武井 義則、後藤 愛、神林 隆一、中瀬古（泉） 寛子、杉山 篤

東邦大・医・薬理学講座

Aging is associated with a progressive decline in cognitive function. While obesity accelerates aging process of the brain,physical activity preserves and improves cognitive performance in the elderly. The expression of a chemokine CX3CL1 has been reported to be increased in both conditions. CX3CL1 is known to chemoattract T cells and monocytes. Moreover, it promotes survival of neurons in the brain and b-cells in the pancreas. In adipose tissues, it is expressed in adipose cells and attenuates effects of obesity-induced chronic inflammation. However, the role of the lifestyle-induced CX3CL1 expression in cognitive aging is unknown. Here, we administered CX3CL1 into the peritoneal cavity of aged mice (15-16 months old) to investigate its impact on the aging process of cognition. In the hippocampus, CX3CL1 increased the number of Type-2 neural stem cells and promoted brain-derived neurotrophic factor (BDNF) expression. This treatment, furthermore, improved novel object recognition memory impaired with advancing age. Intraperitoneal transplantation of peritoneal cells from CX3CL1-treated aged mice improved novel object recognition memory in recipient aged mice. Vagotomy inhibited the CX3CL1-induced increase in BDNF expression. Thus, our results demonstrate that a novel connection among peritoneal cells, the vagal nerve and the hippocampus can reverse the age-associated decline in recognition memory.

IL-34 inhibition attenuates renal fibrosis induced by unilateral ureteral obstruction in mice

Interleukin-34（IL-34）の阻害による片側尿管結紮マウスでの腎線維化抑制効果

○和田 幸寛[1]、本田 浩一[2]、伊藤 義也[1]、細野 加奈子[1]、畑中 畑中[1]、天野 英樹[1]

[1]北里大・医学部薬理学講座、[2]昭和大・医・内科学講座腎臓内科学部門

Introduction: Interleukin (IL)-34, a macrophage (Mø) mediator, is expressed by tubular epithelial cells. However, the influence of IL-34 on tubulointerstitial fibrosis remains to be fully elucidated. We investigated the effect of IL-34 on renal fibrosis caused by unilateral ureteral obstruction (UUO). Material and Methods: 10-week-old male C57BL/6 (B6) mice (n=16) were induced UUO. Groups of animals were given either anti-mouse IL-34 antibody (UUO+anti-IL-34 Ab, 400 ng/kg, n=8) or vehicle (UUO+V, equal volume of saline, n=8) daily by intraperitoneal injection. Four age-matched male B6 mice received sham operation as control. All mice were sacrificed on day 10. Results: Compared to the control, the UUO+V mice exhibited remarkable intrarenal expressions of IL-34 and its two receptors (cFMS and PTP-ζ), which were significantly suppressed by anti-IL-34 Ab treatment. Compared to the UUO+V mice, tubular injury and sirius red positive area were significantly attenuated in the UUO+anti-IL-34 Ab mice. Treatment with anti-IL-34 Ab significantly suppressed the number of $F4/80^+$ Mø and α-SMA^+ myofibroblast in damaged kidneys of UUO. The renal cortical transcript levels of TGF-β, COL-1, TNF-α, IL-6, MCP-1/CCL2, and MIP-1/CCL3 were significantly lower in the UUO+anti-IL-34 Ab mice. Conclusion: Elevated IL-34 expression was related to renal fibrosis. Inhibition of IL-34 with neutralizing Ab suppressed expressions of inflammatory cytokines and fibrogenetic genes via reducing the Mø infiltration, which might lead to attenuate the development of renal fibrosis.

Bipolar effect of cannabinoid CB2 receptors to peripheral neuroinflammation

カンナビノイドCB2受容体は神経炎症を抑制するとは限らない

○野崎 千尋[1]、Zimmer Andreas[2]、柴田 重信[3]

[1]早稲田大・国際理工学センター・Major in Bioscience、[2]University of Bonn・Institute of Molecular Psychiatry、[3]早稲田大・理工・電気・情報生命工学科

It is widely known that cannabinoid type 2 (CB2) receptor deficiency enhances neuroinflammation and pain development in the animal model of nerve injury-evoked neuropathic pain. We previously proposed the upregulated leptin signaling at the peripheral nerve as one of the underlying molecular mechanism, as nerve-injured CB2 receptor knockouts (CB2-KO) displayed robust upregulation of leptin receptors in both injured and non-injured nerve tissue. Those leptin receptors seemed to be expressed on the macrophages which is recruited to the nerve by the tissue injury, indicating the infiltration of leptin receptor-expressing macrophages. Thus, Due to these past results we also hypothesized that lack of CB2 receptor might also enhance the high fat diet (HFD)-induced peripheral neuroinflammation. However, surprisingly, CB2-KOs showed the significant resistance to the HFD-induced neuroinflammation. Namely, 5-week feeding of HFD induced substantial hypersensitivity in WT mice, while tactile sensitivity of HFD-fed CB2-KO remained intact. In the same animals, we further found the significant upregulation of infiltrated macrophages and chemokine receptor CXCR4 expression in HFD-fed WT animals, but not in either HFD-fed CB2 knockout mice or standard fat diet (SFD)-fed WT and CB2-KO controls. Based on these results, we will propose that CB2 receptors might have the bipolar regulatory role to chemokine receptor-mediated inflammatory response, which in the end enhance or inhibit the development of neuroinflammation depending on its cause.

Effect of berberine on dermatitis and itch-related responses in mice with atopy-like dermatitis

アトピー性皮膚炎マウスモデルにおける皮膚炎並びに痒み反応へのberberineの効果

○安東 嗣修[1]、田渕 圭章[2]、清水 忠道[3]

[1]金城学院大・薬・病態薬理、[2]富山大・遺伝子実験施設、[3]富山大・医・皮膚科学

Atopic dermatitis (AD) is a chronic skin disease with severe inflammation and pruritus. Traditional Kampo medicine Orengedokuto improves AD symptom in human patients and atopy-like symptoms in AD mouse model. Berberine is a major component of Orengedokuto. This study investigated the effects and molecular mechanisms of berberine on AD-like symptoms in mice. In NC/Nga mice with atopy-like dermatitis (dermatitis mice), intermittent oral administrations of berberine inhibited skin symptom, itching, cutaneous infiltration of eosinophils and mast cells, and the cutaneous expression of eotaxin, macrophage migration inhibitory factor (MIF) and IL-4. Berberine also inhibited both IL-4/MIF-induced eotaxin in fibroblasts and allergen-induced MIF and IL-4 in mast cells. In mast cells, the GeneChip® microarray analysis showed that antigen increased the expression of EIF3F and MALT1, inhibited by berberine. The regulation of these factors by siRNAs for them showed antigen-induced the expression of MIF and IL-4. These results suggest that berberine inhibits AD-like symptoms through at least downregulation of EIF3F and MALT1 in mast cells.

Acquired oral immune tolerance was overridden by exposure of food antigen via skin to use murine food allergy models

皮膚を介した食物抗原の暴露によって獲得した経口免疫寛容を破たんさせる方法の探索

○山下 弘高[1]、伊波 幸紀[1]、田中 宏幸[2,3]、稲垣 直樹[4]、筒井 正人[1]

[1]琉球大・院医・薬理、[2]岐阜薬科大・院薬・免疫生物、[3]岐阜大・院連合創薬、[4]岐阜医療科学大・薬・薬理

Oral tolerance (OT) was immune regulatory system for foods. Food allergy (FA) patients are induced allergy by inhibition of acquiring OT or overriding OT. Recently, it has been hypothesized that immune tolerance is induced for foods taken orally and that allergy is caused by exposure via skin. In this study, we tried to override OT through exposure of food antigen to skin. We established three murine FA models to analyze the relationship between OT and percutaneous sensitization. IP model: Mice were injected ovalbumin (OVA) intraperitoneally (IP) followed by induction of food allergy. EC model: Mice were pasted a filter paper containing OVA on shaved back skin for epicutaneous sensitization (EC). ID model: Mice were injected OVA intradermally (ID) for sensitization via skin. OT was induced by oral OVA treatment before the sensitization in each model. FA was estimated by drop of body temperature, diarrhea, and OVA-specific IgE level in plasma. In all FA models, we confirmed FA symptoms involving elevation of OVA-specific IgE. OT induction inhibited the increasing in IgE level and suppressed FA in IP model. In EC model with OT induction, we detected increasing in IgE level. In ID model with OT induction, we confirmed FA symptoms and increasing in IgE level. The data indicated that exposure of OVA via skin could override OT.

Effect of Japanese cedar pollen sublingual immunotherapy on allergic rhinitis symptoms during the Japanese cypress pollen dispersal period

スギ花粉舌下免疫療法のヒノキ花粉飛散時期におけるアレルギー性鼻炎症状への効果

○土井−大橋 雅津代[1]、松原 弘季[1]、大久保 公裕[2]、岡本 美孝[3]、前川 祐理子[1]

[1]鳥居薬品（株）、[2]日本医科大学大学院医学研究科、[3]ちば労災病院

Background: The clinical efficacy of Japanese cedar (JC) pollen SLIT tablets for allergic symptoms during Japanese cypress (JCY) pollen dispersal has been controversial in actual clinical settings. JC and JCY both belong to the *Cupressaceae* family and the major allergens of the two species have strong amino acid homology. The efficacy of JC pollen SLIT tablets against seasonal allergic rhinitis during the JC pollen and JCY pollen dispersal periods was investigated.

Methods: A *post-hoc* analysis was conducted in a phase II/III study (JapicCTI no. 142579). Patients with JC pollinosis (aged 5-64 years) were included (placebo n=159, 5000 JAU n=158). Patients in the active 5000 JAU treatment group was treated with JC SLIT tablet daily by self-administration for the duration of the trial. Clinical efficacy was evaluated by the total nasal symptom and medication score (TNSMS) during the peak symptom periods of each pollen season over 3 years.

Results: The daily average TNSMS in the 5000 JAU group was consistently lower than in the placebo group during both the JC and JCY pollen dispersal periods in all three seasons. The larger reduction in TNSMS in the 5000 JAU group compared with the placebo group was observed in a treatment duration-dependent manner.

Conclusions: JC SLIT tablet treatment showed sustained clinical efficacy on allergic symptoms during 3 consecutive JCY pollen seasons. Further studies are required to examine the immunological responses and extent of antibody cross-reactivity towards the homologous major allergens from different *Cupressaceae* family species including JC and JCY.

Involvement of inflammatory cells in the dorsal root ganglion in oxaliplatin-induced neuropathic pain

オキサリプラチンによる神経障害性疼痛における脊髄後根神経節での炎症性細胞の関与

○大澤 匡弘[1]、岩城 杏奈[1]、梅澤 直樹[2]、樋口 恒彦[2]、杉山 洋介[4]、祖父江 和哉[3]、粂 和彦[1]
[1]名古屋市立大・院薬、[2]名古屋市立大・院薬・精密有機反応学分野、[3]名古屋市立大・院医・麻酔科学・集中治療医学分野、[4]名古屋市立大・院医・臨床薬剤学分野

Oxaliplatin is a platinum-based chemotherapeutic agent treating colorectal and gastric cancer. The most common dose-limiting adverse event of oxaliplatin treatment is chronic neuropathic pain, while the detailed mechanisms remain unknown. The present study investigated the molecular mechanisms of microglial regulation of the oxaliplatin-induced neuropathic pain. Intrathecal (I.t.) treatment with inducible nitric oxide synthetase (iNOS) inhibitor 1400W attenuated the oxaliplatin-induced cold and mechanical hypersensitivity. In addition, pharmacological and chemogenetical inhibition of macrophage/microglia also attenuated the oxaliplatin-induced cold and mechanical hyperalgesia. I.t. treatment with STAT3 inhibitor stattic attenuated the oxaliplatin-indued cold and mechanical hypersensitivity. Oxaliplatin induced the increased phosphorylation of neural STAT3 in the dorsal root ganglion (DRG), which was attenuated by i.t. pretreatment with 1400W. Since STAT3 phosphorylation was regulated by PTEN, effects of oxaliplatin on the PTEN activity in the DRG were examined. PTEN expression was decreased by oxaliplatin treatment. Our present study suggested that oxaliplatin induces neural STAT3 activation through the iNOS induction by macrophage/microglial activation in the DRG, which resulted in the oxaliplatin-induced neuropathic pain.

Extrinsic ribosome stimuli triggers development of glioblastoma stem like cells

外因性リボゾームによる膠芽腫がん幹細胞様細胞の発生メカニズムの解明

○城野 博史[1,3]、白川 裕貴[2]、三宅 俊介[1,3]、金丸 歩美[3]、米丸 興[3]、内野 翔太[3]、秀 拓一郎[4]、武笠 晃丈[5]、太田 訓正[6]、齋藤 秀之[1,3]

[1]熊本大・大学病院・薬剤部、[2]順天堂大・院医、[3]熊本大・大学院薬学教育部、[4]北里大・大学病院、[5]熊本大・院生命科学、[6]九州大・基幹教育院

Background: Although glioblastoma (GBM) stem like cells (GSCs), which exhibit chemo-radio resistance and recurrence, are key prognostic factors in GBM patients, molecular mechanisms of GSCs development are largely unknown. Recent studies revealed that extrinsic ribosome incorporation into somatic cells played key roles in cell reprogramming process towards stem cell properties. In this study, we sought to elucidate the mechanisms underlying GSCs development by focusing extrinsic ribosome incorporation into GBM cells.

Results: The ribosome incorporation into GBM cells significantly increased ribosome induced cancer cell spheroid (RICCS) formation, and showed the stem like cell characters. In RICCS, phosphorylation and protein expression of RPS6, an intrinsic ribosomal protein, and STAT3 phosphorylation were involved in regulating cell spheroids formation. Interestingly, glioma-derived extrinsic ribosome also promoted GBM-RICCS formation through the intrinsic RPS6 phosphorylation. Moreover, in glioma patients, RPS6 phosphorylation was observed in higher grade glioma tissues, and predominantly up-regulated in GSCs niches, such as perinecrosis niche and perivascular niche.

Conclusion: Our results suggest the potential biological & clinical significance of extrinsic ribosomal proteins in GSCs development.

Gene regulation during early and late phase of hypoxic response in cancer cells

がんの低酸素応答における早期と長期の遺伝子発現制御機構の解析

○中山 恒

旭川医科大・医・薬理学

Cells in our body are often exposed to hypoxic environment. In such environment, cells regulate respiration and metabolism for adaptation (hypoxic response). Hypoxic response not only plays a key role in maintaining homeostasis, but also in different diseases, such as cancer. Hypoxia-Inducible Factor (HIF) is a central transcription factor in hypoxic response. HIF is promptly up-regulated in hypoxia, and forms adapted cellular state. HIF has multiple target genes, and they coordinately regulate hypoxic response. Recently, HIF specific inhibitor was developed, and it is currently under clinical trial. Alternatively, we have demonstrated that HIF is downregulated, and CREB and NF-kB become activated during chronic phase of hypoxia. However, it remains unclear how the expression pattern of hypoxia-responsive genes changes depending on the timing.
HIF becomes activated by a heterodimer formation of a and b subunits. There are three a subunit isotypes, and they commonly bind to b subunit, ARNT. We have established ARNT knockout (KO) colon cancer HCT116 cells. These cells showed a clear inhibition of typical HIF target gene *LDHA*. In contrast, *MMP1*, which is induced under chronic hypoxia, was equally up-regulated in both wild type and KO cells, but its expression level is significantly reduced in KO cells. Altogether, these results indicate that induction of hypoxic genes is dependent on transcription factors which are activated during chronic phase, however, basal expression of them is mediated by a HIF-dependent machinery.

Inhibition of the mitochondrial shaping protein OPA1 restores lung adenocarcinoma cells sensitivity to gefitinib.

ミトコンドリア形態制御タンパク質OPA1の阻害は肺腺癌細胞のgefitinib感受性を復帰させる

○野口 雅史[1,2]、河野 晋[3]、ペラティエロ アンナ[2]、町田 雪乃[4]、柴田 圭太郎[2]、新谷 紀人[1,5]、河野 隆志[6]、後藤 典子[3,7]、高橋 智聡[3,7]、平尾 敦[3,8]、スコラーノ ルカ[2]、笠原 敦子[3,7,8]

[1]和歌山県立医科大・薬・薬品作用学研究室、[2]パドヴァ大学・生物学部門・ベネト分子医学研究所、[3]金沢大・がん進展制御研究所、[4]日本獣医生命科学大・獣医病理学研究室、[5]大阪大・院薬・神経薬理、[6]国立がん研、[7]金沢大・新学術創成研究機構、[8]金沢大・ナノ生命科学研究所

Despite notable advances in chemotherapy protocols and targeted therapies, ensuing drug resistance limits the efficacy of cancer treatments, calling for the identifying druggable targets that can overcome chemo-resistance. Here we show that the mitochondria-shaping protein OPA1 takes part in the resistance against the tyrosine kinase inhibitor gefitinib in lung adenocarcinoma cells. In gefitinib-resistant lung cancer cells, OPA1 levels were increased, mitochondrial cristae structures were narrower and mitochondrial respiration increased. Genetic and pharmacological OPA1 inhibition in the resistant lung cancer cells sensitized them to gefitinib-induced cytochrome c release and apoptosis. *In vivo*, orthotopic tumors formed by the injection of gefitinib-resistant lung cancer cells were insensitive to gefitinib treatment, but a combination of gefitinib and OPA1 inhibitor reduced tumor size and increased apoptosis. Our data identify the mitochondrial protein OPA1 as a downstream factor that sustains gefitinib resistance and can be targeted to overcome chemo-resistance.

Antitumor Activities by a Defucosylated Mouse–Dog Chimeric Anti-EGFR Antibody in Canine Tumor Xenograft Models

コアフコース欠損イヌ型抗EGFR抗体の抗腫瘍活性の評価

○鈴木 裕之[1]、李 冠傑[1]、浅野 禎三[2]、田中 智大[2]、金子 美華[2]、加藤 幸成[1,2]

[1]東北大・院医・分子薬理学分野、[2]東北大・院医・抗体創薬

The epidermal growth factor receptor (EGFR) contributes to tumor malignancy via gene amplification and protein overexpression. Previously, we developed an anti-human EGFR (hEGFR) monoclonal antibody, EMab -134, which detects hEGFR and dog EGFR (dEGFR) with high sensitivity and specificity by flow cytometry, western blotting and immunohistochemistry. In this study, we produced a defucosylated mouse–dog chimeric anti-EGFR monoclonal antibody, E134Bf. Kinetic analysis of the interactions of E134Bf with the canine osteosarcoma cell line (D-17) and canine fibroblastic cell line (A-72) cells was conducted by flow cytometry. The K_D for the interaction of E134Bf with the D-17 and A-72 cells was 5.5×10^{-10} M and 6.0×10^{-10} M, respectively, indicating that E134Bf exhibits high affinity for D-17 and A-72 cells. Furthermore, E134Bf highly exerted antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity against D-17 and A -72 cells. *In vivo* administration of E134Bf significantly suppressed the development of D-17 and A-72 compared with the control dog IgG in mouse xenografts. These results indicate that E134Bf exerts antitumor effects against dEGFR-expressing canine cancers and could be valuable as part of an antibody treatment regimen for dogs.

Effect of hyaluronic acid nanogels on the drug delivery of cyclosporine administered subcutaneously in rats

ヒアルロン酸ナノゲルを用いたシクロスポリンの徐放化に関する検討

橋本 桂樹[1]、宇佐美 歩樹[1]、森 理人[1]、伊藤 宏輔[1]、薄田 菜々子[1]、曽田 翠[1]、勝又 徹[1]、薮内 昂平[2]、
中井 貴士[2]、○北市 清幸[1]
[1]岐阜薬科大・薬・薬物動態学、[2]旭化成株式会社

Hyaluronic acid (HA) nanogel is derived by the partial modification of hyaluronic acid with cholesterol, causing the formation of self-assembled nanometer-scale hydrogel in water. Thus, HA nanogels might contribute to alter drug delivery of poorly water-soluble drugs. In this study, we tried to investigate the effect of HA nanogels by using cyclosporine (CyA) in rats. HA nanogels (Asahi Kasei Co, 5 mL/kg) containing CyA (2.5-15 mg/kg) were subcutaneously administered in male SD rats (Charles River) and whole blood samples were collected at the designated time-points up to 28 days. The concentrations of CyA were measured using LC-MS/MS 6045 (Shimadzu). In rats administered CyA without HA nanogel (control group), the concentration of CyA was below the detection limit (3 ng/mL) on 7 days after administration. Some formulas of HA nanogel showed the sustained-release properties of CyA. Interestingly, one formula could be detectable up to at least 10 days after administration. HA nanogels would be useful as a tool to alter drug delivery of poorly water-soluble drugs such as CyA.

Establishment of reconstituted depolarization-induced Ca^{2+} release platform for drug discovery of skeletal muscle diseases

骨格筋疾患治療薬開発のための脱分極誘発性Ca^{2+}遊離再構成プラットフォームの構築

○村山 尚[1]、呉林 なごみ[1]、冨田（沼賀） 拓郎[2]、小林 琢也[1]、中田 勉[2]、石田 良典[3]、森 修一[3]、影近 弘之[3]、山田 充彦[2]、櫻井 隆[1]

[1]順天堂大・医・薬理、[2]信州大・医・分子薬理、[3]医科歯科大・生材研

In skeletal muscle, depolarization of the plasma membrane triggers Ca^{2+} release from the sarcoplasmic reticulum (SR),referred to as depolarization-induced Ca^{2+} release (DICR). DICR occurs via the type 1 ryanodine receptor (RyR1), which physically interacts with the dihydropyridine receptor Cav1.1 subunit in specific machinery formed with additional essential components including β 1a, Stac3 adaptor protein and junctophilins. It has recently become clear that mutations in these components cause various skeletal muscle diseases. However, no specific treatment has been developed yet. In this study, we established a high-throughput platform of the reconstituted DICR in HEK293 cells. The essential components were effectively transduced using baculovirus vectors, and Ca^{2+} release was quantitatively measured with R-CEPIA1er, a fluorescent ER Ca^{2+} indicator. High $[K^+]$ depolarization triggered rapid Ca^{2+} release, indicating successful reconstitution of DICR. We tested several known drugs modulating DICR. Whereas RyR1 inhibitors, dantrolene and Cpd1, suppressed DICR, twitch potentiators, e.g., perchlorate, accelerated DICR. These results well reproduced the findings with the muscle fibers and the cultured myotubes. The reconstituted DICR platform will be highly useful for drug discovery for skeletal muscle diseases.

Actomyosin structure in smooth muscle cell treated with arachidonic acid

アラキドン酸作用による平滑筋細胞内アクトミオシン構造の分子形態学的変化

○田中 秀幸[1]、片山 豪[2]

[1]帝京大・医、[2]高崎健康福祉大・人間発達

We have been searching for mechanism to induce smooth muscle contraction that is not associated with phosphorylation of the regulatory light chain (RLC) of smooth muscle myosin. We report that arachidonic acid (AraA) stimulates ATPase activity of unphosphorylated smooth muscle myosin with maximal stimulation (Rmax) of 6.84 + 0.51 relative to stimulation by the vehicle and with a harf-maximal effective concentration (EC50) of 50.3 + 4.2 μM. In presence of actin Rmax was 1.72 + 0.08 and EC50 was 26.3 + 2.3 μM. Our experiments with eicosanoids consisting of the AraA cascade suggested that they neither stimulated nor inhibited the activity. Under conditions that did not allow RLC to be phosphorylated. AraA stimulated contraction of smooth muscle tissue and culture cells with an Rmax of 1.45 + 0.07 and EC50 of 27.0 + 4.4 μM. In addition to the ATPase activities of the myosin, AraA stimulated those of heavy meromyosin, subfragument 1 (S1), S1 from which the RLC was removed, and a recombinant heavy chain consisting of the myosin head. The stimulatory effects of AraA on these preparations were about two fold. The site of AraA action was indicated to be the step-releasing iorganic phosphate (Pi) from the reaction intermediate of the myosin-ADP-Pi complex. The enhancement of Pi relese by AraA was supported by computer stimulation indicating that AraA docked in the actin- binding cleft oh the myosin motor domain. The stimulatory effect of AraA was detectable with both unphosphorylated myosin and the myosin which RLC was fully phosphorylated. The AraA effect on both myosin forms was suggested to cause excess contraction and such as vasospasm.

Regulation of voltage-induced Ca^{2+} release machinery by cell-cell fusion in skeletal myogenesis

骨格筋の細胞融合による電位依存性カルシウム放出機構の制御

○冨田 太一郎、山口 君空、三上 義礼、大島 大輔、赤羽 悟美

東邦大・医・統合生理

One of the most dynamic processes during skeletal myogenesis is cell-cell fusion, which promotes skeletal myoblast to form a large multi-nucleated myofiber equipped with EC-coupling machinery. Recently discovered transmembrane protein Myomixer (Mymx) executes cell-cell fusion, although the physiological significance of Mymx-mediated fusion on myogenic cell differentiation remains largely unknown. The current study focuses on the intracellular Ca^{2+} signaling mechanism to understand the physiological importance of Mymx mediated cell fusion in regulating voltage-induced Ca^{2+}release in the developing skeletal muscle cells. C2C12 cells were used as an in vitro myogenesis model of skeletal muscle cells. We investigated intracellular Ca^{2+} release upon electric field stimulation applied to differentiated Mymx-KO cells and Mymx-rescued cells. We found that the efficiency of Ca^{2+} response was dependent on the Mymx gene expression. Importantly, expression levels of MyoD and myogenin were almost unaltered by the gene rescue of Mymx, suggesting that the Mymx-dependent Ca^{2+} response is regulated independently of these transcription factors. In conclusion, we found a novel regulatory linkage between Mymx expression and the voltage-induced Ca^{2+} release essential for EC coupling.

Systemic administration of lipopolysaccharide derived from *Escherichia coli*, but not *Porphyromonas gingivalis*, inhibits novelty-induced hyperlocomotion in mice

*Porphyromonas gingivalis*とは異なり*Escherichia coli*由来のリポ多糖の全身投与は新環境が誘発したマウスの移所行動の増大を抑制する

○斉藤 幸治[1]、青野 悠里[1]、泉福 英信[2]、三枝 禎[1]

[1]日本大・松戸歯・薬理、[2]日本大・松戸歯・感染免疫

Lipopolysaccharide (LPS), a component of the Gram-negative bacterial cell wall, activates Toll-like receptors (TLRs). *Porphyromonas gingivalis* (Pg) appears to play a role in the development of periodontal disease. *In vitro* studies have suggested that unlike LPS derived from *Escherichia coli* (Ec-LPS), which stimulates TLR4, LPS derived from Pg (Pg-LPS) may inhibit the TLR4. Mice exposed to a novel environment show hyperlocomotion that is inhibited by systemic administration of Ec-LPS. However, whether Pg-LPS influences novelty-induced locomotion is unknown. Therefore, we carried out an open field test to analyze the effects of Pg-LPS. For comparison, effects of Ec-LPS were also studied. Male ddY mouse (25-30 g) were used. The movement of each mouse in the open field was recorded for 30 min using a commercially available behavioural analysis system and the distance travelled (cm) was determined. Each compound was given intraperitoneally 4h before the open field test. Ec-LPS 500 and 840 μg/kg, but not 100 μg/kg, inhibited novelty-induced increases in distance travelled. Inhibition of hyperlocomotion by 840 μg/kg Ec-LPS was counteracted by co-administration of the TLR4 antagonist TAK-242 (3.0 mg/kg). Pg-LPS (100, 500 or 840 μg/kg) failed to alter novelty-induced locomotion. The present results provide *in vivo* evidence that Ec- and Pg-LPS induce different effects. Thus, Ec- but not Pg-LPS inhibits novelty-induced locomotor activity in mice by activating TLR4.

DIF-1 promotes glucose uptake in mouse C2C12 myotube cells

DIF−1はC2C12筋管細胞への糖取込みを促進する

○福永 優子[1]、中西 晃太郎[1]、杉田 光[1]、久保原 禅[2]

[1]千葉科学大・危機管理学部・動物危機管理学科、[2]順天堂大・院スポーツ健康科学研究科・健康生命科学研究室

Differentiation-inducing factor-1 (DIF-1) isolated from the cellular slime mold *Dictyostelium discoideum* has been shown to promote glucose uptake in mouse 3T3-L1 fibroblasts and differentiated 3T3-L1 adipocytes(1). DIF-1 promotes glucose uptake in the cells, at least in part, via an AMP kinase-dependent pathway (a PI-3 kinase/Akt-independent pathway), which is different from the insulin-induced glucose uptake pathway. In this study, we investigated the actions of DIF-1 in skeletal muscle, the largest glucose-metabolizing tissue, using C2C12 myotube cells.

During 15 h of incubation, DIF-1 at 1–20 μM promoted glucose consumption (uptake) in a dose-dependent manner in C2C12 myotube cells, while DIF-1 at 10–20 μM was slightly toxic to the cells. DIF-1 (2 μM)-induced glucose consumption was hardly inhibited with wortmannin (0.1 μM), a PI3K inhibitor, but was partially inhibited with compound C (30 μM), an AMP kinase inhibitor. These results suggest that DIF-1 promotes glucose uptake in skeletal muscle cells via the same mechanisms as those in adipocytes and also that DIF-1 may have therapeutic potential in the treatment of obesity and/or diabetes.

(1) Omata, W. et al. FEBS J. 274, 3392-3404. (2007).

Assessment of learning achievement using self-assessment rubric in the role-play for pharmacological education.

薬理学ロールプレイの学習目標到達度：ルーブリック自己評価を用いた検討

○中村 正帆[1]、木村 武司[2]、長沼 史登[1]、吉川 雄朗[3]、岡村 信行[1]、柳田 俊彦[4]
[1]東北医薬大・医・薬理、[2]京都大学医学部附属病院・総合臨床教育・研修センター、[3]東北大・院医・機能薬理、[4]宮崎大・医看・臨床薬理

Background. The role-play for pharmacological education (RPPE) provides a practical training focused on the basis of drug therapy. Learning achievement in RPPE, however, has not been researched precisely. In this study, we assessed the learning achievement in RPPE by using a self-assessment rubric.
Methods. Participants: Fourth grade medical students at Tohoku Medical and Pharmaceutical University who took the online RPPE (oRPPE) in 2022. Measurements: Self-assessment rubric was presented to participants on the first day of the course. Four following categories were scored on a five-point scale: I) Understanding: a. health problems of the case in charge; b. drug therapy of the case in charge; c. role-play performed by the others, II) Preparation, III) Performance and IV) Discussion and dialogue. Students were directed to assess themselves during preparation and performance of oRPPE. Data collection and analysis: Scores were collected after course completion. All data are presented as the mean ± standard error of the mean (S.E.M.).
Results. The self-assessment showed high scores in the following 3 categories: I) Understanding (a. 4.10 ± 0.06; b. 4.10 ± 0.06; c. 3.94 ± 0.07), II) Preparation (4.32 ± 0.06) and III) Performance (4.21 ± 0.07). These results indicate that RPPE could provide basic practice for drug therapy. It also implies that specification of expected outcomes through rubric beforehand might be useful to guide self- and peer-learning during preparation and performance. On the other hand, the score in IV) Discussion and dialogue (3.75 ± 0.09) might reflect difficulties to discuss the whole process of clinical practice in each case, which requires advanced professional competencies.
Conclusion. The self-assessment rubric is a useful tool for assessing achievement of learning in RPPE.

CaMKII inhibition prevents the Dox-induced mitochondrial dysfunction without the involvement of Drp1 or MCU

○プリヨノ アグン、三明 淳一朗、市原 克則、澤野 達哉、長田 佳子、今村 武史

鳥取大・医・薬理学・薬物療法学

Background: Doxorubicin (Dox), an anticancer drug, is known to induce cardiac toxicity by causing mitochondrial dysfunction. Although CaMKII and its phosphorylation targets, Drp1 to control mitochondrial fission and MCU to control mitochondrial Ca^{2}+ uptake, regulate mitochondrial homeostasis, the involvement of these molecules in the Dox-induced mitochondrial dysfunction remains unclear.

Method: To study the effects of Dox on mitochondrial homeostasis, we evaluated mitochondrial membrane potential (MMP), mitophagy, and mitochondrial Ca^{2}+ content ([Ca^{2}+]m) in H9C2 cells with the following fluorescent dyes, JC-1, Mtphagy, and Rhod2-AM, respectively. To examine the activating effect of Dox on CaMKII, we evaluated the phosphorylation levels of CaMKII by western blotting. To test the involvement of CaMKII, Drp1, and MCU in the Dox-induced mitochondrial dysfunction, the specific inhibitors, KN-93, Mdivi -1, and Ru360, respectively, were used.

Result: Dox treatment dose-dependently reduced MMP and increased the number of cells with mitophagy and [Ca^{2}+]m ($p<0.05$ in all). Dox treatment significantly increased the phosphorylation levels of CaMKII ($p<0.05$). The inhibition of CaMKII suppressed the effects of Dox on the MMP and the mitophagy ($p<0.05$), but not on [Ca2+]m. Contrarily, the inhibition of Drp1 and MCU failed to suppress the decrease in MMP by Dox. Similarly, the inhibition of Drp1 did not reverse the increase in mitophagy by Dox, nor did the inhibition of MCU suppress the elevation of [Ca^{2}+]m by Dox.

Conclusion: These results suggested that activated CaMKII, but not Drp1 and MCU, is involved in the impairment of MMP leading to Dox-induced mitochondrial dysfunction and that the excessive fission by Drp1 and the increased uptake [Ca^{2}+]m by MCU are not the mechanism for the Dox-induced MMP reduction.

Bioimaging analysis of a mouse model of atherosclerosis using radioisotope-labeled oxidized LDL as a probe targeting on foamy macrophages

動脈硬化モデルマウスにおける泡沫化マクロファージを標的とした放射性ヨウ素標識酸化LDLを用いた生体イメージングの解析

○井澤 満[1]、河嶋 秀和[2]、奥野 結衣[1]、中谷 純菜[1]、武田 麻友子[1]、石原 慶一[3]、秋葉 聡[3]、高田 和幸[1]

[1]京都薬大・統合薬科学、[2]京都薬大・放射性同位元素研セ、[3]京都薬大・病態生化学

Atherosclerotic plaques are formed by the accumulation of foamy macrophages, which phagocytose oxidized low-density lipoprotein (LDL) in the intima of blood vessels. Although bioimaging to detect the accumulation of foamy macrophages is thought to be an effective tool for the prevention and treatment of atherosclerosis, it has not yet been established. In this study, we examined the bioimaging of atherosclerotic plaques using human oxidized LDL (oxLDL) labeled with ^{125}I in apolipoprotein E knockout (ApoE-KO) mice, a mouse model of atherosclerosis. Mouse bone marrow-derived macrophages markedly phagocytosed oxLDL but not intact LDL (LDL). The Oil Red O staining revealed that massive amounts of atherosclerosis plaques were formed in the aortic arch and aortic valve in ApoE-KO mice. In the study of single photon emission computed tomography (SPECT), distinct signals were detected in the aorta of ApoE-KO mice treated with ^{125}I-labeled oxLDL but not with ^{125}I-labeled LDL. The local distribution of radioactivity was also detected by autoradiography. We further confirmed the uptake of DiI-labeled oxLDL by macrophages accumulated in atherosclerotic plaques in ApoE-KO mice. A possibility is suggested that bioimaging for the diagnosis of atherosclerosis could be developed with the strategies such as application of radioisotope-labeled oxLDL.

Protective effect of nobiletin against doxorubicin-induced cardiotoxicity in human iPS cell-derived cardiomyocytes.

ヒトiPS細胞由来心筋細胞を用いたドキソルビシンによる心毒性に対するノビレチンによる保護効果の検証

○岩下 賢士郎[1]、藤井 萌功[1]、稲井 誠[2]、坂本 多穂[1]、黒川 洵子[1]

[1]静岡県立大・薬・生体情報分子解析学分野、[2]静岡県立大・薬・医薬品製造化学分野

Human induced pluripotent stem cell-derived cardiomyocytes (hiPS-CMs) are increasingly being used for cardiac safety evaluation, disease modeling, and regenerative medicine. To date, the majority of cardiotoxicity studies have examined the acute drug effects. However, these studies lack information on the chronic effects of cardiotoxic compounds. Here, motion vector prediction (MVP) method was employed to invasively quantify contractile function over 10 days, then to test whether nobiletin has a protective effect against thecardiotoxicity of 8-days exposure to doxorubicin and erlotinib. The MVP method showed that doxorubicin (0.1 - 0.3 μM) significantly reduced contractility compared to erlotinib (0.3 - 3 μM), which has no cardiotoxicity, when administered for more than 5 days, and that the simultaneous addition of 0.03 μM nobiletin significantly reduced this cardiac depression by doxorubicin (0.1 - 0.3 μM). These results suggest that nobiletin is protective against the cardiotoxic effect of doxorubicin, and are promising for future drug discovery applications.

Neurofunctional phospholipids for inhibition of α-synuclein aggregation: A novel therapeutic target for α-synucleinopathies

α−シヌクレインの凝集体形成を抑制する生理活性脂質の探索

○唐木 達哉[1]、羽二生 久夫[2]、松田 佳和[3]、塚原 完[1]

[1]長崎大・院医歯薬・創薬薬理学分野、[2]信州大・医・バイオメディカル研究所、[3]日本薬科大・薬・臨床薬理

Accumulation and aggregation of α-synuclein (α-Syn) are hallmarks of α-synucleinopathies such as dementia with Lewy bodies. Therefore, aggregation of α-Syn is considered a priority target for drug development, and aggregation inhibitors are expected to reduce α-Syn toxicity and serve as therapeutic agents. Here, we report that certain lysophospholipids (LPLs) species behave as inhibitors for α-Syn aggregation. The LPLs are small bioactive lipid molecules characterized by having a single carbon chain and a polar head group. The LPLs we used were extracted from Porcine Liver Decomposition Product (PLDP) which was previously reported to enhance cognitive function in healthy older adults. We found that the LPLs extracted from PLDP (PEL) reduced α-Syn aggregation in cellular model. Especially, four species of LPLs contained in PEL strongly inhibit α-Syn aggregation. Furthermore, we revealed that PEL increased normal cell viability in SK-N-SH cells. Finally we approached the mechanism of the LPLs' inhibitory effect for α-Syn aggregation using *in vitro* assay and evaluated influences to various cellular functions known to be disordered in lesion. Taken all together, these studies indicate that the LPLs would be beneficial as a possible therapeutic target in the treatment of α-synucleinopathies.

Modulation of astrocyte activation by sphingomyelin

スフィンゴミエリンによるアストロサイト活性化調節

○門脇 凌、本田 拓哉、中村 浩之
千葉大・院薬・薬効薬理学

Astrocytes constitute about 20-40% of glial cells, making up the central nervous system (CNS). In the CNS, astrocytes supply neurons with nutritional factors and maintain homeostasis of the extracellular environment through the uptake and efflux of neurotransmitters. In neurodegenerative diseases, astrocytes change their morphology to the activated state and release inflammatory cytokines, which induce CNS inflammation and aggravate neurodegenerative diseases. Sphingolipids, one of the lipids, have been reported as a molecule associated with astrocyte activation, but their involvement remains unclear. In this study, we evaluated astrocyte activity by changing the levels of sphingomyelin (SM), one of the most abundant sphingolipids, using human astrocyte/conditionally immortalized clone 35 (HASTR/ci35). Reduction of SM levels by knockdown of sphingomyelin synthase 1 and/or 2 in HASTR/ci35 attenuated HASTR/ci35 activation by treatment of inflammatory cytokines. Furthermore, selectively reducing SM levels by knockdown of ceramide transport protein also attenuated the activation of HASTR/ci35. On the other hand, increasing the levels of SM by inhibition of neutral sphingomyelinase or addition of SM exogenously promoted activation of HASTR/ci35 by treatment of inflammatory cytokines. These results suggest that SM positively regulates astrocyte activation. Thus, regulating SM levels may provide a therapeutic target for astrocyte-induced CNS inflammation and a new approach to treating neurodegenerative diseases.

Neuroprotection and detection of Aβ by a low molecular weight compound derived from natural product

生薬由来低分子化合物のAβ神経毒性に対する保護作用とAβイメージングに向けた解析

○福田 愛菜[1]、中嶋 聡一[2,6]、尾田 好美[2,6]、西村 周泰[1]、花木 葵[1]、河嶋 秀和[3]、木村 寛之[4]、中村 誠宏[2]、松本 崇宏[5]、高田 和幸[1]

[1]京都薬科大・薬・統合薬科学、[2]京都薬科大・薬・生薬学、[3]京都薬科大・薬・放射性同位元素研セ、[4]京都薬科大・薬・代謝分析学、[5]京都薬科大・薬・公衆衛生学、[6]NPR医薬資源研

In Alzheimer's disease (AD), acetylcholinergic (ACh) neurons are impaired at early pathological stage of AD, and amyloid β (Aβ) oligomers are thought to be a crucial molecule for triggering neurodegenerative processes in AD. In the present study, we first established an *in vitro* Aβ oligomer-induced neuronal cell death model using human induced pluripotent stem cell (iPSC)-derived ACh neurons and *O*-acyl isopeptide of Aβ, which reverts to natural form of Aβ under the neutral pH conditions. In the processes of neurodegeneration in this model, Aβ was tightly attached dendrites and mitochondrial dysfunction was induced. We next identified an Aβ-binding low molecular weight compound, plantainoside B, from the herbal extract of *Bacopa monniera*, which is used for memory enhancement in Ayurvedic medicine. Plantainoside B attenuated mitochondrial dysfunction and exert neuroprotection against Aβ neurotoxicity. Moreover, the ^{125}I-labeled plantainoside B showed a high affinity to brain sections obtained from a model mouse of Aβ plaque formation and Aβ oligomers in gel-loading experiments. Results indicate a possibility for the development of neurotheranostics approach for the strategy of AD treatment.

Impaired dendritic development is a common phenotype observed in primary cultured Purkinje cells expressing various SCA-causing proteins.

樹状突起の発達低下は様々なSCA原因タンパク質を発現させた初代培養小脳プルキンエ細胞で観察される共通の表現型である。

○植田 恵梨香[1]、今野 歩[2]、平井 宏和[2]、倉内 祐樹[1]、香月 博志[1]、関 貴弘[1,3]

[1]熊本大・院生命・薬物活性、[2]群馬大・院医・脳神経再生、[3]姫路獨協大・薬・薬理

Spinocerebellar ataxia (SCA) is a group of autosomal-dominantly inherited ataxia and classified into SCA1-49 by the difference of causal genes, whose mutations include polyglutamine (polyQ)-expanded and missense mutations. Purkinje cells (PCs) are neurons with highly developed dendrites and important for cerebellar functions. We have previously revealed that impaired dendritic development is observed in primary cultured PCs expressing missense mutant SCA14- and SCA21-causing proteins. We assume that various SCA-causing proteins commonly impairs dendritic development in cultured PCs. In the present study, we expressed polyQ-expanded (SCA1, 3 and 6) and missense (SCA34, 38, 41) mutant SCA-causing proteins in primary cultured PCs and evaluated their dendritic development. Cerebellar primary cultures were prepared from E16 embryos of Wistar rats and cultured for 3 weeks. SCA-causing proteins were expressed using adeno-associated viral vector. Compared with wild-type proteins, all 6 SCA-causing proteins, including polyQ-expanded and missense mutants, impaired dendritic development of primary cultured PCs. These findings indicates that impaired dendritic development of cultured PCs is induced by various SCA-causing proteins and would be a common in vitro phenotype of SCA and available for the exploration of novel SCA therapeutics.

Involvement of PRMT5 in the activation of hepatic stellate cells

肝星細胞活性化へのPRMT5の関与

○山下 日菜子[1]、山口 桃生[1]、刀坂 泰史[2]、大岡 央[1]、森本 達也[2]、石川 智久[1]

[1]静岡県立大・薬・薬理学、[2]静岡県立大・薬・分子病態

Liver fibrosis is a significant consequence of chronic liver diseases, where excess deposition of extracellular matrix is caused by the activation of hepatic stellate cells (HSCs). The suppression of HSC activation is therefore regarded as a therapeutic target of liver fibrosis. The present study investigated the involvement of protein arginine methyltransferase 5 (PRMT5), which mediates genome organization and cell cycle regulation, in HSC activation. LX-2 cells, a human HSC cell line, were treated with TGF- β 1 for 48 h in the presence of PRMT5 inhibitors (EPZ015666 and JNJ64619178). The expression of α-smooth muscle actin (α-SMA) and type I collagen α1 (COL1A1), activated HSC markers, were markedly increased by the TGF-β 1 treatment. PRMT5 inhibitors suppressed the increased expression of α-SMA and COL1A1 in a concentration-dependent manner. Knockdown of PRMT5 also suppressed the TGF-β 1-induced COL1A1 expression in LX-2 cells. RNA-sequencing analysis showed that GO terms related to ECM production and SMAD signaling were enriched with RNA of LX-2 cells treated with the PRMT5 inhibitor JNJ64619178. These results suggest that PRMT5 promotes HSC activation, possibly depending on the SMAD signaling pathway, and therefore might be a target for the prevention and treatment of liver fibrosis.

Establishment of assessment system for anti-fibrotic activity using an ex vivo hepatic fibrosis model

肝線維化ex vivoモデルを用いた抗線維化活性評価系の確立

○髙橋 亮汰、山口 桃生、森野 純鈴、岡部 磨幸、大岡 央、石川 智久

静岡県立大・薬・薬理

Currently, there is no effective treatment for liver fibrosis. *In vitro* studies on the activation of hepatic stellate cells (HSCs), which is responsible for liver fibrosis, have been used as a drug screening system for it. However, even if some drug shows an inhibitory effect on HSC activation, its anti-fibrotic effect is required to be confirmed in liver fibrosis animal models, which further requires a lot of time and cost. In the present study, we tried to establish an *ex vivo* model of liver fibrosis using precision-cut liver slices (PCLSs) to solve such problems. PCLSs of 250 μm thickness were prepared from male C57BL/6J mice using a vibratome and cultured in RPMI medium in a 5% CO_2 incubator. Although cellular ATP content was decreased on day 1 compared to day 0, it was then maintained until day 5, suggesting that the ex vivo model is viable for at least 5 days. Treatment with Et-OH (50, 100 mM), one of the liver injury stimuli, for 5 days increased mRNA expression of Acta2 and Col1a1, liver fibrosis markers, in PCLSs. DIF-1 (50, 100 μM), which has an anti-fibrotic effect, significantly suppressed the Et-OH-induced increases in the markers. These results suggest that the *ex vivo* model using PCLSs is useful as a drug screening system for the development of drugs for treatment of liver fibrosis.

The development of biologics inhibiting IL-33 signaling on the basis of the signal transduction mechanisms

シグナル伝達機序に基づくIL−33/ST2シグナル抑制分子の作製

○山本 里彩[1]、安藤 智暁[1]、貝谷 綾子[1]、伊沢 久未[1]、前原 明絵[1]、北浦 次郎[1]

[1]順天堂大・院医・アトピー疾患研究センター、[2]順天堂大・医・5年生

IL-33 contributes to the pathogenesis of allergic diseases. Upon the binding of IL-33 to a membrane receptor ST2, IL-1 receptor accessory protein (IL-1RAcP) is recruited to form a heterodimeric receptor complex which transmits a signal. On the other hand, a soluble form of ST2 acts as an endogenous inhibitor of IL-33 signaling. Here, we aimed to develop the biologics inhibiting IL-33 signaling. First, we generated IL-33 reporter cell lines, in which IL-33 stimulation induced NFkB-driven expression of DsRed. We then generated IL-33trap-Fc which is composed of extracellular domains of IL-1RAcP and ST2 fused to the Fc portion of human IgG. Notably, IL-33trap-Fc more strongly suppressed IL-33-stimulated DsRed expression in the reporter cells than ST2-Fc which is composed of an extracellular domain of ST2 fused to human IgG Fc. Consistently, IL-33trap-Fc remarkably inhibited IL-33-stimulated IL-6 production of bone marrow-derived mast cells as compared with ST2-Fc. Moreover, intraperitoneal administration of IL-33trap-Fc efficiently inhibited IL-33-stimulated eosinophil accumulation in vivo. Taken together, these results indicated that IL-33trap-Fc was effective in inhibiting IL-33 signaling. We also attempt to generate the biologics that bind to ST2 but fail to recruit IL -1RAcP, thereby blocking IL-33 signaling.

L-Carnitine supplementation attenuates lenvatinib-induced muscle impairment without diminishing its anti-angiogenesis efficacy

L-カルニチンの補充はレンバチニブによる筋障害を血管新生抑制効果の減弱なしに軽減する

○靖 崢[1]、射場 智大[2]、内藤 尚道[2]、許 平平[1]、盛重 純一[1]、長田 直人[1]、大久保 裕直[3]、安藤 仁[1]
[1]金沢大・院医薬保健・細胞分子機能学、[2]金沢大・院医薬保健・血管分子生理学、[3]順天堂大学医学部附属練馬病院・消化器内科

[Aim] Lenvatinib (LEN), an oral tyrosine kinase inhibitor, is widely used to treat several types of advanced cancers but often causes muscular adverse reactions. Our previous study revealed that LEN reduces L-carnitine (L-CAR) content, expression of carnitine-related genes, and mitochondrial function in the skeletal muscle. Therefore, the present study aimed to investigate whether L-CAR supplementation prevents LEN-induced muscle impairment without affecting its anti-angiogenesis effect.
[Methods] Eight-week-old male Wistar rats were divided into four groups and administrated orally once daily for 2 weeks with vehicle, LEN (2 mg/kg/day), LEN + L-CAR (150 mg/kg/day), or LEN + L-CAR (300 mg/kg). In the in vitro studies, differentiated C2C12 myocytes, HUVECs, and mouse aorta were treated with LEN (0.1 and/or 1 μM) and L-CAR (1.6, 6.4, and 25.6 mM).
[Results] L-CAR supplementation significantly attenuated LEN-induced deleterious effects on L-CAR content, expression of carnitine-related (OCTN2, CPT1, CACT, and CPT2) and OXPHOS genes in the skeletal muscle of rats. In addition, L-CAR prevented LEN-induced reductions in mitochondrial function (ATP content and membrane potential) in C2C12 myocytes. Furthermore, L-CAR did not affect the anti-angiogenesis action of LEN assessed by the tube formation and ring assays.
[Conclusion] These results suggest that L-CAR supplementation can alleviate the adverse reactions of LEN in the skeletal muscle without reducing its antineoplastic effect.

Development of fast-dissociating recombinant antibody probes for multiplexed super-resolution molecular mapping

多重高密度超解像による分子マッピングのための迅速解離リコンビナント抗体プローブの開発

○張 千里[1]、宮本 章歳[1]、有森 貴夫[2]、高木 淳一[2]、渡邊 直樹[1,3]

[1]京都大・院生命科学・分子動態生理学、[2]大阪大・蛋白質研究所・分子創製学研究室、[3]京都大・院医・神経薬理学

We report a mutagenesis strategy that can effectively increase the dissociation rate of antibodies by orders of magnitude without compromising the binding specificity. Single-molecule localization microscopy greatly surpasses the diffraction limit of conventional optical microscopy. The imaging fidelity and labelling density, however, are limited by spatial interference between bulky antibodies in a confined resolved area. IRIS has overcome the problem using exchangeable probes that transiently bind to endogenous targets. In our previous research, generation of fast-dissociating IRIS probes has been challenging. In the present study, we have developed a new mutagenesis strategy that make it feasible to generate IRIS probes from the repository of off-the-shelf antibodies. We successfully generated dozens of IRIS probes and demonstrate multiplexed localization of endogenous proteins in primary neurons that visualizes small synaptic connections. Our fast-dissociating probes achieved 4-fold higher label density than conventional super-resolution approaches. Thus, IRIS could visualize the feature of synaptic components with higher fidelity. In addition, the mutagenesis strategy will provide more applications for high affinity antibodies developed in pharmaceutical research, such as super-resolution imaging based disease diagnosis and biomarker identification.

Effects of 2-carba- cyclic phosphatidic acid derivatives on IL-1β-stimulated human chondrocytes

ヒト軟骨細胞における2−カルバ環状ホスファチジン酸の抗炎症作用について

○吉井 雅起[1]、諸星 俊郎[2]、塚原 完[1]

[1]長崎大・院医歯薬・創薬薬理学分野、[2]SANSHO(株)

Osteoarthritis (OA) is a common joint disease characterized by the breakdown of subchondral bone and cartilage damage, most often affecting middle-aged and elderly people. Although the etiology of OA is still unknown, some reports suggest that inflammatory factors such as interleukin (IL)-1β mediate the progression of OA. In order to investigate the effect of IL-1β and the possibility of treatment for OA, we used 2-carba-cyclic phosphatidic acid (2ccPA) and its derivatives on human chondrocytes. 2ccPA is a synthesized phospholipid based from a bioactive phospholipid mediator: cyclic phosphatidic acid (cPA). It is previously reported that 2ccPA exhibits anti-inflammatory and chondroprotective effects on an OA animal model. 2ccPA and its ring-opened body (ROB) derivative significantly suppressed IL-1β -induced upregulation of IL-6, matrix metalloproteinase-13, and cyclooxygenase-2, as well as the degradation of type II collagen and aggrecan. However, the other two derivatives, the deacylated body and the ring-opened deacylated body showed little effect on IL-1β -exposed human chondrosarcoma cell-line. These data suggest that acyl chain of 2ccPA and ROB is essential for anti-inflammatory effect on OA. Taken together, this study provides evidence that 2ccPA and ROB would be a novel therapeutic agent for OA.

Can Cannabidiol affect the peripheral circadian clock in PER2::LUC mice?

カンナビジオールが末梢時計に与える影響

○WANG HSIAO HSIEH[3]、野崎 千尋[1]、柴田 重信[3]

[1]早稲田大・国際理工学センター・Major in Bioscience、[2]University of Bonn・Institute of Molecular Psychiatry、[3]早稲田大・理工・電気. 情報生命工学科

Cannabidiol (CBD) is the second major cannabinoid which is often said to improve anxiety and sleep with no psychoactive effect. It is said that CBD can promote the production of wake-related neurotransmitters such as dopamine. Further, co-injection of CBD and THC increased sleeping time in rats. However, no study has been done for the in-vivo effect of CBD to circadian clock. Present study aimed to elucidate whether CBD can modify peripheral circadian rhythm.

PER2::LUC knock-in female mice were used to determine the effect of CBD to the peripheral clock. Mice were divided into six groups: CBD isolate (99% crystalline) in MCT oil, CBD isolate in 5% EtOH/5% cremophor/water, and water-soluble CBD nanopowder in water, with respective vehicle controls. Each drug was orally administered at ZT4 or ZT16 for three days, then PER2 gene expression in the liver, kidney, and submandibular gland is observed by in-vivo imaging.

We found that phase advance in the liver and the submandibular gland only happens when MCT or CBD in MCT (CBD/MCT) was administered at ZT4. However, the phase advance did not differ between the MCT and CBD/MCT groups. Furthermore, neither the vehicle nor the CBD affected the peripheral clock when administered at ZT16.

This study suggested that not CBD but rather MCT oil affect the circadian clock in mice. As MCT oil is commonly used as a base for CBD products, we propose that MCT might be the possible factor that affects the circadian clock and cause rhythm improvement. Therefore further detailed studies on the effect of CBD products will be needed.

Derivatized-imaging mass spectrometer revealed the effect of theanine for monoamine metabolism

テアニン摂取による脳内カテコールアミンの変動とうつ病予防効果の検証

○武藤 誠[1]、鹿野 仁美[1]、安部 綾[2]、小関 誠[2]、平 修[1]

[1]福島大学・農学群・食農学類、[2]太陽化学株式会社・ニュートリション事業部

L-Theanine (LTE) is a derivative of glutamic acid, which is abundant in tea leaves and contributes to the umami and sweetness of tea. In recent years, its effects on the brain, such as relaxation, have been attracting attention. In this study, we used imaging mass spectrometry (IMS) to visually analyze the changes in the catecholamine system in the brain after the administration of LTE. In IMS, we applied derivatization reagent to improve detection limit. Simultaneous imaging of catecholamines, LTE and g-aminobutyric acid (GABA) is particularly useful to understand a metabolic pathway. We investigated whether symptom of depression is improved or not by free drinking of LTE water. The mice that are freely drinking of theanine (Group 1), symptoms of depression was milder than that of drinking of water (Group 2). IMS showed dopamine (DA) marginally produced from caudate putamen from Group 2, but DA was produced from the Group 1. Interestingly, GABA increased at hypothalamus nucleus paraventricularis (PVN), which controls eating amount, from Group 1 compared with Group 2. We hypothesized an increasing of GABA at PVN works an appetite stimulation. Practically, an appetite of mouse in Group 1 was not decreased compared with that in Group 2. IMS visually gives us the pathway of catechol amine and improvement of depression by theanine.

The Hippo pathway kinase Lats1/2 inhibition increases slow- and fast-twitch fibers via activation of Tead cofactors.

Hippo経路キナーゼLats1/2阻害はTead調節分子を活性化して遅筋と速筋を増加させる

○堀 直人、鈴木 孝行、荒木 拓郎、石川 千夏、南須原 杏律、高野 博之、山口 憲孝

千葉大・院薬・分子心血管薬理学研究室

【Background】

Skeletal muscles are composed of slow-twitch fiber and fast-twitch fiber. Transcriptional factor Tead induces muscle differentiation with its cofactor Taz. However, whether Tead controls slow- and fast-twitch fiber is unknown. Thus, we focused on Vgll3, another cofactor of Tead, and investigated its role in muscle differentiation.

【Result】

We established Vgll3 expressing C2C12 cells and differentiated these cells. Vgll3 expressing cells showed upregulation of muscle differentiation markers and an increase in slow-twitch fiber marker Myh7 with a decrease in fast-twitch fiber marker Myh4. These results suggest that Vgll3 increases slow-twitch fiber. Recent studies reported Taz activation induces Vgll3 expression. Thus, we used the Lats1/2 inhibitor, which induces Taz activation. Lats1/2 inhibitor induces Vgll3 expression and upregulation of Myh7 and Myh4. This result suggests that Lats1/2 inhibition induces Taz activation to increase fast-twitch fiber and Vgll3 expression to increase slow-twitch fiber simultaneously.

【Conclusion】

Our results suggested that Vgll3 increases slow-twitch fiber, and Lats1/2 inhibition increase slow- and fast-twitch fiber via activation of Vgll3 and Taz. Aging significantly decreases fast-twitch fiber and causes older adults injury. Thus, Tead and its cofactors are important for aging treatment.

Identification of novel effects of Eliglustat and investigation its affects against idiopathic pulmonary fibrosis (IPF)

○久留宮 慧臣、本田 拓也、中村 浩之

千葉大・院薬・薬効薬理学

【Background, Purpose】

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, irreversible, and ultimately fatal lung disease. IPF occurs due to TGF-β1/Smad signaling activation.

Previously, we observed that eliglustat, glucosylceramide synthase inhibitor exhibited anti-fibrotic effects against nomal human lung fibroblast. So, we checked whether eliglustat exhibits anti-fibrotic effects also against IPF patient-derived cells (IPF cells), and tried to elucidate its mechanism.

【Results, Discussion】

First, we tested the anti-fibrotic effects of eliglustat in IPF cells. Treatment of IPF cells with eliglustat similarly suppressed the up-regulation of fibrotic proteins such as α-SMA and collagen by TGF-β1. Eliglustat had no effects on phosphorylation and translocation to the nucleus of Smad. The knockdown of glucosylceramide synthase did not inhibit the up-regulation of α-SMA by TGF-β1. These results suggest that eliglustat inhibits fibrotic protein transcription by Smad independently of its inhibitory effect against glucosylceramide synthase.

Next, we focused on sterol regulatory element-binding protein2 (SREBP2) to elucidate the anti-fibrotic mechanism of eliglustat. SREBP2 regulates intracellular cholesterol levels and is known to inhibit the transcript activity of Smad. Treatment of IPF cells with eliglustat induced translocation of SREBP2 to the nucleus and up-regulation of downstream genes of SREBP2. Inhibition of SREBP2 attenuated the eliglustat-induced down-regulation of α-SMA expression. These results suggest that eliglustat exhibits anti-fibrotic effects through activation of SREBP2.

Regulation of neuron-astrocyte communication via ATP/P2Y1 signaling by microglia

ミクログリアによるATP/P2Y1シグナルを介したニューロン–アストロサイト間情報伝達の制御

○鈴木 秀明[1,2]、繁冨 英治[1,2]、平山 幸歩[1]、高橋 由香里[3]、池中 一裕[4]、田中 謙二[5]、加藤 総夫[3]、尾藤 晴彦[6]、小泉 修一[1,2]

[1]山梨大・院医・薬理、[2]山梨大・院医・山梨GLIAセンター、[3]慈恵医大・神経科学、[4]生理研・分子神経生理、[5]慶應大・医・先端研・脳科学、[6]東京大・院医・神経生化学

P2Y1 receptor is upregulated in astrocytes in many neurological diseases. We have previously shown that elevated P2Y1 receptor expression in astrocytes causes neuronal hyperexcitability by enhancing neuron-astrocyte communication in the hippocampal CA1 region. However, contribution of microglia to the astrocytic P2Y1 receptor-mediated neuron-astrocyte communication is not known, despite the fact that microglia are also activated in such pathological conditions. To this end, we attempted to investigate the role of microglia in astrocyte P2Y1 receptor signaling by depleting microglia with a CSF1 receptor antagonist, PLX5622. The results are summarized in the following two points: 1) Microglia depletion increased *P2ry1* gene expression in astrocytes and enhanced Ca^{2+} signal via P2Y1 receptor, indicating that microglia would have a role to inhibit P2Y1 receptor expression in astrocytes. 2) Microglia depletion prolonged the time required for degradation of exogenously applied ATP. Because microglia highly express an ATP degrading enzyme CD39, they would play a central role in shutting-off of P2Y1 receptor signals by metabolizing ATP. Taken together, it is suggested that microglia would also play an important role in neuron-astrocyte communication via 2 different modes, i.e., inhibition of P2Y1 receptor expression and degradation of extracellular ATP.

Mechanistic analysis for suppression of neuropathic pain in circadian clock gene deficient mice

時計遺伝子の機能不全マウスにおける神経障害性疼痛の発症抑制メカニズムの解析

○山川 稚葉[1]、安河内 冴[1]、鶴留 優也[4]、牛島 健太郎[4]、鶴田 朗人[1,2]、松永 直也[3]、小柳 悟[1,2]、大戸 茂弘[1]
[1]九州大・院薬・薬剤学、[2]九州大・院医薬・グローカルヘルスケア、[3]九州大・院薬・薬物動態学、[4]山口東京理科大・薬・薬剤学，製剤学

Because the expression of up to 10% of genes is under the control of the circadian machinery consisting of clock genes, it should not come as a surprise that the disfunction of clock gene affects the onset and/or state of various disease. Diurnal variations in pain hypersensitivity are common in chronic pain disorders, but pathological relevance of clock genes in neuropathic pain hypersensitivity remains unknown. In this study, we investigated the threshold of mechanical pain hypersensitivity in peripheral sciatic nerve-ligated (PSL) animals and found that clock gene deficient mice (*Per2*$^{m/m}$ mice) failed to develop the neuropathic pain hypersensitivity. As observed in wild-type mice, PSL- *Per2*$^{m/m}$ mice also activation of glial cells in the dorsal horn of the spinal cord, as well as increased expression of pain-related molecules. On the other hand, the descending pain suppressor system and endocannabinoid system were upregulated in *Per2*$^{m/m}$ mice, suggesting that the suppression mechanism against neuropathic pain is enhanced by dysfunction of clock gene. Therefore, *Per2*$^{m/m}$ mice are less likely to develop pain hypersensitivity even when peripheral nerves are injured. These findings indicate that endogenous pain suppression system are under the control of circadian clock. Identification of circadian clock controlled pain suppressor molecule would be a therapeutic target for treatment of neuropathic pain.

A subset of spinal dorsal horn inhibitory interneurons crucial for analgesic effect associated with spinal noradrenaline

脊髄後角ノルアドレナリンに関連した鎮痛効果に重要な脊髄後角抑制性神経サブセット

○末藤 大智[1]、石橋 忠幸[1,2]、吉川 優[1]、山浦 健[2]、津田 誠[1]

[1]九州大・院薬・薬理学分野、[2]九州大・院医・外科学講座　麻酔・蘇生学

Pain information transmission/processing in the spinal dorsal horn (SDH) is strongly controlled by descending neurons from the brain. One of the major neurotransmitters of descending pathways is noradrenaline (NA). Descending NAergic neurons from the locus coeruleus (LC) is known to produce analgesic effects via activation of inhibitory interneurons in the SDH. However, the identity of the inhibitory interneuron subset in the SDH is poorly understood. Recently, we have found a subset of the SDH inhibitory interneurons captured by adeno-associated viral (AAV) vectors incorporating a neuropeptide Y promoter (AAV-NpyP^{+}) that is crucial for neuropathic allodynia. Here, we showed that this neuronal subset is a major target of spinal NA to inhibit pain information transmission/processing. Whole-cell patch-clamp recordings using spinal cord slices revealed that NA predominantly depolarizes AAV-NpyP^{+} neurons. This effect was suppressed by a pharmacological blockade and genetic knockdown of α_{1B}-adrenoceptor (AR) in AAV-NpyP^{+} neurons in the SDH. Furthermore, we found that the analgesic effect of duloxetine on neuropathic pain which is associated with an increase in the spinal NA level by inhibiting NA reuptake into presynaptic terminals is reduced by AAV-NpyP^{+} neuron-selective knockdown of α_{1B}-ARs. These results indicate that α_{1B}-ARs expressed in AAV-NpyP^{+} neurons would be a target of spinal NA presumably from descending LC neurons and contribute to the analgesic effect of duloxetine. Thus, spinal α_{1B}-ARs could be a new therapeutic target.

Paclitaxel-induced peripheral neuropathic pain formation contributes to synaptic plasticity in the spinal dorsal horn.

Paclitaxel誘発末梢性神経障害性疼痛の形成には脊髄後角におけるシナプス可塑的な変化が寄与している

○山田 果琳、澤幡 雅人、久米 利明、歌 大介

富山大・院医薬・応用薬理学研究室

Purpose: Paclitaxel (PTX) is a typical anticancer drug that induces peripheral neuropathy and significantly reduces patients' quality of life. Previous our study showed gabapentinoids, such as mirogabalin, attenuate PTX-induced peripheral neuropathic pain by acting on the spinal dorsal horn. PTX-induced peripheral neuropathy has so far focused on peripheral inflammation, but it is possible that changes in the spinal dorsal horn may contribute to PTX-induced peripheral neuropathy. In this study, we investigated the mechanism of synaptic plasticity in the spinal dorsal horn by using electrophysiological and immunohistochemical analysis.

Methods: We administered a single intraperitoneal dose of PTX 5 mg/kg to C57BL/6NCr mice. We analyzed the frequency of spontaneous and von Frey filament (vFF; 0.69 mN) evoked firing in spinal dorsal horn neurons by using *in vivo* extracellular recording. Immunohistochemical staining was performed on spinal cord (L4-6) slices.

Results: Electrophsiological data showed the frequency of spontaneous and vFF evoked firing in spinal dorsal horn neurons were significantly enhanced in PTX model mice.

The levels of the neuronal activation marker c-fos were increased with mechanical allodynia formation in PTX model mice. Glia-associated makers Iba1 and GFAP also showed chronological changes after PTX-treatment.

Conclusion: The present study suggests that synaptic plastic changes occur not only in the periphery but also in the spinal dorsal horn in the PTX-induced peripheral neuropathic pain model.

The effect of REV-ERB agonist on nociceptive hypersensitivity in monoiodoacetate-induced osteoarthritis model

MIA誘発変形性膝関節症モデルにおけるREV-ERB agonistの鎮痛効果の検討

○橋爪 宥樹、元成 初寧、中村 庸輝、中島 一恵、森岡 徳光

広島大・院医系（薬）・薬効解析

Osteoarthritis (OA) is characterized by pain caused by inflammation and degradation of cartilage matrix in joint. Although the number of patients is expected to increase due to the aging of the population, the use of anti-inflammatory drugs, which is the main therapeutics, may not be effective in pathology of OA. REV-ERBs are one of nuclear receptors involved in a wide range of physiological functions. We have previously shown that REV-ERBs were expressed in primary cultured chondrocytes, and REV-ERB agonist suppresses the upregulation of proinflammatory cytokines and matrix degradation enzymes in these cells under inflammatory conditions. However, the role of REV-ERBs in pathogenesis of OA is not clear. Thus, we investigated the effect of REV-ERB agonist on nociceptive hypersensitivity in monoiodoacetate (MIA)-induced OA model. SR9009, a REV-ERB agonist, was administered intraarticularly twice a week, starting 3 days after MIA administration. Mechanical thresholds were measured by the von Frey test. MIA induced mechanical hypersensitivity from day 3 after administration, which persisted at least until day 28. Administration of SR9009 significantly ameliorated mechanical hypersensitivity from day 14 after MIA administration. These results suggest that activation of REV-ERB might induce an analgesic effect on OA pain.

A prototype of the microsensing system for i*n vivo* drug monitoring in the skin with diamond electrode

マイクロダイヤモンド電極を用いた皮膚内薬物動態のin vivo計測システムの開発

○Ahmad Norzahirah Binti[1]、澤村 晴志朗[1]、緒方 元気[2]、栄長 泰明[2]、日比野 浩[1]

[1]大阪大・院医・統合薬理学、[2]慶應義塾大・理工・化学

Monitoring of plasma drug concentrations is required for effective pharmacotherapy. Repetitive collection of whole blood followed by analysis of plasma samples with conventional methods delays representation of crucial results. Skin is an easily accessible organ; a portion of systemically circulating drug molecules is diffused to the dermal interstitial fluid. Thus, the compound's pharmacokinetics (PK) in the fluid mirrors the plasma PK. To approach such local dermal space, here we describe a microsensing system with a needle-type boron-doped diamond (BDD) electrode, which detects chemical compounds by redox reaction. As a test analyte we chose an anticancer drug, doxorubicin. In an in vitro experiment with a BDD microsensor, doxorubicin elicited a current in response to applied negative potential. Calibration curve covered the therapeutic window (10－100 nM). The sensor's performance was also tested in the collected interstitial fluids. Finally, the sensor was inserted into the dermis layer in anesthetized live rats; after doxorubicin was intravenously injected, the local PK was tracked for >1 hour with the C_{max} and T_{max} 3.1 ± 1.4 nM and 33.6 ± 20.6 mins, respectively (n = 7). By combining a formula linking the local measurements to plasma data, this microsensing system may be applicable to real-time monitoring of systemic PK.

Three-dimensional structural analysis of pharmacokinetics-related membrane protein P-glycoprotein using cryo-electron microscopy

クライオ電子顕微鏡を用いた薬物動態関連膜タンパク質P糖タンパク質の三次元立体構造解析

○濱口 紀江[1,2]、安達 成彦[3]、守屋 俊夫[3]、川崎 政人[3]、安田 賢司[2]、千田 俊哉[3]、小笠原 諭[2]、村田 武士[2]

[1]千葉大・院医薬・薬理学、[2]千葉大・理・構造生物学、[3]高エネ機構・物構研・構造生物

P-glycoprotein (P-gp) is mainly found in the cell membrane of the small intestine and blood-brain barrier in vivo, and is responsible for the extracellular transport of cytotoxic hydrophobic compounds. P-gp is known to transport many pharmaceutical compounds as substrates. If we can understand the substrate recognition mechanism of P-gp, it will be possible to design pharmaceutical compounds that are not recognized by P-gp. Recently, the complex structures of human P-gp with substrates and inhibitors have been reported by single-particle analysis using Cryo-EM, and the differences in the binding pockets of substrates and inhibitors have been clarified. However, a detailed understanding of how P-gp can identify compounds as substrates or inhibitors has not been achieved. In this study, we aim to elucidate the detailed substrate recognition mechanism by elucidating and comparing multiple complex structures of P-gp and compounds. First, we established a system for expression and purification of human P-gp. Further, we have established a simple system for reconstitution into Nanodisc. Recently, we succeeded to obtain the 3D structure at the highest resolution (2.93 Å) as human P-gp. In this presentation, we will introduce the expression, purification, and Nanodisc reconstruction systems of P-gp and the obtained 3D structures.

Caveolin-1 regulates ATP signaling mediated by P2X7 receptor in pro-inflammatory macrophages.

炎症性マクロファージにおいて、カベオリン1はP2X7受容体を介するATPシグナルを調節する。

○澤井 優輝、鈴木 良明、近藤 るびい、今泉 祐治、山村 寿男

名古屋市大・院薬・細胞分子薬効解析

[Background] Macrophage (Mϕ) plays crucial roles in immunity and its dysfunction leads to the chronic inflammatory diseases such as arteriosclerosis. Several Mϕ functions are modulated by the activation of ionotropic purinergic P2X7 receptor. Caveolin-1 (Cav-1) enables effective intracellular Ca^{2+} signaling by accumulating ion channels within caveolae domain. In this study, we analyzed the functional coupling between Cav-1 and P2X7 receptor using Cav-1 knockout (Cav-1 KO) mice.

[Methods] In murine bone marrow-derived Mϕ (BMDM), the expression of Cav-1 was analyzed by real-time PCR and Western Blotting. Interaction of Cav-1 and P2X7 receptor was analyzed by proximal ligation assay. Ca^{2+} influx, K^+ efflux and reactive oxygen species (ROS) production were measured with confocal microscopy. Cell death was analyzed by LDH assay.

[Results] The expression of Cav-1 was increased by LPS (lipopolysaccharide)-induced inflammatory stimulation in BMDM. Cav-1 was interacted with P2X7 receptor. Thereafter, ATP-evoked Ca^{2+} influx and K^+ efflux were increased in Cav-1 KO BMDM. ROS production and cell death evoked by ATP were also enhanced in Cav-1 KC BMDM.

[Conclusion] Cav-1 suppresses the activation of P2X7 receptor and modulates immune responses in Mϕ. This study may lead to the development of novel drugs for chronic inflammatory diseases.

Pore opening, not voltage sensor movement, underpins the voltage-dependence of facilitation by a hERG blocker.

電位センサーの移動ではなく、細孔の開口がhERG阻害剤による促進作用の電位依存性を生み出している

○河野 諒太朗[1]、古谷 和春[1,2]、一藁 南[1]、足立 亮[1]、Clancy Colleen[2]、Sack Jon[2]、喜多 紗斗美[1]

[1]徳島文理大・薬・薬学科、[2]カリフォルニア大学デービス校・医・生理学

A drug that blocks the cardiac myocyte voltage-gated K^+ channels encoded by the hERG carries a potential risk of long QT syndrome and life-threatening cardiac arrhythmia. Interestingly, certain hERG blockers can also facilitate hERG activation to increase hERG currents, which may reduce proarrhythmic potential. However, the molecular mechanism remains unclear. The hallmark feature of the facilitation effect by hERG blockers is that a depolarizing preconditioning pulse shifts voltage-dependence of hERG activation to more negative voltages. Here we utilize a D540K hERG mutant to study the mechanism of the facilitation effect. D540K hERG is activated by not only depolarization but also hyperpolarization. With D540K hERG, we find that nifekalant, a hERG blocker and Class III antiarrhythmic agent, blocks and facilitates not only current activation by depolarization but also current activation by hyperpolarization, suggesting a shared gating process upon depolarization and hyperpolarization. Moreover, in response to hyperpolarizing conditionings, nifekalant facilitates D540K hERG currents but not wild-type currents. Our results indicate that induction of facilitation is coupled to pore opening, not voltage per se. We propose that gated access to the hERG central cavity underlies the voltage-dependence of induction of facilitation.

Development of a novel drug targeting TRPC3/C6 channels

TRPC3/C6 チャネルを標的とした新規阻害剤の開発

○白戸 真美子

京都大・大学院工学研究科・合成生物・化学専攻

A group of TRPCs, TRPC3, TRPC6, and TRPC7, form Ca^{2+}-permeable channels directly activated by diacylglycerol (DAG) and play important roles in regulating neuronal survival and dendritic growth, cardiovascular fibrosis *in vitro* and *in vivo* through regulation of Ca^{2+}signaling. Various compounds targeting these TRPC channels have been developed for the treatment of serious diseases such as sudden pulmonary fibrosis and chronic nephropathy. However, none of these compounds have yet reached clinical application, and therefore development of new TRPC3/C6/C7 inhibitors has been much-needed. Here, we have developed a piperazine derivative targeting TRPC3/C6 channels. This compound suppressed receptor-activated Ca^{2+}influx in a dose-dependent manner in human embryonic kidney cells 293 expressed with human TRPC3 or TRPC6 (TRPC3, IC_{50} = 0.086: TRPC6, IC_{50} = 0.034μM). This drug showed no significant inhibitory or stimulatory effect on other TRPs including TRPC7. Interestingly, during isolation of human TRPC7, we obtained a new splice variant of human TRPC7; we are in the process to characterize biophysical and pharmacological properties of the variant that has a deletion in one of the functionally critical domains.

TMEM16A-mediated Ca^{2+}-activated Cl^- currents is increased in portal vein smooth muscle cells from caveolin 1-deficient mice

カベオリン1の欠損はTMEM16Aチャネルを介した門脈Cl_{Ca}電流を増大させる

○川田 成紀、近藤 るびい、鈴木 良明、山村 寿男

名古屋市大・院薬・細胞分子薬効解析

In vascular smooth muscles, the activity of Ca^{2+}-activated Cl^- (Cl_{Ca}) channels regulates the membrane excitability and myogenic tone. TMEM16A channels are predominantly form Cl_{Ca} currents in vascular smooth muscles including portal vein smooth muscles (PVSMs). Caveola is a cholesterol-rich membrane invaginations and structurally contributes to effective and efficient signal transduction. Caveolin 1 (Cav1) is accumulated in the caveolin and plays a key role in forming the functional complex among enzymes, receptors, and ion channels. In this study, the functional roles of Cav1 on the expression and activity of TMEM16A Cl_{Ca} channels were examined in portal vein smooth muscle cells (PVSMCs) from wild-type (WT) and Cav1-knockout (KO) mice. Spontaneous contractions of PVSMs were recorded using an isotonic transducer. TMEM16A-mediated Cl_{Ca} currents were recorded by whole-cell patch-clamp configurations. The expression of TMEM16A channels was quantitatively analyzed by real-time PCR. The amplitude of spontaneous contractions of PVSMs was larger in Cav1-KO mice than WT mice. Whole-cell Cl_{Ca} currents were also larger in Cav1-KO PVSMCs than WT PVSMCs. Importantly, Ani9 (a specific blocker for TMEM16A channels)-sensitive currents were increased in Cav1-KO PVSMCs compared to WT PVSMCs. The expression of TMEM16A channels was higher in Cav1-KO PVSMs than WT PVSMs. The present data strongly suggest that the caveola structure formed by Cav1 negatively regulates the expression and activity of TMEM16A-mediated Cl_{Ca} channels in vascular smooth muscle cells.

The L-DOPA receptor GPR143 in the indirect pathways regulates an anxiety-like behavior through GPR143-DRD2 coupling

マウス線条体間接路におけるL-DOPA受容体GPR143はD2Rとの連関を介して不安様行動を制御する

○田近 伶、増川 太輝、内村 放、五嶋 良郎

横浜市立大・院医・薬理

We propose that L-DOPA by itself is a neurotransmitter. Recently, a G-protein coupled receptor GPR143, a gene product of ocular albinism1, was identified as a receptor for L-DOPA. In this study, to identify the physiological role of GPR143, we performed phenotypic analysis using Gpr143-gene deficient (GPR143-KO) mice. To assess anxiety- and exploration-related behaviors, we employed zero-maze test, and found that time spent in open arms was decreased in GPR143-KO mice when compared to wild-type (WT) mice. The time spent in open arms was also decreased in striatal indirect pathway specific GPR143-KO mice. To investigate the involvement of endogenous L-DOPA, we examined the effect of alpha-methyl-para-tyrosine, a synthetic inhibitor of L-DOPA on mouse behavior. We found that administration of α-MPT at the dose of 3mg/kg (i.p.) decreased the release of L-DOPA without affecting that of dopamine from the dorsal striatum. The administration of α-MPT decreased the time spent in open arms in WT mice, while this effect was not observed in GPR143-KO mice. Furthermore, intraventricular administration of a synthetic peptide, which inhibited the interaction between GPR143 and dopamine D2 receptor (DRD2), increased anxiety-like behavior. These results suggest that L-DOPA regulates anxiety-like behavior through GPR143 and DRD2 coupling in the striatal indirect pathway.

Inhibition of amino acid transporter LAT1 drastically suppresses the transport of large neutral amino acids and induces the downregulation of global translation in cancer cells

アミノ酸トランスポーターLAT1の阻害はがん細胞における大型中性アミノ酸輸送を顕著に低下させグローバルな翻訳抑制を誘導する

○西窪 航[1]、大垣 隆一[1,2]、岡西 広樹[1]、奥田 傑[3]、徐 旻憲[1]、遠藤 仁[4]、金井 好克[1,2]
[1]大阪大・院医・生体システム薬理、[2]大阪大・先導的学際研究機構・生命医科学融合フロンティア研究部門、[3]東京大・院農学生命科学・食品生物構造、[4]ジェイファーマ

Nutrient uptake is essential for maintaining the enhanced growth and proliferation of cancer cells. LAT1 (SLC7A5), which preferentially transports large neutral amino acids, is highly expressed in various cancers. LAT1 inhibitors are preclinically shown to suppress the cancer cell proliferation and tumor growth, and a representative compound JPH203 is under clinical evaluation. However, detailed pharmacological influence of LAT1 inhibition on the overall uptake of large neutral amino acids and the protein synthesis in cancer cells that are thought to be crucial for its anti-cancer effects have not been elucidated yet. Here, we showed that JPH203 dramatically inhibits the uptake of all the large neutral amino acids in multiple pancreatic cancer cell lines. We also found that JPH203 significantly inhibits the amino acid uptake even in cell culture media containing high concentrations of various amino acids. Analyses of the protein synthesis activity based on the binding state of mRNA with ribosomes (Polysome analysis) and the incorporation of puromycin into nascent polypeptides (SUnSET) revealed that JPH203 suppresses global translation. These results advance our understanding of pharmacological activities underlying the anti-cancer effects of LAT1 inhibitors, further supporting the adequacy of cancer treatments targeting LAT1.

Iron tablets delay gastric emptying, which is ameliorated by 5-HT_3 receptor antagonist.

鉄剤は胃排泄を遅延させ、5−HT_3受容体阻害薬により遅延は改善する。

○山田 舜己[1]、三原 大輝[1]、丸山 彩[2]、水戸部 裕子[2]、大橋−土井 雅津代[2]、堀 正敏[1]、黒澤 珠希[1]、岸 和寿[1]、神﨑 萌絵[1]

[1]東京大・農・獣医薬理、[2]鳥居薬品

Gastrointestinal symptoms, including nausea and vomiting, are common adverse effects of oral iron tablets, but the mechanism of iron-induced nausea and vomiting is not yet known. Studies have shown that there are close relationships between gastrointestinal motility and gastrointestinal symptoms such as nausea, vomiting, and diarrhea, with more than 90% of patients with delayed gastric emptying experiencing nausea and vomiting. However, the effect of iron on gastrointestinal motility has not yet been investigated. In the present study, we aimed to elucidate the effects of iron on gastrointestinal motility using sodium ferrous citrate (SFC), the most commonly used iron tablets. Gastric emptying in mice was assessed by ^{13}C-octanoic acid breath test to examine the effect of SFC (3-30 mg Fe/kg, p.o.) with or without the 5-HT_3 receptor antagonist, palonosetron hydrochloride (5 mg/kg. s.c.). Colon transit was also measured by the beads method. The results showed that SFC delayed the gastric emptying, which was ameliorated by administration of palonosetron hydrochloride. It was also confirmed that ingredients of the tablets had no effect on gastric emptying. SFC also had no effect on the colon transit *in vivo*. These results lead the possibility that the iron-induced delayed gastric emptying may be mediated through nausea and vomiting.

Regulatory system of cannabinoid type 1 receptors in the basolateral amygdala on the place preference and anxiolytic-like behaviors

場所嗜好性および抗不安様行動に対する基底外側扁桃体カンナビノイド1型受容体の制御システム

○徳竹 伯洸、浅野 昂、中家 茂俊、宮西 肇、泉尾 直孝、新田 淳美

富山大・大学院医学薬学研究科・薬物治療学研究室

Cannabis is the most widely used addictive drug following alcohol and tobacco. However, the mechanisms involved in the mental effects and dependence formation are unclear. Δ9-tetrahydrocannabinol (THC), the main active substance in cannabis, binds and affects cannabinoid type 1 receptors (CB1R) in the brain. The mice were *i.p.* administered arachidonylcyclopropylamide (ACPA), a CB1R-selective agonist, and then two behavioral experiments were performed. Treatments of ACPA induced the anxiolytic-like behavior in the elevated plus maze test. ACPA increased place preference in the conditioned place preference test. The BLA of mice highly expresses CB1R in the GABAergic interneurons. We aimed to reveal the role of CB1R in BLA for ACPA-induced behaviors. AM251, a CB1R selective antagonist, was administered intra-BLA before *i.p.* administration of ACPA. Intra-BLA administration of AM251 inhibited ACPA-induced anxiolytic-like behavior and place preference. Furthermore, in vivo microdialysis was performed to measure basal GABA levels in the BLA. Acute administration of ACPA had significantly increased basal GABA levels. Chronic administration of ACPA did‘t affect basal GABA levels. These results suggest that CB1R in the BLA contributes to behavior disorders caused by the acute or chronic use of cannabis and these behaviors maight be through a complex control system involving GABA. This study suggests that CB1R in the BLA may lead to new therapeutic targets in the treatment of cannabis-induced adverse effects.

The involvement of the medial prefrontal cortex in nicotine-induced facilitation of object recognition memory retrieval

ニコチンによる物体認知記憶の想起促進に対する内側前頭前野の関与

○江崎 博仁[1]、北中 貴紀[2]、西谷 直也[1,2]、出山 諭司[1,2]、金田 勝幸[1,2]

[1]金沢大・院薬・薬理、[2]金沢大・薬・薬理

We have previously reported that nicotine (Nic) facilitates object recognition memory (ORM) encoding via activation of excitatory neurons in the medial prefrontal cortex (mPFC) in mice. In this study, we investigated whether Nic also facilitates ORM retrieval using the novel object recognition test (NOR). Male C57BL/6J mice (7 – 12 weeks) received Nic before the test session, which was performed 24 hours after the training session of the NOR. Systemic administration (0.1 mg/kg; s.c.), but not intra-mPFC infusion (0.3 μg/side), of Nic enhanced ORM, suggesting that Nic facilitates ORM retrieval by acting on brain regions other than the mPFC. However, suppression of mPFC neuronal activity with inhibitory DREADD hM4Di, which was specifically expressed in excitatory neurons using an AAV vector, significantly inhibited the systemic Nic-induced facilitation of ORM retrieval. Moreover, activation of mPFC excitatory neurons with excitatory DREADD hM3Dq significantly facilitated retrieval of ORM. These data suggest that Nic facilitates ORM retrieval through the indirect activation of mPFC excitatory neurons.

Involvement of cannabinoid receptor type 2 (CB2) to dendritic cell population on inflammatory and allergic response

カンナビノイドCB2受容体は2型古典的樹状細胞を介して炎症およびアレルギー反応を制御する

○細木 春花[1]、野崎 千尋[2]、古谷 彰子[1,3]、柴田 重信[1]

[1]早稲田大・理工・電気・情報生命工学科、[2]早稲田大・国際理工学センター・Major in Bioscience、[3]愛国学園短期大学・家政科

Cannabinoid receptor type 2 (CB2) is one of the major receptors for cannabis, which is expressed all over the body especially on the immune-related cells. It is considered that CB2 can work as a regulator in the immune system, particularly to suppress inflammatory responses in macrophages and microglias when activated. However no other immune cells have been studied whether and how they get modulated by CB2 activity. The objective of this experiment is to search for the other immune cell that can be affected by CB2 activity, and reveal the detailed mechanism of CB2-involved immune regulation.

To characterize the CB2-regulated cells under the inflammatory state, we injected LPS (1 mg/kg, i.p.) to CB2 deficient mice (CB2-KO) and wild-type (WT) controls. Spleen has been harvested from these mice 2 hours after the LPS administration, and immunophenotyping by flow cytometry has been conducted. In this experiment, we found the particular increase of the type 2 classical dendritic cell (cDC2) population in LPS-treated CB2-KO. As past studies reported that cDC2 recruits helper T cells when the body is exposed to an allergen, we further induced allergic rhinitis by OVA sensitization to investigate in-vivo effect of cDC2 increase in CB2-KO and WT animals. As expected, CB2 deficiency resulted as the significant exacerbation of allergic symptoms compared to WT mice. These results suggest that CB2 activity may suppress the allergic response by the reduction of cDC2 recruitment. Pharmacological effect of CB2 agonists to these symptoms will be studied as the future experiment.

A novel T-type Ca^{2+} channel inhibitor, KTtp38, developed by structural modifications of pimozide, a typical antipsychotic agent: Evaluation of the channel selectivity, electrophysiological characteristics and analgesic activity

定型抗精神病薬pimozideの構造展開により開発した新規T型Ca^{2+}チャネル阻害薬KTtp38：チャネル選択性、電気生理学的特徴、鎮痛活性の評価

○笠波 嘉人[1]、高島 康宏[1]、木野 貴博[1]、石川 千浩[2]、長南 百香[3]、豊岡 尚樹[2,3]、関口 富美子[1]、坪田 真帆[1]、川瀬 篤史[4]、大久保 つや子[5]、吉田 繁[6]、岡田 卓哉[2,3]、川畑 篤史[1]

[1]近畿大・薬、[2]富山大院・理工、[3]富山大・工・生命工学、[4]近畿大・薬・生物薬剤、[5]福岡看護大・基礎・基礎看護、[6]近畿大・理工・生命科学

In this study, we examined the selectivity, electrophysiological properties and analgesic activity of KTtp38, a novel inhibitor of T-type Ca^{2+} (Ca_v3) channels, developed by structural modification of pimozide, a typical antipsychotic agent. The IC_{50} value (μM) of KTtp38 was 0.0934 and 1.109 for inhibiting $Ca_v3.2$-dependent currents in response to a test pulse of -20 mV from holding potentials (HPs) of -80 and -110 mV, respectively, indicating a state dependency. The IC_{50} of KTtp38 for inhibiting $Ca_v3.1$-depedent currents caused by the test pulse from HP of -80 mV was 0.217 μM. Pimozide, but not KTtp38, at 1 μM completely inhibited the specific bindings of [^{3}H]-spiperone to D_2 and D_3 receptors in rat striatal membrane fractions. In isolated rat jugular vein rings, the 5-HT_2 receptor-mediated contraction was inhibited by pimozide, but not KTtp38, at 10 μM. In mice, i.p. administration of pimozide, but not KTtp38, caused catalepsy. KTtp38 abolished somatic and visceral pain caused by an H_2S donor, known to enhance $Ca_v3.2$ activity, in mice. KTtp-38 also reversed oxaliplatin-induced peripheral neuropathy in wild-type, but not $Ca_v3.2$-null, mice. The $T_{1/2}$ (h) of KTtp38 and pimozide in the blood was 2.42 and 2.47, respectively. Collectively, KTtp-38 is considered a state-dependent, selective Ca_v3 inhibitor and useful as an analgesic.

Ameliorating effects of corosolic acid in monocrotaline-induced pulmonary hypertensive rats

コロソリン酸によるモノクロタリン誘発性肺高血圧症ラットの病態改善効果

○川出 有希子[1]、山村 彩[2]、近藤 るびい[1]、鈴木 良明[1]、山村 寿男[1]

[1]名古屋市大・院薬・細胞分子薬効解析、[2]愛知医大・医・生理

Pulmonary arterial hypertension (PAH) is a progressive and fatal disease of the cardiovascular system. PAH is characterized by thickening of the pulmonary artery wall (remodeling) and often causes inflammation around the pulmonary artery. It has been reported that corosolic acid (CRA), is a pentacyclic triterpene acid contained in banaba leaves, has anti-inflammatory, anti-diabetic, and anti-cancer effects. In the present study, the effects of CRA on the pathogenesis of PAH were examined using monocrotaline (MCT)-induced pulmonary hypertensive (PH) rats. Male SD rats (4 weeks-old) were injected subcutaneously with vehicle (saline; control) or MCT (60 mg/kg). CRA (1 mg/kg) was administered intraperitoneally daily from 1 week after MCT injection. At 3 weeks after MCT injection, the effects of CRA on the *in vivo* parameters of PAH pathogenesis were analyzed. Hematoxylin and eosin (H&E) staining revealed that CRA clearly improved PAH remodeling in MCT-induced PH rats. The treatment with CRA also reduced the Fulton ratio (an index of right ventricular hypertrophy) in MCT-induced PH rats. Furthermore, CRA significantly lowered right ventricular systolic pressure (RVSP) in MCT-induced PH rats. In contrast, CRA did not affect these parameters in control rats. Taken together, CRA may be useful as a novel therapeutic candidate for PAH.

Honokiol Preserves Mitochondrial Sirtuin 3 And Suppresses Hypoxia-reoxygenation Injury in Cultured Myocytes

心筋細胞低酸素再酸素化障害に対するホノキオールのサーチュイン3を介した保護効果

○谷島 旭、小原 幸、鳥羽 裕恵、中田 徹男

京都薬科大・薬・病態薬科学系臨床薬理学分野

[Background] Honokiol is a small-molecule polyphenol isolated from the genus Magnolia and known as an activator of sirtuin 3, a mitochondrial deacetylase. We examined whether honokiol attenuates mitochondrial injury, leading to the attenuation of cell death in hypoxia-reoxygenation (H/R) injury in cardiomyocytes. [Methods and Results] Neonatal rat cultured cardiomyocytes were subjected to 5 hours of hypoxia followed by 30 minutes of reoxygenation in the presence or absence of honokiol (30 μmol/L). Lethal myocyte injury was assessed by LDH activity in culture medium and myocyte apoptosis was examined by nuclear staining with DAPI and caspase 3 activity. H/R significantly increased LDH activity and apoptotic myocytes, and treatment with honokiol significantly attenuated these indices of myocyte death. In mitochondrial apoptotic pathway, reduction of mitochondrial membrane potential plays critical roles, and ATP is mainly produced by mitochondria. After H/R mitochondria lost their membrane potential, detected by TMRM fluorescence, leading to reduction of ATP content in myocytes, and honokiol recovered them. After H/R protein expression of sirtuin 3 was significantly restored by honokiol. Sirtuin 3 is known to deacetylate Mn-SOD. After H/R honokiol decreased acetylation levels of Mn-SOD and tended to attenuate mitochondrial hydrogen peroxide production. [Conclusion] These results indicate that Honokiol protects mitochondria via enhancement of sirtuin 3, leading to attenuation of H/R-induced myocyte lethal injury.

Vascular endothelial growth factor inhibitor increases the incidence of aortic dissection in mice

血管新生阻害剤はマウスモデルにおいて大動脈解離発症リスクを高める

○辻中 海斗[1,2]、石澤 有紀[3]、大峯 航平[1]、西 穂香[1]、吉岡 俊彦[1,2]、近藤 正輝[2]、糸数 柊人[1]、新村 貴博[4]、相澤 風花[2]、八木 健太[4]、合田 光寛[1,2]、石澤 啓介[1,2]

[1]徳島大・院医薬・臨床薬理学分野、[2]徳島大学病院・薬剤部、[3]徳島大・院医薬・薬理学分野、[4]徳島大学病院・総合臨床研究センター

Aortic dissection is highly lethal, and the risk factors such as hypertension, aging, and atherosclerosis are thought to contribute to its onset. Recently, there has been increasing reports that vascular endothelial growth factor (VEGF) inhibitors can induce the aortic dissection as an adverse event. However, the association between VEGF inhibitors and aortic dissection has been unclear. Therefore, we investigated if VEGF inhibitor increases the onset of aortic dissection using acute aortic dissection model mice (AAD mice).

Sunitinib (100 mg/kg/day) was administered orally for 28days to AAD mice induced by nitric oxide inhibitor, angiotensin II, and lysyl oxidase inhibitor. Blood pressure was measured every week. After 28days, the incidence rate of AAD was estimated. For in vitro study, human umbilical vein endothelial cells (HUVEC) were treated by sunitinib for 24 hours. Then, mRNA expressions of intracellular cell adhesion molecule-1 (ICAM-1) and endothelin-1 (ET-1) were measured.

Sunitinib increased systolic blood pressure (182 mmHg vs 288 mmHg with sunitinib ; $p < 0.01$) and the incidence of AAD (40% vs 59% with sunitinib; $p=0.26$). Moreover, sunitinib increased mRNA expressions of ICAM-1 and ET-1 in HUVEC. These results suggested that VEGF inhibitors induced high blood pressure and developed AAD via endothelial damage.

Eucommia ulmoides oliver leaf extract and geniposidic acid improve hypoxia-induced pulmonary arterial hypertension

杜仲葉エキスおよびゲニポシド酸は低酸素誘発肺高血圧症を改善する

○谷 和佳奈[1]、披田 真里[1]、松原 匠弥[1]、田頭 秀章[2]、喜多 知[2]、根本 隆行[2]、平田 哲也[3]、岩本 隆宏[2]、喜多 紗斗美[1,2]

[1]徳島文理大・薬・薬理、[2]福岡大・医・薬理、[3]小林製薬(株)・中央研究所・研究開発

Pulmonary arterial hypertension (PAH) is a severe and progressive disease that causes right heart failure. The pathogenesis of PAH is generally characterized by persistent high pulmonary arterial resistance and pulmonary arterial remodeling. In this study, we investigated the effects of Eucommia ulmoides oliver leaf extract (ELE) on hypoxia-induced PAH in mice. We observed that Eucommia ulmoides oliver leaf extract (ELE) improve right ventricular systolic pressure (RVSP) and pulmonary vessel muscularization. To identify an active ingredient, geniposidic acid (～1 mg/kg, ～5 mg/kg), a major component of ELE, were orally administered to C57BL/6J mice during exposure to hypoxia for 4 weeks. Geniposidic acid significantly suppressed the elevation of RVSP in hypoxia-induced PAH mice. In addition, hypoxia-induced pulmonary arterial muscularization was slightly attenuated in geniposidic acid-treated mice. In human pulmonary artery smooth muscle cells (HPASMC), endothelin-1-induced intracellular Ca^{2+} elevation was attenuated by geniposidic acid (200 μM). Furthermore, geniposidic acid (50 – 200 μM) increased the maximal respiration in HPASMC. These findings suggest that geniposidic acid may be active ingredient of ELE which effectively improve the development of hypoxia-induced PAH by preventing the vascular remodeling and mitochondrial dysfunction of pulmonary artery.

Cardiotoxicity assessment of EGFR-TKI using human iPS cell-derived cardiomyocytes

ヒトiPS細胞由来心筋細胞を用いたEGFRチロシンキナーゼ阻害薬の心毒性評価

○柳田 翔太[1,2]、佐塚 文乃[1]、林 紗代[1]、小野 敦[2]、諫田 泰成[1]

[1]国立医薬品食品衛研・薬理部、[2]岡山大・院医歯薬

【Introduction】Tyrosine kinase inhibitors (TKIs) have improved the survival of patients with various types of cancer. Growing evidence suggest that cancer therapy-related cardiotoxicity has become important as the serious adverse event associated with many TKIs. Epidermal growth factor receptor-TKI (EGFR-TKI), which has demonstrated efficacy in patients with non-small-cell lung cancer, has been reported to have a risk of cardiac dysfunction. However, cardiotoxicity of EGFR-TKIs has not been fully understood. Here we evaluated the effects of EGFR-TKIs on contractility using human iPS cell-derived cardiomyocytes (hiPSC-CMs).

【Methods】We used iCell cardiomyocyte 2.0 (FCDI). Motion analyses were performed using a cell motion imaging system (SI8000, Sony). Real-world pharmacovigilance data were analyzed by a reporting odds ratio from FDA Adverse Event Reporting System (FAERS).

【Results】We found that several EGFR-TKI decreased contraction velocity in a concentration-dependent manner, while other EGFR-TKIs did not. To confirm the*in vitro* data, we analyzed the cardiotoxicity risk of EGFR-TKIs by the real-world pharmacovigilance data from FAERS. EGFR-TKI, which decreased contraction velocity in hiPSC-CMs, was significantly associated with cardiac failure and decreased ejection fraction.

【Conclusion】Thus, contractile analysis of hiPSC-CMs would be useful to assess TKI-induced cardiac dysfunction in human. We are planning to evaluate other types of TKIs with hiPSC-CMs.

Generation of induced pluripotent stem cells derived from a pair of dizygotic twins.

創薬に向けた二卵性双生児男女由来iPS細胞の樹立

○若林 聖士[1]、長田 千尋[1]、齋藤 桜子[1]、坂本 多穂[1]、山口 賢彦[1]、砂川 陽一[2]、諫田 泰成[3]、森本 達也[2]、黒川 洵子[1]
[1]静岡県立大・薬・生体情報分子解析学分野、[2]静岡県立大・薬・静岡県大薬・分子病態（静岡県立大学 薬学部 分子病態学分野）、[3]国衛研・薬理部

In many cardiovascular diseases, differences between women and men have been well described in epidemiology, pathophysiology, clinical manifestations, treatment efficacy, and outcomes, and have attracted attention as an aspect of personalized medicine. Recently, the use of induced pluripotent stem cells (iPS) cells to reflect individual differences in the drug discovery and the toxicological assay has been attracting attention, but a screening assay system for human cells to evaluate sex differences has not yet been established. Thus, we here propose to develop an *in vitro* system using iPS cell lines derived from a pair of dizygotic twins. We report the progress of three pairs of clones selected for future sex-differences analysis; no sex differences were observed in the expression of the three germline differentiation markers. As a next step, we plan to examine sex differences in gene expression in twin pairs of undifferentiated and differentiated cardiomyocytes to obtain data that will form the basis for future functional analysis of iPS cell-derived cardiomyocytes, including cardiotoxicity assessment.

Evaluation of the efficacy of the mitochondrial mitogen inhibitor Mdivi-1 using non-alcoholic steatohepatitis (NASH) liver organoids

非アルコール性脂肪肝炎（NASH）肝臓オルガノイドを用いたミトコンドリア分裂因子阻害剤Mdivi-1の有効性の評価

○望月 まりあ[1]、田邊 究[2]、臼井 達哉[3]、佐々木 一昭[4]

[1]東京農工大・農・獣医薬理、[2]東京農工大・農・獣医薬理、[3]東京農工大・農・獣医薬理、[4]東京農工大・農・獣医薬理

Non-alcoholic steatohepatitis (NASH) is a disease in which fatty liver develops independently of alcohol intake and progresses to cirrhosis and liver cancer. Currently, no effective treatment for NASH has been found, and new approaches to elucidate the pathogenesis of the disease are needed. It's also been suggested that NASH is associated with functional abnormalities in mitochondria, which are involved in lipid metabolism. Our laboratory has successfully established liver organoids from NASH model mice that can reproduce the fibrotic pathology of NASH, and electron microscopic images of NASH liver organoids showed lipid accumulation, mitochondrial deformation, and aggregation compared to normal liver organoids. Therefore, we analyzed the morphological changes and expression levels of fibrosis-related markers in NASH liver organoids upon treatment with Mdivi-1, a mitochondrial mitogen (DRP1) inhibitor. The results showed that Mdivi-1 suppressed the expression of dendritic morphology in NASH liver organoids and decreased the mRNA expression levels of Collagen-I and α-SMA. In addition, when Mdivi-1 was administered long-term to mice fed a NASH-inducing diet, improvement of fatty liver was observed compared to the solvent-fed group. These results suggest that Mdivi-1 may be useful as a therapeutic agent to improve NASH pathology.

Immunoglobulin therapy improves the lysolecithin-induced demyelination of mouse sciatic nerve via anti-inflammatory macrophage accumulation

免疫グロブリン療法は抗炎症性マクロファージを集積しマウス坐骨神経のリゾレシチン誘導脱髄を改善する

○瀬戸口 潔[1]、林 明子[1]、河田 紋実[1]、柳岡 大悟[1]、笠井 清花[1]、山口 宜秀[1]、石橋 智子[1]、馬場 広子[2]、大滝 博和[1]

[1]東京薬科大・薬・機能形態、[2]新潟医療福祉大・リハビリテーション・作業療法

Immunoglobulin (IgG) therapy is a strategy for treatment of autoimmune, immunodeficiency and acute infectious diseases. Chronic inflammatory demyelinating polyneuropathy (CIDP) is a rare and refractory autoimmune disorder of the peripheral nervous system, characterized by symmetric weakness, impaired sensation and damaged myelin (demyelination). Although intravenous immunoglobulin (IVIg) preparation is used for the therapy, the mechanism of this therapy on demyelination has not been understood. In this study, we examined the effect of human IgG on the lysolecithin-induced demyelination in the mouse sciatic nerve. Lysolecithin was injected into sciatic nerves of the ICR mice (day 0) to induce demyelination and 20 mg (i.v., day 1) and 10 mg (i.p., day 3) of IVIg preparation or the same volumes of saline for control group were administered. Demyelination area and infiltrated macrophages were evaluated with the longitudinal sciatic nerve sections on the day 7 and 14 by immunostaining. The demyelination areas of the IVIg-treated group were significantly less than those of the control group. $CD68^+$ macrophages infiltrated in the lesions and $CD68^+$ $CD206^+$ macrophages were more prominent in IVIg-treated group.

The results suggest that IgG therapy decreased demyelination areas possibly through the accumulation of M2-type macrophages.

Activated protein C suppresses neuropathic pain through activation of proteinase-activated receptor 1 (PAR1)

活性化protein Cはproteinase-activated receptor 1 を介して神経障害性疼痛を抑制する

○圓尾 賢悟[1]、池田 裕哉[1]、坪田 真帆[1]、王 登莉[2]、西堀 正洋[3]、南 達郎[4]、伊藤 彰敏[4]、川畑 篤史[1]

[1]近畿大・院薬・病態薬理学研究室、[2]岡山大・院医歯薬・薬理、[3]岡山大・院医歯薬・創薬研究推進、[4]旭化成ファーマ・医薬研究センター・薬理

The prevention of oxaliplatin-induced peripheral neuropathy (OIPN) by thrombomodulin alfa (TM α) involves thrombin-dependent activation of protein C (PC) and thrombin-activatable fibrinolysis inhibitor (TAFI), in addition to inactivation of high mobility group box 1 (HMGB1). We have demonstrated that complement C5a, degradable by activated TAFI (TAFIa), known as carboxypeptidase B (CPB), is involved in OIPN development. In the present study, we examined the effect of APC on OIPN as well as surgically induced neuropathic pain in mice, and asked whether proteinase-activated receptor 1 (PAR1) would participate in the effects of APC in those neuropathic pain models, given that APC is an unbiased or biased agonist of PAR1. The OIPN in mice was prevented fully by TM α, an anti-HMGB1-neutralizing antibody (HAb) or TAFIa/CPB, and, to a lesser extent, by APC. Vorapaxar, a PAR1 antagonist, completely and partially canceled the anti-OIPN effects of APC and TM α, respectively. Interestingly, the neuropathic allodynia caused by partial sciatic nerve ligation was also abolished by TM α, and reduced by HAb or APC, and the effect of APC was reversed by vorapaxar. Our data suggest that PAR1 is involved in the preventive effects of APC and, in part, of TM α on OIPN and surgically induced neuropathic pain.

Macrophage-Schwann cell communication promotes peripheral nerve regeneration

マクロファージとシュワン細胞のコミュニケーション介した神経再生メカニズム

○大島 絵莉、林 良憲、篠田 雅路

日本大・歯・生理

Recent studies indicate the importance of the signal relay from macrophages towards Schwann cells in axon regeneration after peripheral nerve injury. However, the molecular mechanisms underlying axon regeneration via macrophage-Schwann cell communication remain largely unclear. Here, we explored macrophage-derived molecules relevant to axon regeneration. After inferior alveolar nerve transection (IANX), the rats showed hypoesthesia in the lower lip, which was recovered from 10 days after IANX by an intrinsic regeneration capacity. In contrast, macrophage ablation caused delayed nerve regrowth. Furthermore, c-Jun-positive Schwann cells, a repair phenotype, disappeared after the removal of macrophages. Cathepsin S (CTSS) from macrophages promoted recovery from hypoesthesia and cleaved ephrin B2 on fibroblasts. EphB2, a receptor of ephrin B2, was expressed in Schwann cells. Accelerated recovery from hypoesthesia after IANX following CTSS treatment was prevented by neutralization of ephrin B2. These results suggest that CTSS from macrophages liberates ephrin B2 which in turn facilitates axon regeneration in the orofacial regions. Our results lead to the development of novel therapeutics for hypoesthesia caused by nerve injury targeting CTSS.

Increased levels of circulating cell-free DNA in COVID-19 patients with respiratory failure

呼吸不全を伴ったCOVID−19患者における血液中遊離DNAレベルの上昇

○若山 勝紀[1]、中平 毅一[1,2]、田中 明彦[3]、福田 陽佑[3]、趙 晶[1]、大田 進[3]、相良 博典[3]、吉栖 正典[1]
[1]奈良県立医科大・医・薬理学講座、[2]Weill Cornell Medicine・Department of Medicine、[3]昭和大学病院・呼吸器アレルギー内科

Background: Cell-free DNA (cf-DNA) is known to be released from injured cells and act as a critical activator of inflammation and the immune system. Patients with COVID-19 could develop respiratory failure and therefore require oxygen therapy. In this study, we hypothesized that circulating cf-DNA level could reflect the severity of COVID-19.

Methods: Analyses of cf-DNA levels were performed on serum samples from 95 hospitalized-patients with confirmed COVID-19 at Showa University Hospital (Tokyo, Japan). Cf-DNA levels were assessed by measuring the copy number of mitochondrial DNA (mtDNA) and nuclear DNA (nDNA) using quantitative real-time PCR.

Results: Patients were grouped into Moderate, Severe, and Critical using the severity criteria by the National Institutes of Health in U.S.A. There was no significant difference on both cf-DNA levels between Moderate and Severe groups, and between Severe and Critical groups. Meanwhile, both of the levels were significantly higher in Critical group than Moderate group. Patients were also grouped by their respiratory treatment. Both cf-DNA levels significantly increased in patients with oxygen-supplementation and patients with intubation, compared to those with no oxygen supplementation and with non-intubation, respectively. There was negative association between oxygen saturation (SpO_2) and cf-nDNA levels, not cf-mtDNA.

Conclusion: These results suggest that serum cf-DNA could serve as a useful biomarker to help determining therapeutic management for respiratory failure in COVID-19.

Mild electrical stimulation and heat shock can suppress acute kidney injury (AKI) to chronic kidney disease (CKD) transition

微弱パルス電流及び温熱の同時印加は急性腎障害から慢性腎臓病への移行を抑制し得る

○津波古 遥希、寺本 啓祐、加世田 将大、Piruzyan Mariam、岩上 長巨、荒川 知南、Suico Marry Ann、首藤 剛、甲斐 広文

熊本大・院医薬・遺伝子機能応用学分野

AKI is considered as a “curable disease”, but recent epidemiological studies and meta-analysis have revealed that AKI is a risk factor for CKD. Therefore, it is necessary to establish a treatment that can control the AKI to CKD transition. We have studied mild electrical stimulation (MES) and heat shock (HS; 42°C) that promotes effective biological responses. Interestingly, in adriamycin (ADR)-induced nephrotic syndrome (NS) mouse model, MES +HS significantly suppressed albuminuria and proteinuria, which are characteristics of NS. We also investigated the effects of MES+HS on AKI to CKD transition in a mouse model of bilateral ischemia reperfusion injury (Bi-IRI). The renal function of Bi-IRI mouse model was rapidly decreased and then recovered over time. However, tubular damage, inflammation and fibrosis were observed even after recovery of renal function. MES+HS promoted the recovery of renal function in this model. Moreover, MES+HS significantly suppressed tubular damage, inflammation, and fibrosis, which are indicators of AKI to CKD transition on day 14 after Bi-IRI. It has been reported that a subpopulation of failed-repair proximal tubular cell (FR-PTC) emerges after AKI and is involved in the development of chronic disorders. We found that MES+HS reduced the number of Vcam1-positive tubular cells, a marker of FR-PTC, suggesting that MES+HS promotes normal tubular repair. Together, MES+HS can suppress AKI to CKD transition by regulating inflammation, fibrosis and also the emergence of FR-PTC involved in the chronicity of the renal disorder.

The involvement of inflammasomes on the learning and memory impairment in a mouse model of embolic stroke

塞栓性脳梗塞モデルマウスの学習記憶障害におけるインフラマソームの関与

○野村 彩衣[1]、黒川 和宏[1]、髙橋 浩平[1]、宮川 和也[1]、持田（齋藤） 淳美[1]、武田 弘志[2]、辻 稔[1]

[1]国際医療福祉大・薬・薬理、[2]国際医療福祉大・福岡薬・薬理

Post-stroke cognitive impairment (PSCI) is one of the major complications after a stroke and affects quality of life. Recently, several studies demonstrated that elevated brain proinflammatory cytokines such as TNF-α, IL-6 and IL-1β can induce cognitive impairment. However, the mechanisms underlying PSCI remain unclear. In the present study, we investigated whether post-stroke inflammasomes are involved in the development of PSCI using acetic acid-induced embolic cerebral infarct mice. Long-term learning and memory assessed by the passive avoidance test was impaired on days 7 and 14 after stroke, whereas short-term learning and memory assessed by Y-maze test showed no changes. Also, the expression of the phosphorylated AMPA receptor subunits GluR1 at serine 831 and serine 845 in the dorsal hippocampus were significantly decreased. Under these conditions, inflammasome-related proteins, including caspase-1, ASC/TMS1, IL-1β, TNF-α and IL-18, were significantly increased in the dorsal hippocampus 14 days after stroke. The present findings suggest that a decrease in phosphorylated GluR1 at ser831 and ser845 via the inflammasome activation pathway in the dorsal hippocampus may be involved in the development of learning and memory impairment after embolic stroke.

Phase-specific synchronization of basolateral amygdalar neurons with neocortical slow oscillations

扁桃体基底外側核と皮質徐波の位相特異的な同期活動

○佐藤 由宇[1]、池谷 裕二[1,2]

[1]東京大・院薬・薬品作用、[2]東京大・Beyond AI研究推進機構

The basolateral amygdala (BLA) shows firing activity synchronized with neocortex slow oscillations, a form of <1-Hz oscillations that occur dominantly during slow-wave sleep or under anesthesia and is believed to contribute to the formation of emotional memories. Despite its importance for elucidating the neural circuits involved in the formation of emotional memories, the mechanism of the synchronization has remained unclear, mainly because it is difficult to record neuronal membrane potentials in deep brain regions, such as the BLA. We recorded membrane potentials of BLA neurons using a new method that enables whole-cell recording from deep brain regions in vivo. We found that BLA neurons transiently depolarized at late active phases in the slow oscillations. To determine the neural source of the depolarization of BLA neuros, we focused on the medial prefrontal cortex, whose axons projecting to the BLA is known to contribute to the formation of fear memories, as a candidate region that depolarizes BLA neurons during slow oscillations. BLA-projecting neurons were retrogradely labeled with channelrhodopsin-2 using retrograde adeno associated virus, and their firing activity was recorded using an opto-tagging method. These results provide insight into the neural mechanism that synchronizes the slow oscillations between the BLA and the neocortex and lead to the elucidation of the mechanism underlying the formation of emotional memories.

Involvement of ceramide kinase and study as a therapeutic target in Niemann-Pick disease type C

ニーマン・ピック病C型におけるセラミドキナーゼの関与と治療標的としての検討

○田内 理紗子、中村 浩之、本田 拓也、内海 直也、橋本 直宏

千葉大・院薬・薬効薬理学

(Background and purpose)

Niemann-Pick disease type C (NPC) is a genetic disorder in which patient cells have endosomal/lysosomal accumulation of cholesterol. No approved drug improves cholesterol accumulation, and the creation of new therapeutic drugs are desired. Currently, there are many relationships between sphingolipids and NPC have been reported. This study investigated the relationship between NPC and ceramide-1-phosphate, produced by phosphorylating ceramide, using NPC1-null mice.

(Results and discussion)

NPC is characterized by clinically affecting the brain and liver; premature death invariably results.

We generated double-knockout (DKO) mice lacking NPC1 and CerK and compared the phenotypes of NPC mice and DKO mice in these tissues. In the brain, cholesterol accumulation and Purkinje cell survival were improved in DKO mice compared with those in NPC1-null mice. In the liver, cholesterol accumulation and liver disorder were improved in DKO mice compared with those in NPC1-null mice. Administration of a CerK inhibitor to NPC1-null mice delayed the onset of clinical signs and prolonged the lifespan. These results suggest that CerK may be helpful as a novel therapeutic target for NPC.

Hippocampal mitochondrial dysfunction induces anxiodepressive-like behaviors in mice with neuropathic pain.

海馬のミトコンドリア機能障害は神経障害性疼痛マウスに不安・うつ行動を誘発する

○吉本 夏輝、中島 一恵、中村 庸輝、森岡 徳光

広島大院医系科学・薬効解析

Neuropathic pain (NP) is frequently accompanied by anxiodepressive-like behaviors, yet the mechanisms remain unclear. Mitochondrial dysfunction induces neuroinflammation and has been implicated in various neurological diseases, including depression. However, the relationship between mitochondrial dysfunction and anxiodepressive-like behaviors in the NP state is unclear. The current study examined whether mitochondrial dysfunction is involved in anxiodepressive-like behaviors in mice with NP. NP was induced by partial sciatic nerve ligation (PSNL) of male ddY mice. Anxiodepressive-like behaviors were evaluated by forced swim test, social interaction test, and novelty suppressed feeding test. Mitochondrial dysfunction was assessed by quantifying mitochondrial DNA in the cytoplasmic fraction. The expression of type Ⅰ interferon mRNA was analyzed by real-time PCR. Curcumin was orally administered to inhibit mitochondrial dysfunction. PSNL induced anxiodepressive-like behaviors with accompanying mitochondrial dysfunction and increase of type I interferon mRNA in the hippocampus at 8 weeks post-injury. Curcumin suppressed mitochondrial dysfunction and improved anxiodepressive-like behaviors. The current study suggests that mitochondrial dysfunction in the hippocampus could be involved in anxiodepressive-like behaviors under NP state.

Ceramide kinase, a lipid-metabolizing enzyme, is involved in the pathophysiology of schizophrenia and Parkinson's disease by regulating the levels of extracellular dopamine.

脂質代謝酵素セラミドキナーゼは細胞外ドパミンレベルを調節することで、統合失調症およびパーキンソン病の病態に関与する

○金原 拓哉、安江 匡隆、本田 拓也、中村 浩之

千葉大・院薬・薬効薬理学

Dopamine (DA) is a critical neurotransmitter which modulates motor functions, learning and motivation. Abnormal DA signaling is related to neuronal diseases such as schizophrenia (SZ) and Parkinson's disease (PD). The symptoms of SZ are classified as hyperDAergic positive symptoms and hypoDAergic negative symptoms. PD is a common progressive neurodegenerative disorder characterized by the loss and degradation of DAergic neurons. Current drugs poorly treat both diseases.

Ceramide kinase (CerK) is an enzyme which phosphorylates ceramide, a central metabolite of sphingolipids to produce ceramide-1-phosphate (C1P). CerK/C1P pathway is reported to be involved in extracellular homeostasis of some neurotransmitters *in vitro*, however, the involvement with SZ or PD remains unclear.

In this study, we created SZ and PD model in mice genetically deleted *Cerk* and compared their phenotypes with wild-type mice. We found that *Cerk* deficiency exacerbated the positive symptoms of SZ partly due to an increase in the extracellular DA levels. In contrast, negative symptoms of SZ and motor dysfunction of PD were partly improved by *Cerk* deficiency.

These results suggest that CerK may be a new therapeutic target for hypoDAergic diseases such as negative symptoms of SZ and PD by regulating extracellular DA levels.

Identification of two distinct neuronal subpopulations encoding parenting and aggressive behaviors toward pups in the population of amygdalohippocampal area neurons projecting to the medial preoptic area using projection-specific and activity-dependent labelling

内側視索前野へ投射する扁桃体海馬野ニューロンは養育行動と攻撃行動をコードする異なる2つのニューロン集団を有する

○佐藤 圭一郎、南 雅文、天野 大樹

北海道大・院薬・薬理

Although parenting behavior is necessary for development of infants, underlying neural mechanisms remain unclear. The amygdalohippocampal area (AHi) neurons projecting to the medial preoptic area (MPOA), a key region for parenting, were shown to be activated by both parenting and aggression toward pups using retrograde tracer and c-Fos immunostaining. We labeled MPOA-projecting AHi neurons in a projection-specific and activity-dependent manner using a retrograde adeno-associated virus vector expressing Cre recombinase activity-dependently and mice expressing tdTomato Cre-dependently. As a result, we observed the presence of two functionally distinct subpopulations of parenting and aggressive response neurons.

We next performed scRNA-seq to determine whether the two populations were molecularly distinct and found 395 genes exhibiting higher expression levels in the parenting subpopulation than in the aggression subpopulation, and 755 genes showing the opposite pattern. Among them, we focused on 5-HT7 receptor because of high expression of *Htr7* in the parenting subpopulation. Intraperitoneal administration of LP44, 5-HT7 receptor agonist, resulted in activation of parenting-labeled, but not aggression-labeled, neurons. Finally, microinjection of LP44 into the AHi 15 min before behavioral test promoted parenting behavior.

In conclusion, we identified distinct subpopulations of MPOA-projecting AHi neurons encoding parenting and aggressive behaviors toward pups. The results demonstrated that parenting AHi neurons expressed 5-HT7 receptors and were activated by administration of 5-HT7 receptor agonist, which suppressed aggressive behavior toward pups.

Arcadlin induction affects dendritic spine density in the hippocampal dentate gyrus after cerebral ischemia

脳梗塞後の海馬歯状回において誘導されるArcadlinが樹状突起スパイン密度に与える影響

○中澤 秀真、井上 耀介、井上 翔太、山口 菜摘、中谷 仁、澤野 俊憲、田中 秀和

立命館大・院生命科学・薬理学研究室

Dendritic spine morphological changes occur in the brain after cerebral ischemia. Arcadlin, a non-clustered protocadherin δ2, is induced in a neuronal activity and reduces the dendritic spine density via endocytosis of N-cadherin. Cerebral ischemia induces neuronal activity but the expression of Arcadlin and its role in the ischemic brain are not clear. In this study, we analyzed Arcadlin expression and dendritic spine changes after cerebral ischemia using a highly reproducible mouse model of middle cerebral artery occlusion (MCAO). We found that *Arcadlin* mRNA was significantly upregulated in the hippocampal dentate gyrus (DG) at 4 hours after MCAO. Dendritic spine density in the ipsilateral DG was lower than in sham mice. These results suggest that Arcadlin may be involved in the reduction in the dendritic spine density. We are performing the comparison of dendritic spine density between MCAO and Sham using *Arcadlin* KO mice.

Dendritic morphology of the pyramidal cells in the piriform cortex of *Arcadlin*$^{-/-}$ mice

Arcadlin$^{-/-}$マウスの梨状皮質錐体細胞の樹状突起形態

○北川 貴士[1]、河前 なつみ[1]、重松 成秋[1]、高山 晃行[1]、上村 健士郎[1]、山形 要人[1]、杉浦 弘子[2]、澤野 俊憲[1]、中谷 仁[1]、田中 秀和[1]

[1]立命館大・院生命科学・薬理学研究室、[2]東京都医学総合研・脳・神経科学研究分野

Arcadlin (Acad/Protocadherin-8) is a non-clustered d2-protocadherin of the cadherin superfamily. Acad is induced quickly by neural activity and is known as a molecule that reduces spine density in the hippocampal neurons in vivo and in vitro. Pyramidal neurons in the layer II of the piriform cortex strongly expresses *Acad* mRNA. The piriform cortex is often kindled electrically for seizure generations. We hypothesize that the neural activity of the piriform neurons induces Arcadlin, which in turn modulates their spine morphology. In order to investigate this possibility, we examined the dendritic morphology of the pyramidal neurons in the piriform cortex in *Acad*$^{-/-}$ mice. Contrary to our expectation, *Acad*$^{-/-}$ mice showed a lower spine density than WT mice in the pyramidal cells of piriform cortex. The change in spine density was most obviously observed in thin spines, and in the dendritic zone distal to the cell body. The data suggest that Acad does not suppress the piriform pyramidal cells at least in unkindled status. We will further examine whether the induced Acad suppresses the piriform pyramidal cells under kindled condition.

Elucidation of the pathogenesis of short-term memory impairment in a mouse model of lipopolysaccharide-induced inflammation

リポ多糖惹起性炎症モデルマウスにおける短期記憶障害の発症機序解明

○森本 康平、江口 遼太、乙黒 兼一

北海道大・院獣医

Diseases with peripheral inflammation, such as sepsis and peritonitis, are associated with an increased risk of central nervous system diseases. Injection of lipopolysaccharide (LPS) into mice has been widely used as a disease model for peripheral inflammation and exhibit cognitive dysfunction. Although glial cells have been implicated in the pathogenesis of cognitive dysfunction, the detailed mechanisms remain unclear, and most studies have been conducted in young or old mice. In this study, we examined the effects of LPS on short-term memory in adult mice to elucidate the mechanisms of pathogenesis.

C57BL/6N mice (male, 11-13 weeks old) were injected with LPS (3 mg/kg, i.p.). At 7 days after injection, the novel object recognition test was conducted, and LPS decreased the discrimination index. At 7 days after injection, the number of c-Fos+ cells, a marker of neuronal activation, decreased in the hippocampal CA1 region, and the percentage of immature spines detected by Golgi-Cox staining was increased. The gene expression of several inflammatory factors peaked at 1-3 days after LPS injection and recovered to pre-injection levels at 7 days after injection.

These results indicate that the short-term memory impairment was observed at 7 days after LPS injection. Inhibition of hippocampal CA1 neuron activation and the decrease in synaptic strength due to immature spines may be involved in the short-term memory impairment.

取り下げ

What factors contribute to diosgenin-induced memory recovery?

記憶障害改善の要因は何か.

○永田 朋也、楊 熙蒙、東田 千尋

富山大・薬・和漢医薬学総合研究所　神経機能学領域

Alzheimer's disease (AD) is a neurodegenerative disorder characterised by deposition of Aβ and hyperphosphorylated tau in the brain. In addition, hyperactivation of glial cells, cerebrovascular damage, and structure changes of white matter are also observed in AD brain. We previously found that diosgenin regenerated axons in the brain and improved memory impairment in AD model mouse (5XFAD). However, other beneficial effects of diosgenin leading to memory recovery remain unknown. This study aimed to investigate the effect of diosgenin on morphology of glial cells, blood vessels, and myelin in 5XFAD mouse brains.

Vehicle solution or 0.1 μ mol/kg diosgenin were orally administered to wild-type and 5XFAD mice for 14 days. Diosgenin administration significantly improved object recognition memory in 5XFAD mice. The brain slices were served for immunohistochemistry. Diosgenin administration did not remarkably influence the areas of $GFAP^+$ astrocytes, $Iba1^+$ microglia and $CD31^+$ blood vessels at least in the prefrontal cortex, hippocampus, and perirhinal cortex in 5XFAD mice. We are currently evaluating myelin formation and neurogenesis.

This study indicated that diosgenin administration didn't provide no numerical changes of astrocytes, microglia, and blood vessels in the 5XFAD mouse brains. Therefore, we are now narrowing down the most contributing factor to memory improvement by diosgenin.

Food-derived amino acid ergothioneine inhibits histamine metabolizing enzyme and promotes anti-inflammatory M2 microglia polarization

食物由来アミノ酸 ergothioneineによるヒスタミン分解酵素阻害と抗炎症性M2ミクログリアへの分極促進

○上田 優花、石本 尚大、勝部 諒、増尾 友佑、加藤 将夫

金沢大・薬学系

Oral administration of a food-derived amino acid ergothioneine (ERGO) enhances cognition in mice although molecular mechanisms remain unclear. Our comprehensive molecular targeting assays showed inhibition by ERGO of histamine N-methyltransferase (HNMT), a main histamine-metabolizing enzyme in the brain (J Funct Foods 95, 105165, 2022). We here attempted to characterize the inhibitory effect of ERGO on HNMT-induced methyl transfer from a methyl donor S-adenosyl-methionine (SAM) to histamine. A radioenzymatic assay using a [^{3}H]SAM showed that mouse brain homogenate increased radioactivity of the [^{3}H]N-methylhistamine, while incubation with ERGO or an HNMT inhibitor metoprine significantly suppressed the radioactivity, suggesting that ERGO inhibits murine brain HNMT. Lineweaver-Burk analysis using human recombinant HNMT showed that the inhibition by ERGO was competitive with histamine. Quantification of N-methylhistamine by LC-MS/MS showed that human recombinant HNMT produced N-methylhistamine in a time-dependent manner, whereas ERGO and metoprine significantly suppressed the production. We then examined the activation of microglia, immune cells in the brain, because histamine is an important molecule in the immune system. Immunochemical analysis showed that HNMT was expressed in primary cultured microglia (PCM). Exposure of PCM to ERGO or metoprine significantly increased mRNA expression of CD206, a marker for anti-inflammatory M2 microglia. Thus, the HNMT inhibition by ERGO may be associated with microglial polarization in the brain.

Inhibitory effect of mirogabalin for various pruritogens-induced acute itch

種々の起痒物質により誘発される掻き動作に対するミロガバリンの効果

○堀 圭汰[1]、澤幡 雅仁[2]、久米 利明[2]、歌 大介[2]

[1]富山大・院医薬・応用薬理、[2]富山大・院医薬・応用薬理

Itch is defined as an irritating sensation that triggers a desire to scratch. The signaling pathways of itch fall into two main categories, histaminergic and non-histaminergic pathway. Mainly, C fibers transmit itch information in both pathways. Since it is known that $\alpha_2\delta$ subunits composing voltage-gated Ca^{2+} channels are expressed in C fibers, mirogabalin, a novel gabapentinoid that targets these subunits, may be applicable as an antipruritic drug. In this study, using male ICR mice, we showed effect of mirogabalin on scratching behavior induced by several pruritogens (histamine, chloroquine and compound 48/80). Scratching bouts increased by these pruritogens are decreased by oral administration of mirogabalin (10 mg/kg). The oral administration of mirogabalin (10 mg/kg) exhibited no sedation. In addition, the scratch behavior was inhibited by intracisternal injection of mirogabalin, but not local intradermal injection. These results suggest that mirogabalin is effective against itch transmitted through both histaminergic and non-histaminergic pathway and also that central nerve system, especially spinal cord, are involved in the antipruritic effect of mirogabalin.

Lysophosphatidic acid (LPA) improves glomerular histology in a murine model of SLE

リゾホスファチジン酸（LPA）がSLEモデルマウスの糸球体腎炎を改善させる

○永田 亘、小泉 明穂、中川 慶一、髙橋 さやか、石塚 俊晶

防衛医科大・院医・薬理学講座

Objective: Lupus nephritis is a typical clinical manifestation of SLE. We previously reported that LPA treatment improves depressive-like behavior and microglial activation in MRL/lpr mice (an animal model of SLE). Thus, we examined the effects of LPA on renal function and glomerulonephritis in MRL/lpr mice. Method：18-week-old MRL+/+ mice (n=12) were treated with vehicle and 18-week-old MRL/lpr mice (n=24) were treated with vehicle or LPA (1 mg/kg) for 2 weeks. After the treatment, urine and blood samples were collected, and histological examinations were performed. Results and Discussion: The significant increases in daily urinary albumin levels in MRL/lpr mice were lost by LPA treatment. Creatinine in plasma was not significantly different between the three groups. The significant increases in plasma dsDNA antibody levels in MRL/lpr mice were lost by LPA treatment. The increases of CD68-positive cells in the glomerulus were found in MRL/lpr mice and the increases were suppressed by LPA treatment. The PAS-positive rates in MRL/lpr mice were significantly increased compared to MRL+/+ mice and the increases were significantly suppressed by LPA treatment. These results suggest that LPA treatment improves glomerulonephritis and proteinuria in MRL/lpr mice.

The efficiency of leucine administration on the maintenance of systemic immune function in a sarcopenia model

サルコペニアマウスへのロイシン投与による免疫恒常性維持への影響

○望月 万莉井、細田 佑樹、稲葉 奏介、劉 爽、茂木 正樹

愛媛大・院医・薬理学

It is well known that chronic inflammation causes the sarcopenia. Whether the efforts to prevent skeletal muscle loss benefit the immune homeostasis remains unclear. In the present study, the efficacy of leucine supplementation on the maintenance of systemic immune function was evaluated. A sciatic nerve denervation-induced sarcopenic model was established and the volume and thickness of skeletal muscle on the hindlimb were evaluated by magnetic resonance imaging. Oral administration of leucine was carried on for 56 weeks. The skeletal muscle mass and the subsets or function of immune cells were analyzed.

In leucine-treated sarcopenic mice, skeletal muscle mass on the hindlimb was significantly increased compared to that in non-treated sarcopenic mice. Leucine treatment repaired the mitochondria dysfunction in splenocytes from sarcopenic mice both *in vitro* and *in vivo*. In sarcopenic mice, an increase of the PD-1 expression was observed in CD4+ and CD8+ T cells. However, oral leucine administration restored the expression of PD-1 in the lymphocytes to the level of non-sarcopenic mice.

In conclusion, the administration of leucine exhibits beneficial effects on sarcopenia and may influence the anti-cancer immune responses via adaptive immune resistance mechanism.

Roles of CCR5 in development of fibrosis in severe asthma

重症喘息の線維化におけるCCR5の役割

○霜良 勇人、長谷 雪乃、羽口 天太、髙森 伊富、松田 将也、北谷 和之、奈邉 健
摂南大・薬・薬効薬理学研究室

Mechanisms underlying development of lung fibrosis in severe asthma have been unclear. In our murine models of mild and severe asthma, lung fibrosis was significantly developed only in the severe asthma model. RNA-seq analyses revealed that not only genes of collagen type 1 but also genes of matrix metalloproteinases (MMPs) and activins, TGF-b family cytokines were found to be highly expressed in the lung of the severe asthma model compared with that of the mild model. More interestingly, pathway analyses showed that pathway of CCR5 and the ligands were up-regulated in the severe model. In this study, roles of CCR5 in the development of lung fibrosis in the severe model were analyzed. Treatment with a CCR5 antagonist, maraviroc but not dexamethasone exerted significant inhibition of the development of lung fibrosis. Interstitial macrophages (IMs) that expressed CCR5 were markedly increased in the lung, and the degree was significantly higher in the lung of severe asthma model than that of the mild asthma model. Real-time RT-PCR analyses revealed that IMs derived from the lung of severe model expressed higher mRNA levels of MMPs and activins than those of mild asthma model. From these results, it was strongly suggested that $CCR5^{+}$ IMs were possibly involved in the development of the lung fibrosis in severe asthma.

Inhibitory effect of selective serotonin reuptake inhibitors (SSRIs) on Toll like receptor-dependent and -independent production of IL-6

Toll様受容体依存的/非依存的IL-6産生に対する選択的セロトニン再取り込み阻害薬（SSRI）の抑制効果

○竹中 洋平[1]、田中 隆[2]、北畠 和己[1]、倉持 幸司[2]、青木 伸[1]、月本 光俊[1]

[1]東京理科大・院薬、[2]東京理科大・院理工

Inflammatory diseases lead to excessive proinflammatory cytokine production (cytokine storms). The cytokine storms are highly lethal, so establishing an effective treatment is desirable. Recently, some of selective serotonin reuptake inhibitors (SSRIs), which are used to treat depression, have been reported to be effective in treating various inflammatory diseases, such as COVID-19. In this study, in order to elucidate which SSRI would be the most suitable as an anti-inflammatory drug, we investigated that the effect of 5 SSRIs on the production of inflammatory cytokine (Interleukin-6; IL-6) induced by macrophage activation induced in a Toll-like receptor (TLR)-dependent manner, and by T cell activation induced in a TLR-independent manner. In J774.1 murine macrophage cells, pretreatment with the SSRIs significantly suppressed IL-6 release induced by TLR3, TLR4, and TLR9 agonist. On the other hand, these SSRIs are also significantly suppressed IL-6 release induced by T cell activator in murine splenic lymphocytes. Our results show that fluoxetine has potent inhibitory effect on IL-6 production induced by various stimuli and low cytotoxicity among the 5 SSRIs. An examination of the structural requirements indicated that the *N*-methyl group of fluoxetine has a critical role in the inhibition of IL-6 production. Overall, our findings suggest that fluoxetine might be one of the preferred SSRI for further evaluation as an anti-inflammatory drug to treat cytokine storms.

Activation of regulatory T cell through prostaglandin E_2-EP4 signaling

○松浦 竜真、Thumkeo Dean、成宮 周

京都大・院医・創薬医学講座

Prostaglandin (PG) E_2, a bioactive lipid biosynthesized from arachidonic acid, exerts its functions through 4 cognate receptors EP1-4. Our previous studies suggested that PGE_2 attenuates antitumor immunity in the tumor microenvironment by the recruitment and activation of regulatory T cells (Tregs), which are a subset of T cells specialized in immunosuppression. However, whether such actions of PGE_2 on Treg is a direct or indirect action remains largely unknown. To address this question, we generated induced Tregs (iTregs) from purified splenic $CD4^+$ T cells by CD3/28 stimuli in the presence of TGF-β *in vitro* and examined the direct effect of PGE_2. Using flow cytometry analysis, we found that the expression level of Foxp3, a master transcription factor of Treg, and 4-1bb, a coactivator of Treg, were both significantly enhanced upon PGE_2 treatment. Furthermore, these effects were notably suppressed in the presence of EP4 antagonist, suggesting that PGE_2 contributes to Treg activity through the EP4 receptor. In addition, RNA-seq revealed that not only *4-1bb*, but other Treg coactivator genes such as *Ctla4*, *Gitr*, and *Ox40,* were greatly increased in the PGE_2 treatment group. Given that previous studies reported that high expression level of these genes in intratumoral Tregs is positively correlated to Treg immune suppression activity, we speculated that PGE_2 produced in the tumor microenvironment may directly induce the activated phenotype of intratumoral Treg through EP4 receptor.

The effects of dasatinib on muscle regeneration

ダサチニブの筋再生への影響

○石田 七海[1]、黒澤 珠希[2,3,4]、後藤 もも[2]、堀 正敏[2]

[1]東京大・農・獣医薬理学教室、[2]東京大・院農学生命科学・獣医薬理、[3]徳島大・院医歯薬・生体栄養学分野、[4]文部科学省・日本学術振興会・特別研究員PD

As there are no effective treatments for muscular dystrophy (MD), identifying systemically acting small-molecule therapeutics is desirable. Tyrosine phosphorylation of β-dystroglycan, which occurs via tyrosine kinase in dystrophin-deficient muscles, has been reported to induce muscle damage, and several tyrosine kinase inhibitors have been researched as potential therapeutic agents for MD. Nilotinib, a second-generation tyrosine kinase inhibitor, was potentially effective in MD by reducing muscle fibrosis. However, there was a problem that its direct effect on satellite cells inhibited muscle differentiation. Dasatinib, a third-generation tyrosine kinase inhibitor, is also expected to be effective in MD, but its effect on muscle regeneration is unknown.
Here, we investigated the effects of dasatinib on muscle, with a focus on muscle regeneration. We administered dasatinib to mice whose tibialis anterior muscle was damaged by cardiotoxin. In the dasatinib-treated mice, abnormal myofibers were observed, and muscle regeneration may be impaired. The effect on muscle differentiation was examined using the myoblast cell line C2C12. There was abnormal cell fusion, and this abnormality differed from that previously described for nilotinib. Further research is currently underway into mechanisms causing the abnormal muscle regeneration.

Mechanisms for anti-apoptotic effects of nobiletin in pancreatic β-cells

ノビレチンの膵β細胞に対する抗アポトーシス作用機序の解析

○梶 萌、金子 雪子、石川 智久

静岡県立大・院薬・薬理

The chronic hyperglycemia in type 2 diabetes causes deterioration of pancreatic β-cell dysfunction due to decreases in insulin secretory response and β-cell mass. Nobiletin, a citrus flavonoid, has been reported to improve hyperglycemia and insulin resistance in type 2 diabetic model mice. We previously showed that nobiletin, a citrus flavonoid, exerts insulinotropic and anti-apoptotic effects in pancreatic β-cells through the elevation of cAMP levels. In the present study, we investigated mechanisms for anti-apoptotic effects of nobiletin in INS-1 cells, a rat β-cell line. Endoplasmic reticulum stress was induced by thapsigargin, tunicamycin, or chronic high glucose exposure. The expression of apoptosis-related proteins in INS-1 cells was analyzed by Western blotting. Nobiletin significantly suppressed the elevation of cleaved caspase-3 expression induced by these proapoptotic stimulations. The expression of thioredoxin interacting protein (TXNIP), a regulator of cellular oxidative stress, was also suppressed by nobiletin treatment. Nobiletin slightly restored the decrease in phosphorylated Akt levels induced by thapsigargin or tunicamycin treatment. These results suggest that nobiletin suppresses apoptosis induced by endoplasmic reticulum stress via the degradation of TXNIP, which might be mediated by Akt.

Efficacy of an alkali extract of *Sasa* sp. in a mouse model of acute kidney injury

クマザサアルカリ抽出液の急性腎障害モデルマウスに対する効果の検討

○小関 優太朗[1,2]、柴田 佳太[1,2]、藤澤 知弘[3]、野部 浩司[1,2]

[1]昭和大・院薬・薬理、[2]昭和大・薬理科学研究セ、[3]大和生物研

Acute kidney injury (AKI) is a catastrophic disease with high morbidity and mortality in hospitalized patients and contributes to the pathogenesis of chronic kidney disease. However, there are no approved effective treatment for AKI. There are many causes of AKI, including ischemia, hypoxia, and nephrotoxicity. The primary cause is ischemia reperfusion injury, due to trauma, shock, sepsis, and renal transplantation. Previous studies have shown radical scavenging activity and anti-inflammatory activity of an alkali extract of *Sasa* sp. The present study aimed to evaluate the efficacy of an alkali extract of *Sasa* sp. in AKI model mice. AKI was induced by temporary vascular clamping of the left kidney for 45 min followed by reperfusion, two weeks after removal of right kidney. We have measured the renal function using urine and serum, and morphological assessment. The levels of inflammation markers in the kidney have also been measured by ELISA. An alkali extract of *Sasa* sp. improved the renal function, the damage of renal tubules and exhibited anti-inflammatory in the kidney. These findings indicate that an alkali extract of *Sasa* sp. protects kidney in AKI model mice.

Effects of *Cistanche tubulosa* extract on cervical spondylotic myelopathy

頚椎症性脊髄症に対するニクジュヨウエキスの効果

○須山 真聡、東田 千尋

富山大・院医薬・薬科学専攻

In cervical spondylotic myelopathy (CSM), degenerative changes affecting the vertebrae, intervertebral disks and ligaments compress the spinal cord. Subsequent neuronal damage leads to motor and sensory dysfunctions. Although standard treatment of CSM is surgical decompression, neurological impairment sometimes remains or recurs. Therefore, fundamental therapy that recovers neuronal damage is required.

Our previous study clarified that *Cistanche tubulosa* (CT) extract improved motor dysfunction in spinal cord injury. Since we supposed that the extract might be effective also for CSM, this study aimed to investigate therapeutic effects of CT extract on CSM.

30% ethanol CT extract was orally administered to CSM model mice. Motor and sensory functional changes were observed. Recovery of spinal axons were also evaluated by visualization using neuronal tracers. To clarify functional mechanism of CT extract to axons, we focused on acteoside and echinacoside that are main components in CT extract. After oral administration of CT extract, echinacoside transferred to the spinal cord and brain. Acteoside also transferred to the spinal cord. Therefore, we investigated effects of these components on primary cultured cortical and spinal neurons. Acteoside increased axonal and dendrite densities.

This study showed that acteoside transferred to the spinal cord after oral administration of CT extract, and extended axons *in vitro*. Experiments of CT extract on functional recovery and axonal repairing in CSM model mice are now under investigating.

Metabolic changes in hypothalamic glial cells under pathological conditions of cancer cachexia

がん悪液質病態下視床下部グリアにおける代謝変動

○渡邊 諒[1]、須田 雪明[1,2]、成田 道子[2,3]、松山 富貴子[1,4]、濱田 祐輔[1,2]、葛巻 直子[1,2]、成田 年[1,2]

[1]星薬科大・薬・薬理、[2]国立がん研セ・研・がん患者病態生理、[3]東京医科大・医学総合研・分子細胞治療、[4]日本小動物医療セ

Cancer cachexia affects many patients with terminal cancer. It significantly reduces their quality of life and also affects their survival rate. Biological homeostasis helps the body adapt to stimuli such as stress and environmental changes, and a breakdown of this control mechanism is thought to lead to the development of various diseases. To better understand the fluctuations of biological homeostasis in these disease pathologies, it is important to understand cellular metabolomics. In this study, we focused on metabolic changes in hypothalamic glial cells under conditions of cancer cachexia. As a result, we found that the expression of several mRNAs involved in glycolysis and the TCA cycle were markedly altered in glial cells isolated from the hypothalamic region of cancer cachexia model mice. Based on these results, we speculate that metabolic abnormalities may be induced in hypothalamic glial cells under the pathological condition of cancer cachexia. We are currently investigating the details by a comprehensive metabolomic analysis.

Elucidation of interaction factors between COPD and lung cancer

COPD−肺がん合併症の病態メカニズム解明

○林 恵[1]、中嶋 竜之介[1]、髙橋 宜暉[1,2]、岸本 朋樹[1]、福山 絢美[1]、小笠原 長耀[1]、スイコ メリーアン[1]、甲斐 広文[1]、首藤 剛[1]

[1]熊本大・薬・遺伝子機能応用学分野、[2]熊本大・院医薬・博士課程リーディングプログラム「HIGO」

Chronic obstructive pulmonary disease (COPD) is the third leading cause of death in the world and is characterized by inflammation, emphysema and respiratory dysfunction. The development of radical treatment and the achievement of long-term disease management are urgent issues for COPD. Because COPD is highly complicated with lung cancer and this complication leads to poor prognosis, it is very important to elucidate its pathological mechanism and develop a novel therapeutic strategy. Previous research showed the relationship between lung cancer and COPD-derived inflammation, but not emphysema. Therefore, I selected elastase model and induced lung cancer using tobacco-specific carcinogenesis (NNK) for lung cancer initiation stage model (COPD-NNK) and lewis lung carcinoma (LLC) for lung cancer exacerbation stage (COPD-LLC). In COPD-NNK model, incidence of lung tumors was increased but COPD phenotypes were not exacerbated. Moreover, I found that α7nAChR-p-Akt pathway was activated in these tumors. Additionally, the survival rate was decreased and intratumor T cells and immune checkpoint protein PD-L1-positive macrophages were increased in COPD-LLC model. In contrast, the survival rate was increased in anti-PD-L1 antibody-treated COPD-LLC model. These results suggested that PD-L1-positive macrophages inhibited T cell activity and decreased the survival rate. In this study, I revealed changes in tumor microenvironment and exacerbation mechanism in COPD-lung cancer complication.

Establishing a mouse model of cervical spondylotic myelopathy and effect of acteoside on neurological disabilities in the model

頚椎症性脊髄症のマウスモデルの確立とacteosideによる改善効果

○羽柴 圭悟、東田 千尋

富山大・和漢研

Cervical spondylotic myelopathy (CSM) is the most common cause of neurological disability in worldwide, which is caused by chronic compression of the spinal cord. Mainstay of treatment is surgical decompression, conservative treatment, or symptomatic analgesics. However, many patients end up with substantial residual disability. Disabilities of motor and sensory function are caused by neuronal damage and axonal loss. Therefore, we supposed that accomplishment of axonal growth is the most critical therapeutic strategy for CSM. Since our previous study suggested acteoside as a facilitator of axonal growth in spinal cord injury, this study aimed to investigate effects of acteoside on functional disability in CSM model mice.

The animal models of CSM reported so far have experimental disadvantages, such as difficulties of controlling compressive intensity, and unstable and too long time-course for appearance of motor dysfunction. First, we established a new mouse model of CSM to solve the problems. After laminectomy at C3-C5, the cervical cord was compressed by a mini screw. Motor functions of forelimbs and hind limbs decreased depth-dependently of the compression. The dysfunction was obvious at least 9 days after the compression. At the compressed center, GFAP-positive astrocytes increased, and NF-H positive axonal density decreased compared to sham mice. Since we succeeded establishing CSM model, acteoside was administered orally from 7 days after compression. Motor and sensory functions and histological evaluation are now under investigation.

Effective drug for optic nerve growth in a mouse model of normal tension glaucoma and it‘s mechanism

正常眼圧緑内障モデルマウスにおける視神経伸長を促す薬物とそのメカニズム

○渋江 省吾、東田 千尋

富山大・和漢医薬学総合研究所・神経機能学領域

Glaucoma is a major cause of irreversible blindness worldwide, and is evoked by degeneration and loss of retinal ganglion cells (RGC). Current drug treatments are focused on lowering intraocular pressure, which don‘t accomplish vision recovery. Our laboratory previously found axonal regeneration activity of Drug A (name is closed due to the patent). Therefore, this study aimed to investigate effects of Drug A on optic nerve growth in an optic nerve crush model.

Based on our previous data showing that intravitreal injection of Drug A increased optic nerve density in the optic nerve crush model, this study aims to investigate oral treatability and potency of Drug A.

At first, we evaluated brain penetration of Drug A after p.o. administration. Drug A was detected in the retina, the optic nerve and whole brain at least 6h after p.o. administration.

Immediately after optic nerve crushing, Drug A or vehicle solution was administered orally for 3 weeks. Intraocular pressure measurements and a behavioral vision test were conducted on the day of sacrificing. After that, the retina, optic nerves and brain were dissected and served for histochemical evaluation. The number of retinal ganglion cells and optic nerve termination to the lateral geniculate nucleus were increased by Drug A.

Drug A treatment to primary cultured RGC for 4 days significantly enhanced axonal length. The mechanism of Drug A for axonal growth of RGC is under investigation.

Sleep homeostasis of a monophasic sleep in reptiles

単相性睡眠をとる爬虫類を用いた睡眠恒常性維持機構の解明

○羽鳥 聖七、山口 翔、松井 双葉、周 至文、乗本 裕明

北海道大・医・細胞薬理学教室

Sleep is essential for human beings to live a healthy life and to build a healthy society. However, there are few efficient drugs or treatments for sleep disorders since the detailed neuronal mechanisms associated with sleep are unknown. In particular, monophasic sleep at night as human is less understood due to a lack of model organisms. For example, most rodents used for life science research are nocturnal and take a polyphasic sleep. Recently, we introduced a reptile, Australian bearded dragon *Pogona vitticeps* as a new model organism for sleep research. *Pogona* is diurnal and takes a monophasic sleep at night with periodic slow wave sleep (SWS) and REM sleep alternation. Furthermore, electrophysiological experiments and pharmacological treatment can be applied without much difficulty as in rodents. It may be suitable for sleep research, however, the basic properties of sleep in *Pogona* remain elusive. In this study, we examined whether sleep homeostasis is observed in response to sleep deprivation (SD). 7 hours of SD by light and gentle handling at night showed delayed wake-up time compared with a control group, suggesting the presence of sleep homeostasis in *Pogona*. Furthermore, LFP analysis showed that SD affected the periodic oscillation of SWS and REM sleep, as in humans.
These results indicate that the features of sleep, especially sleep homeostasis in each sleep stage are similar between reptiles and mammals. Observation/manipulation of reptilian sleep may open a new avenue for understanding the fundamental functions and circuit mechanisms of monophasic sleep.

Construction of minimal neural circuits by iPSC-derived astrocytes for glial drug discovery

神経変性疾患の病態解明に資するiPS細胞由来アストロサイトによる最小神経回路の構築

○内野 鉱也[1]、田中 泰圭[2,3,4]、川口 紗果[1]、窪田 香織[1]、渡辺 拓也[1]、桂林 秀太郎[1,4]、廣瀬 伸一[3,4,5]、岩崎 克典[1]
[1]福岡大・薬・臨床疾患薬理学教室、[2]第一薬科大・薬・薬物治療学分野、[3]株式会社iONtarget、[4]福岡大・てんかん分子研究所、[5]福岡大・医・総合医学研究センター

Astrocyte is a type of glial cell involved in synaptic transmission and the formation and maturation of synapses. Currently, the establishment of induced pluripotent stem cells (iPSCs) allows the differentiation of stem cells into various types of cells while preserving the patient phenotype. Therefore, patient iPSCs replace animal models in pathological analysis and drug discovery. Technological advances have provided access to human iPSC-derived astrocytes (HiAs). Furthermore, neurons co-cultured with pathological astrocytes have been used to study their morphology, protein levels, and spontaneous synaptic responses. However, these studies did not investigate detailed synaptic functions such as synaptic transmission evoked by electrical stimulation and morphological analysis at the single neuron level. In this study, we established autaptic cultures with HiAs (HiAs Autaptic Cultures, HiAACs), single neuron cultures grown in isolation on microislands of HiAs that form synapses exclusively with themselves. We found that neurons in HiAACs develop morphologically by co-culture with HiAs and form functional synapses that exhibit excitatory postsynaptic currents. Although we used healthy astrocytes in this study, HiAACs can be used to study various diseases by using patient-derived astrocytes in the future.This work was supported by funding from JSPS, AMED, MEXT, the Science Research Promotion Fund and The Fukuoka University Fund, and Kyushu University Hospital.

Cellulose rich food induces intestinal disturbance, anxiety-like behavior and amygdalar dopaminergic hyperactivity in mice.

セルロースは腸内環境の悪化を引き起こし、マウスの不安様行動および扁桃体におけるドーパミン神経系の作用を亢進する

○伊東 楓[1]、原口 敦嗣[1]、野崎 千尋[1,2]、柴田 重信[1]

[1]早稲田大・先進理工・電気・情報生命、[2]早稲田大・国際理工学センター・Major in bioscience

It is indicated that the intestinal environment affects the brain, which is known as the gut-brain axis. In fact, improvement of intestinal environment is reported to suppress anxiety-like behavior in mice model of depression and schizophrenia. However, its detailed mechanisms remain unclear, particularly in context of intestinal environment effects on emotion in non-disease mice. The object of this experiment is therefore to verify the effect of intestinal environment on anxiety-like behavior and its detailed physiological mechanisms. We previously found that cellulose rich food (AIN-93M) suppresses the production of short-chain fatty acid and exacerbate the intestinal condition. In the present study, we fed mice with AIN-93M to modify the intestinal environment, or the mouse chow that can maintain a favorable intestinal environment (MF). After 8-weeks feeding, AIN-93M-fed animals displayed the significant increase of marble-burying behavior compared to MF-fed group, suggesting that dysfunction of intestine lead by AIN-93M enhanced anxiety-like behavior. Furthermore, dopamine release as well as dopamine receptor expression has been increased in amygdala of AIN-93M fed mice but not in MF animals, indicating that enhanced anxiety-like behavior in AIN-93M animals is due to such modification of amygdalar dopaminergic system.
These results suggest that cellulose rich food may exacerbate intestinal environment, which may enhance the anxiety-like behavior and overactivation of dopaminergic system.

Circulating apolipoprotein B-100 promotes scar formation after spinal cord injury

血中apolipoprotein B−100による脊髄損傷後の瘢痕形成促進作用

○米津 好乃[1,2]、田辺 章悟[1]、三澤 日出巳[2]、村松 里衣子[1]

[1]国立精神・神経医療研究セ・神経研・神経薬理、[2]慶應義塾大・院薬・薬理学

Spinal cord injury (SCI) causes severe neurological dysfunction depending on the neuronal network disruption. Neuronal network regeneration is prevented by the scar tissue formed around the lesion; therefore, inhibition of scar formation is considered to contribute to neuronal network regeneration, resulting in functional recovery. It is known that the scar is composed of several cell types. Among them, recent studies indicated that pericytes act as key players in scarring triggered by their proliferation. Thus, understanding the molecular mechanism regulating pericyte proliferation may be useful to promote neuronal network regeneration after SCI; however, the mechanism is not fully elucidated. Here we focused on the disruption of vascular barrier, a histological feature around the lesion, and found that circulating apolipoprotein B-100 (ApoB-100) promotes pericyte proliferation which contributes to the scar formation after SCI. CRISPR-Cas9 knockout screens with primary mouse pericytes identified that ApoB-100/low-density lipoprotein receptor (LDLR) signal promotes pericyte proliferation. ApoB-100 knockout mice exhibited a decrease in pericyte-derived scar formation and a significant improvement of motor function after SCI. These results suggest that circulating factors, especially ApoB-100, could be a novel therapeutic target for treating SCI.

Chronic social stress-induced transition of microglial transcriptome states in mice

マウスの慢性社会ストレスによるミクログリアの遺伝子発現状態遷移

○三島 零、谷口 将之、松下 和敏、古屋敷 智之

神戸大・院医・薬理

Microglia-derived neuroinflammation has been associated with the stress pathology of mental illness. However, the nature of microglial stress responses remains poorly understood. Using single-cell transcriptome analyses, we found that acute and chronic social defeat stress altered the transcriptome of microglia in multiple brain areas, including the medial prefrontal cortex (mPFC), primary motor and sensory cortices, hippocampus, nucleus accumbens, and hypothalamus, in mice. Despite some brain region-specific patterns, individual variability of stress-susceptibility emerged broadly across the brain areas. We further analyzed transcriptomes of mPFC microglia, the activation of which are essential for chronic stress-induced depression-like behavior, and identified several transcriptomic states of microglia, through which chronic stress promoted the transition from a homeostatic state to a distinct state with brain region-specificity and stress susceptibility signature. These findings demonstrate multiple states of microglia reflecting brain area specification and stress susceptibility, the transition of which may contribute to the stress pathology of mental illness.

The Use of an Amyloid beta Intracerebroventricular Model to Investigate the Interaction between Alzheimer's Disease and Circadian Dysfunction

アミロイドベータ脳室内投与モデルによるアルツハイマー病と概日リズム障害の相互作用の検討

○ラベル リンダ[1]、野崎 千尋[2]、田原 優[3]、柴田 重信[1]

[1]早稲田大・理工・電気・情報生命工学科、[2]早稲田大・国際理工学センター・Major in Bioscience、[3]広島大学大学院・医系科学研究科・公衆衛生学

There are 40 million people worldwide living with Alzheimer's Disease (AD), and by 2050 the prevalence is expected to increase to 150 million people, thus resulting in a 1 in 2 chance of having AD by the age of 85. An increase in amyloid-b plaques (Ab) and hyperphosphorylated tau are widely accepted as the core indicators of the disease. These indicators of AD onset and progression are becoming increasingly attributed to the disturbance of optimal sleep/wake cycles. Additionally, AD progression exacerbates normal sleep/wake cycles, resulting in a cyclical worsening of AD pathology, circadian rhythm, and cognitive dysfunction. By using an Ab intracerebroventricular (ICV) injection mouse model, mice can present an AD-like pathology within days to weeks after injection, allowing for the expedited examination of the disease. This model can serve as a tool to investigate the mechanism behind the disturbance of the biological clock and AD progression, and therefore find therapeutic targets to delay, prevent or cure the disease.

Food-derived hydrophilic amino acid ergothioneine prevents cognitive decline in Alzheimer‘s disease model mice at its clinically relevant plasma concentrations.

食物由来水溶性アミノ酸ergothioneineはヒトで到達しうる血中濃度でアルツハイマー病モデルマウス記憶障害を予防する

○松尾 雄介[1]、石本 尚大[1]、増尾 友佑[1]、笹栗 弘貴[2]、齊藤 貴志[2]、西道 隆臣[2]、加藤 将夫[1]

[1]金沢大・薬学系、[2]理研・脳神経科学研究セ

Progressive pathology of Alzheimer's disease (AD) including aggregation of amyloid beta (Aβ) appears decades before the onset of dementia. Therefore, early intervention before the appearance of the symptoms is crucially important. We focused on food-derived hydrophilic amino acid ergothioneine (ERGO) because oral ERGO administration enhances cognitive function in normal mice. The purpose of the present study is to elucidate the preventive effect of ERGO on cognitive decline in the AD model, App^{NL-G-F} mice expressing humanized Aβ. App^{NL-G-F} mice were orally administered ERGO or vehicle weekly between 5 weeks to 7 months of age. Novel object and spatial recognition tests showed that ERGO administration significantly improved the cognitive declines in the App^{NL-G-F} mice. ERGO concentration in plasma of App^{NL-G-F} mice at 3 months of age reached a steady state around 10 μM, which was close to that reported in humans orally administered ERGO. Proteome analysis of the hippocampus samples showed that 71 and 91 proteins were significantly up- and down-regulated more than two-fold by ERGO treatment, respectively. Further enrichment analysis revealed that neurogenesis-related proteins were significantly enriched. Immunochemical analysis in the hippocampus showed that area of newborn neuron marker doublecortin-positive cells in the ERGO-treated App^{NL-G-F} mice was significantly higher than that in vehicle-treated mice. These results suggest that ERGO would prevent cognitive decline at least partially via the promotion of neurogenesis in App^{NL-G-F} mice.

Synaptotagmin 4 contributes to spontaneous regeneration of neural networks after spinal cord injury

*Synaptotagmin 4*による脊髄損傷後の神経回路の修復

○樋口 京香[1,2,3]、田辺 章悟[1]、成田 年[3,4]、村松 里衣子[1]

[1]国立精神・神経医療研究セ・神経薬理研究部、[2]東京医科歯科大・院医歯・NCNP脳機能病態学、[3]星薬科大・薬・薬理、[4]国立がん研・がん病態生理

Spinal cord injury (SCI) causes severe neurological dysfunction such as motor deficits and sensory impairments caused by the disruption of neuronal networks. Injured neurons in the central nervous system spontaneously regenerate their neuronal network, resulting in promoting functional recovery. However, the regenerative capacity in the adult central nervous system is limited, therefore, understanding the mechanism that promotes regeneration of the neuronal network is required to establish a strategy for restoring motor function after SCI. In this study, we explored the factor that promotes neurite elongation using *in vitro* siRNA-screening and found that *Synaptotagmin 4* (*Syt4*) has a potential to promote neurite elongation. We detected that *Syt4* is mainly expressed in neurons in the brain compared with other organs. To ask the function of *Syt4* in vivo, we injected adeno-associated virus 9 (AAV9) encoding shRNA against *Syt4* into the motor cortex and found that suppression of *Syt4* prevented regeneration of neuronal network and functional recovery in the mice with SCI. These results indicate that *Syt4* expressing in neurons sustains the spontaneous regeneration of the neuronal network in the adult central nervous system.

Interaction of Munc13-1 and RIM that contributes to the formation/maintenance of synaptic vesicle release sites

シナプス小胞放出サイトの形成に寄与するMunc13-1とRIMの相互作用

○小島 佑介、坂本 寛和、並木 繁行、廣瀬 謙造

東京大・院医・細胞分子薬理学

Neurotransmitter release is regulated by several proteins localized at the active zones of presynaptic terminals. Munc13-1 is a multi-domain active zone protein which redundantly interacts with other active zone proteins including RIM. We have previously shown that Munc13-1 forms supramolecular nanoassemblies that function as the synaptic vesicle release sites. Here, we aimed to clarify a more detailed molecular mechanism of the formation/maintenance of Munc13-1 nanoassemblies by intervening in the interaction of Munc13-1 with RIM. For this purpose, we isolated the Zn^{2+} finger domain of RIM (RIM-ZF), which interacts with the C_2A domain of Munc13-1 and expressed it in neurons to competitively inhibit the binding of endogenous Munc13-1 and RIM. Glutamate imaging revealed that the expression of RIM-ZF in cultured neurons caused a significant decrease in neurotransmitter release, although a previous study showed that the RIM-ZF partially rescues suppressed neurotransmitter release in RIM knock-out neurons. Furthermore, quantitative immunocytochemical analysis with super-resolution microscopy revealed that the expression of RIM-ZF altered the nanoscale distribution of Munc13-1 molecules at the active zones. Thus, our results suggest that a direct interaction of RIM-ZF with Munc13-1 itself is incomplete for the appropriate formation of synaptic vesicle release sites, and that cross-linkage of RIM-ZF to the other domains of RIM is necessary for the precise positioning of Munc13-1 at the active zone.

Skeletal muscle atrophy-induced hemopexin accelerates cognitive dysfunction in 5XFAD mice

骨格筋萎縮により分泌が増加するヘモペキシンは5XFADマウスの認知障害発症を加速する

○井城 綸沙、東田 千尋

富山大・和漢医薬学総合研究所・神経機能学領域

Physical inactivity is one of risk factors for Alzheimer's disease (AD). Performing physical exercise is difficult at old age, and thus, decline in physical movement may be a cause of age-associated lowering of the brain function. This study aimed to elucidate the onset of the skeletal muscle atrophy-induced acceleration of AD and its molecular mechanism.

Presymptomatic AD model (5XFAD) mice were used. The bilateral hindlimbs were immobilized by cast-attachment for 14 days. Wet weight of hindlimbs muscles were significantly lower in cast-attached 5XFAD mice than those in non-cast mice. At the same time, object recognition memory in the cast-attached 5XFAD mice was impaired. The hindlimb muscles were organ cultured. And the conditioned media (CM) was separated by 2D-PAGE and analyzed by MALDI-TOF MS. The most increased spot in the cast-attached muscle CM was hemopexin. Hemopexin levels in the skeletal muscle, plasma, and hippocampus were increased in cast-attached 5XFAD mice. Continuous i.c.v. infusion of hemopexin for 2 weeks induced memory deficits in young 5XFAD mice. Gene microarray analysis of the hippocampus was performed to investigate the molecules involved in memory deficit. Lipocalin-2 (Lcn2) mRNA, neuroinflammation-associated factor, was increased in the hippocampus in hemopexin-infused 5XFAD mice compared to in control mice.

These findings provide new evidence indicating that skeletal muscle atrophy has an unbeneficial impact on the occurrence of memory impairment in 5XFAD mice, which is mediated by skeletal muscle atrophy-driven hemopexin.